32nd Symposium and Annual Meeting of the

International Society for Ceramics in Medicine

BOOK OF ABSTRACTS

bioceramics|32

SEPTEMBER 20-23, 2022 - VENICE *MESTRE*, ITALY

The Venice photos present in the Book of Abstracts are edited by Giacomo Scarpa, *@venezialayout*

WELCOME TO BIOCERAMICS 32

Dear Colleagues and friends,

it is a great pleasure for me to open the 32nd Edition of BIOCERAMICS congress, which is the first meeting after COVID 19 crisis and a long period of isolation where our contact of collaboration and friendship was confined within virtual tools.

Indeed, Pandemic has greatly influenced our life, our countries, our families and penalized all research institutions, industrial sectors, and communities engaged to tackle the crisis.

The top priority in the last years has been to ensure citizens' health and safety and to secure materials supplies into medical, environmental and food supply chains amidst border closures and lockdown measures. From such an experience we have become aware of the importance in supporting global co-operation and global markets and in this respect, it was even more evident that Materials are everywhere! Besides the great challenges the crisis has also created opportunities for re-directing worldwide the R&D&I efforts and the Materials community has a paramount importance offering a variety of solutions towards a new global economic model: the most important area of investigation now are:

- Materials modelling for the design of antibacterial and antiviral surfaces, coatings, and paints; for the development of vaccines and drugs; for the understanding and predicting the behaviour of SARS-CoV-2 Virus and accelerated materials development.
- Digitalisation and Artificial Intelligence; in times of confinement and social distancing, our technological ability to support digital working has enabled organizations to maintain activities, minimizing traveling while carrying out substantial work.
- Circular economy: materials recovery & waste re-/upcycling technologies are necessary while providing safe solutions for residues. Additive manufacturing technologies will be useful for recycled, re-designed and reshaped materials manufacturing.

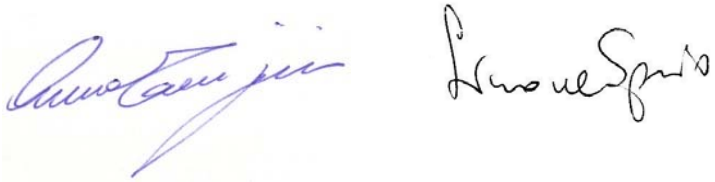
One major lesson learned from the Corona crisis is the need to create a less dependent, more resilient economy by guaranteeing raw materials supplies, by ensuring higher materials durability, higher energy efficiency, higher degrees of materials re-cycling and re-use, and by materials-saving through optimized products "by design".

In this scenario Biomaterials are very important specifically addressing healthcare challenges, such as: a) long-lasting and selectively-active paints, coatings, surfaces to avoid infection; b) improved air and water filtration, c) biodegradable selective packaging materials with antimicrobial properties, d) low cost, ultra-fast scalable detection methods for viral particles, e) enhanced personal protection equipment (PPE) including facemasks, f) diagnostic devices integrated in the form of a patch.

Starting from this considerations, we have introduced new emerging topics into Bioceramics32 giving to the conference enhanced transdisciplinary character.

Our main goal has been to highlight the key role of ceramic-based biomaterials in fostering new sectors of Medicine, particularly for Tissue Regeneration, Nanomedicine for Theranostics, and new emerging topics including *"Antibacterial Materials"*, *"Nanosafety"*, *"Skin Care"* and *"Circular Economy"* caring the presence of material scientists and clinicians from all over the world, and including special Scientific Sessions, Workshops and Round Tables organized by companies and EC-funded projects. Particularly, we will host a workshop focusing on Bioceramics in Neurosurgery, as well as other two European Workshops: the first held by the project AIMed, focused on Antimicrobial Integrated Methodologies, the second on Nanosafety, dealing with Safe and Sustainable by Design Paradigms applied to (Nano) materials.
As a most relevant innovation, we have introduced a new concept of presentations, in the form of Plenary Relay Sessions, where clinicians and material scientists share the same podium, describing clinical needs and giving adequate solutions to them.
Our intent was to push the communication between material scientists and clinicians, as the most effective way to pursue research objectives able to really meet the clinical needs of our society.
With this, I trust that the conference will be a great opportunity for all of us to start or rekindle fruitful scientific relations.

Anna Tampieri and Simone Sprio



Summary

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Alberto BALLARDINI (GreenBone ortho srl, Italy)
Giovanni BARBANTI-BRODANO (Rizzoli Orthopedic Institute, Italy)
Aldo BOCCACCINI (University of Erlangen-Nürnberg, Germany)
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KEYNOTE SPEAKERS

Simeon AGATHOPOULOS (University of Ioannina, Greece)
Matthias AHLHELM (Fraunhofer Institute for Ceramic Technologies and Systems, Germany)
Iulian ANTONIAC (Polytechnic University of Bucharest, Romania)
Conrado APARICIO (Universitat International de Catalunya, Spain)
Bikramjit BASU (Indian Institute of Technology, Bangalore, India)
Marc BOHNER (RMS Foundation, Switzerland)
Giulia BRUNELLO (Universitätsklinikum Düsseldorf**,** Germany**)**
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ORGANIZERS AND SPONSOR

Anna Tampieri, **Chair of the Conference**, has 30 years of experience in the development of innovative nature-inspired processes and various biomimetic materials from bioceramic nanoparticles to 3-D scaffolds, addressed to bone regeneration and nanomedicine. Anna was project coordinator or WP Leader of 15 EC-funded projects, author of over 300 scientific papers and inventor of 15 international patents. Establishing close relationship with orthopaedic clinicians and biomedical companies, Anna sustained the translation of various innovative and effective biomedical products to the clinics, particularly new hydroxyapatite implants for large cranial reconstruction and hybrid scaffolds for osteochondral regeneration.

Co-Chairs:
Simone Sprio, Senior Researcher at the Institute of Science and Technology for Ceramics (ISTEC-CNR)
Corrado Piconi, Associate Researcher at the Institute of Science and Technology for Ceramics (ISTEC-CNR)

The National Research Council of Italy **(CNR)** is a national public body with the mission of carrying out, promoting, disseminating, transferring, and enhancing research activities in the main areas of knowledge development and their applications for the scientific, technological, economic and social development of the country. **ISTEC-CNR** is the largest Italian institute specifically addressed to the global study of ceramic materials.

ACKNOWLEDGMENTS

The Organizing Committee would like to acknowledge particularly the financial supporters (JECS Trust, TATASteel, Finceramica, Greenbone Ortho, RMS, TOSOH Bioscience, QI technologies, Zeiss, Frontiers Journal and LITHOZ) and the institutions which helped us at different levels: The National Research Council of Italy (CNR): Institute of Science and Technology for Ceramics (ISTEC-CNR), CNR Department of Chemical Sciences and Materials Technology (DSCTM-CNR), CNR Department of Biomedical Sciences (DSB-CNR), the International Society for Ceramics in Medicine (ISCM), the European Ceramic Society (ECerS), the American Ceramic Society (ACerS), the European Society for Biomaterials (ESB), Società Italiana Biomateriali (SIB), Processes (an Open Access Journal by MDPI), the Italian Society of Arthroscopy, Knee, Upper Limb, Sports, Cartilage and Orthopaedic Technologies (SIAGASCOT), the European Orthopaedic Research Society (EORS), the Italian Society of Orthopaedics and Traumatology (SIOT), and the European Society for Clinical Investigation (ESCI).

GENERAL INFORMATION

BADGE

Badges denoting registration status will be given to all participants upon their check-in at the registration desk. Participants will not be admitted to any scientific session without their badge.

OFFICIAL LANGUAGE

The official language of the Congress is English. Simultaneous translation will not be provided.

TIMING

Oral presentations will last 15 minutes, 12 of talk plus 3 minutes of Q&A.
Keynote presentations will last 20 minutes, 17 of talk plus 3 minutes of Q&A.
Plenary presentations will last 30 minutes.
Posters will be exposed for the whole duration of the conference and they will be open for discussion during coffee breaks and lunch time.

PASSPORT AND VISAS

All foreign visitors must have valid passport. No visas are required for visitors from Western European countries, the United States, Japan, and many Latin American and British Commonwealth countries. Citizens from other countries must have the required visas. However, it is advisable to contact the Italian Consulate or Embassy in your country for information on visas and health certificates if required.

LETTERS OF INVITATION

Requests for Letters of Invitation for VISA purposes should be directed to the Organizing Secretariat.
Please note that such letter cannot grant any financial support. The Letter will be sent after the payment of the registration fee.

INSURANCE

The Organizing Secretariat cannot accept responsibility for accidents or damage to participants or accompanying persons before, during or after the Congress. Participants are urged to make their own arrangements with respect to travel and health insurance.

WI-FI

Free internet Wi-Fi connection will be provided everywhere in the Conference venue areas.

MOBILE PHONES

Participants are kindly requested to keep their mobile phones in off position in the rooms where scientific sessions are being held.

CERTIFICATE OF ATTENDANCE

Participants can print their own certificate of attendance at self-service kiosks in the Congress center.

NOTICE OF FILMING AND PHOTOGRAPHY

It is strictly forbidden to take pictures and/or video during the Congress Presentations. This be permitted only during the Gala Dinner, the Opening Ceremony and during the Awards Ceremony.

AUDIO-VISUALS AIDS

Each room will be equipped with personal computers for the video presentation. The slides should be prepared with the program Windows PowerPoint – **not** Macintosh / Apple – with 800×600 or 1024×768 resolution. The presentations should be delivered on USB pendrive. It is not allowed to use your own computer.
The delivery of the slides to the Slide Center will be made in time before the corresponding session.

IMPORTANT NOTE

In order for the conference program to be respected, strict compliance with all the times indicated is essential. Speakers are therefore requested to strictly follow the assigned times. The Chairmen, in addition to the thanks for having accepted such a heavy burden, should also be prayed to be inflexible in enforcing the scheduled hours.

PRIVACY POLICY

Aristea International S.r.l. handles all personal data according to the laws of the EU-Data Protection Regulation (EU-GDPR) and the Italian Legislative Decree no. 196/2003.
For your registration to the Congress we have to collect, save and process your personal data, and we need you consent.
For our privacy policy, please consult the following link: Privacy and Cookie Policy | BIOCERAMICS 32

REFRESHMENT AND MEALS

Lunches as well as coffee breaks will be served at the scheduled times, in the form of a buffet.

GALA DINNER

At the time of registration to the conference (on Tuesday), the participants will have the opportunity to register for the Gala Dinner on site.

TECHNICAL INFORMATION

The three Conference rooms will be opened before sessions in order to allow you to download your power point or PDF presentation with enough anticipation with the help of the chairperson assistants. All presentation will be removed at the end of the Congress.

Poster boards will be numbered according to the Book of abstracts. The posters will not be removed until the last coffee break on September 23rd. Posters left on the boards after the closing ceremony will be removed by the conferences staff and trashed. Material for posting your poster will be offered by the congress organization

AWARDS

HIRONOBU OONISHI MEMORIAL AWARD OF ISCM

At this 32nd edition of BIOCERAMICS the fifth "*Hironobu Oonishi Memorial Award*" will be delivered.

Dr. Hironobu Oonishi was one of the founders of the International Society for Ceramic in Medicine (ISCM) and the Co-chairperson of the 1st annual meeting of ISCM held to Kyoto (Japan), in the 1988. He was an excellent hip surgeon and was well known as having discovered that irradiated polyethylene exhibited resistance against wear in joint replacements. After serving as Director of the clinic "Hironobu Oonishi Memorial Center for Joint Replacement and its Research" in Osaka, Japan, he passes away on November 1st, 2014 at the age of 79.

BEST ORAL/POSTER AWARDS

JECS Trust has made available funds to cover travel and accommodation costs for students. Part of these funds will be granted to students presenting the best 3 orals and 3 posters. The best 3 oral and 3 poster presentations will be anyway awarded with a certificate. If one or more winners are already entitled to receive the *JECS Trust* funding, the financial contribution from *JECS Trust* will be given to the next runners up.

PROGRAM AT A GLANCE

	TUESDAY, SEPTEMBER 20, 2022		
	ROOM 1	**ROOM 2**	**ROOM 3**
10.00	Registration open		
14.00	Opening ceremony		
14.15	***Chairs: S. Sprio & K. Ishikawa***	**PLENARY 1** – *Y. Tabata*	
14.45	**PLENARY 2** – *H. Zreiqat*		
	S1_CaP BIOCERAMICS FOR REGENERATIVE MEDICINE ***Chairs: S. Sprio & K. Ishikawa***	**S2_ADDITIVE MANUFACTURING OF BIOCERAMICS** ***Chairs: L. Ambrosio & S. Farè***	**S3_CELL-MATERIALS INTERACTION AND CO-CULTURES** ***Chairs: S. Panseri & Y. Tabata***
15.15	***Keynote*** – *C. Drouet*	***Keynote*** – *M.P. Ginebra*	***Keynote*** – *T. Ibrahim*
15.35	***Keynote*** – *J. Chang*	***Keynote*** – *M. Ahlhelm*	***Keynote*** – *M. Kawashita*
15.55	M. Merle	F. E. Weber	Z. Vuslat Parlak
16.10	A. El-Ghannam	A. F. Montero	D. Shepherd
16.25	Coffee Break / Poster Session / Exhibition		
	Chairs: C. Drouet & M. Iafisco	***Chairs: J. Rau & C. Combes***	***Chairs: M. Kawashita & F.E. Weber***
16.45	***Keynote*** – J. Locs	***Keynote*** – R. Gadow	**16.45** ***Keynote*** – *S. Farè*
17.05	M.G. Raucci	L. Johansson	**17.05** ***Keynote*** – *S. Panseri*
17.20	C. Stähli	J. Homa	**17.25** R. Manser
17.35	K. Ishikawa	L. Del-Mazo-Barbara	
17.50	Y. Maazouz	A. C. Sousa	**17.55** E. Cichoń
18.05	K. Späth	I. Bouakaz	***18.10*** A. Abélanet
18.20	L. Degli Esposti	M. Montanari	**18.25** F. Tibourtine
18.35	V. Kumar Dewangan		**18.40** M. R. Iaquinta
18.55	**WELCOME RECEPTION**		
20.00	End of the first day		

WEDNESDAY, SEPTEMBER 21, 2022

08.20	***Chairs: M. Bohner & A. Tampieri***	**PLENARY 3** – *M. Marcacci*		
	PLENARY RELAY SESSION			
08.50	**Cranial reconstruction**	*F. Servadei*	*M. Dapporto*	
09.30	**Osteochondral regeneration**	*E. Kon*	*M. Alini*	
10.10	Coffee Break / Poster Session / Exhibition			
10.30	**Long bone regeneration**	*P. Giannoudis*	*A. Ballardini*	
11.10	**Spinal fusion**	*G. Barbanti-Brodano*	*S. Leeuwenburgh*	
11.55	**Tata Steel Medical Materials Business**			

	ROOM 1	ROOM 2	ROOM 3	ROOM 4
	S1_ CaP BIOCERAMICS FOR REGENERATIVE MEDICINE ***Chairs: M.P. Ginebra & J. Locs***	**S5_SMART NANOSYSTEMS & ANTIBACTERIAL DEVICES** ***Chairs: B. Basu & C. Vitale-Brovarone***	**S9_BIOCERAMICS FOR DENTAL APPLICATIONS** ***Chairs: MHP da Silva & J. Chevalier***	**WORKSHOP POROUS HYDROXYAPATITE CRANIOPLASTY: EFFICACY AGAINST INFECTION AND PAEDIATRIC INDICATIONS** ***Sponsored by: FINCERAMICA***
12.20	***Keynote*** *– M. Bohner*	***Keynote*** *– P. Ducheyne*	***Keynote*** *– J. Tartsch*	**11.00** *Chair: F. Servadei*
12.40	***Keynote*** –A. Leriche	***Keynote*** *– J. Rau*	***Keynote*** *– S. Leeuwenburgh*	**11.10** *L. Visai*
13.00	C. Ohtsuki	H. Mabroum	D. Bomze	**11.35** *M. Ganau*
13.15	T. Yokoi	C. Combes	N. Hosseinabadi	**12.00** *P. Frassanito* **12.25** *P. Costantino* **12.50** *Discussion*
13.30	Lunch / Poster Session / Exhibition			
15.00	***Chair: B. Basu***	**PLENARY 4** – *L. Visai*		

	S4_ANTIBACTERIAL BIOCERAMICS FOR SMART PROSTHETIC APPLICATIONS – ***Sponsored by ACERS*** ***Chairs: R. Narayan & I. Antoniac***	S5_SMART NANOSYSTEMS & ANTIBACTERIAL DEVICES ***Chairs: P. Ducheyne & M. Alini***	S9_BIOCERAMICS FOR DENTAL APPLICATIONS ***Chairs: C. Mortellaro & M. Del Fabbro***
15.30	***Keynote*** *– A. El-Ghannam*	***Keynote*** *– G. Maccauro*	**15.30** ***Keynote*** *- S. Taschieri*
15.50	G. Graziani	G. Baldi	**15.50** ***Keynote*** *- G. Tartaglia*
16.05	V. Müller	E. Cansever Mutlu	**16.10** ***Keynote*** *- G. Brunello*
16.20	S. Begand	M. Iglesias-Fernandez	**16.30** A. Kocjan
16.35	S. Fournier	N. Hassani Besheli	**16.45** A. A. Porporati
16.50		A. Marfoglia	
17.05	Coffee Break / Poster Session / Exhibition		

	S1_CaP BIOCERAMICS FOR REGENERATIVE MEDICINE ***Chairs: J. Gomez-Morales & N. Döbelin***	S5_SMART NANOSYSTEMS & ANTIBACTERIAL DEVICES ***Chairs: P. Palmero & A. El-Ghannam***	S9_BIOCERAMICS FOR DENTAL APPLICATIONS ***Chairs: S. Leeuwenburgh & J. Tartsch***
17.25	***Keynote*** *– I. Cacciotti*	**17.25** ***Keynote*** *– M. Iafisco*	***Keynote*** *– C. Aparicio*
17.45	***Keynote*** *– C. D'Arros*	**17.45** A. Barroug	***Keynote*** C. Knabe
18.05	A. Fantou	**18.00** F. Vergnaud	H. Abdelrazik
18.20	K. Hurle	**18.15** A. D'Urso	K-H. Yoo
18.35	L. Cuypers	**18.30** E. Cianflone	S. Kamakura
18.50	I. Touaiher	**18.45** B. Parra-Torrejon	M. Di Foggia
19.20	End of the second day		

THURSDAY, SEPTEMBER 22, 2022

	ROOM 1	ROOM 2	ROOM 3
08.30	***Chair: A. Boccaccini***	**PLENARY 5 –** *J. Chevalier*	
	S4_ANTIBACTERIAL BIOCERAMICS FOR SMART PROSTHETIC APPLICATIONS – *Sponsored by ACERS* ***Chairs: C. Piconi & E. Champion***	**S6_HYBRID AND COMPOSITE BIOCERAMICS FOR BONE & OSTEOCHONDRAL REGENERATION** ***Chairs: M. Alini & M. Sandri***	***Workshop H2020 ITN Project AIMed – Antimicrobial Integrated Methodologies***
09.00	***Keynote*** *– B. Basu*	***Keynote*** *– J. Gomez-Morales*	***9.00*** ***Chair*** *- A. Stamboulis*
09.20	***Keynote*** *– P. Palmero*	***Keynote*** *– C. Vitale-Brovarone*	***9.05*** *Invited*
09.40	N. Döbelin	L. Sabio	***9.25*** *Invited*
09.55	W. Xia	S. Meille	***9.45*** *Invited*
10.10	K. Hans	S. Skibiński	***10.05*** *Invited*
10.25	A. Daskalova	N. Elahpour	***10.25*** *Round Table (**Chair**: C. Drouet,* on-stage participants: A. Boccaccini, B. Basu, A. Braum, A. Stamboulis, D. Grossin)
10.40	A. Corozzi		
11.00	Coffee Break / Poster Session / Exhibition		
	Chairs: A. Adamiano & R. Pullar	***Chairs: H. Zreiqat & M. Ahlhelm***	***S12_NANOSAFETY TOOLS IN SUPPORT TO REGULATION*** ***ASINA 1st Stakeholder Workshop: Safe and sustainable by design Paradigms applied to (Nano)materials***
11.20	***Keynote*** *– I. Antoniac*	**11.20** F.J. Acebedo-Martinez	
11.40	**Keynote** *– T. da Ros*	**11.35** F. Banche Niclot	
12.00	K. Schickle	**11.50** J. Lao	
12.15	M. Imariouane	**12.05** E. Campodoni	**11.00** Welcome - **chair**: *A. L. Costa*
12.30	M. Pavarini	**12.20** R. Rodriguez-Gonzalez	**11.10** *ASINA Project: A. L. Costa*
12.45	T. Oberbach	**12.35** H. Mabroum	**11.20** *SAbyNA Project: S. Vàzquez-Campos*
13.00	Lunch / Poster Session / Exhibition		**11.30** *SABYDOMA Project: T. Chamberlain*
14.30	***Chairs: J. Jones & S. Sprio*** **PLENARY 6** *– A. Boccaccini*		**11.40** SbD4Nano *Project: C. Fito*
15.00	**PLENARY 7** *– B. Fadeel*		**11.50** HARMLESS *Project: W. Fransman*
15.30	**Plenary Talk – H. Oonishi Award –** G.Daculsi		**12.00** SUNSHINE *Project: D. Hristozov*
	S7_Commemorative Session: BIOCERAMICS AND GLASS TECHNOLOGY ***Chairs: S. Agathopoulos & M. Lasgorceix***	**S8_INNOVATIVE PROCESSES AND CERAMICS** ***Chairs: A. Tampieri & C. Piccirillo***	**12.10** DIAGONAL *Project: S. Resch* **12.20** SusNanoFab *Project: M. Cioffi* **12.30** NanoFabNet *Project: S. Friedrichs* **12.40** IRISS *Project: C. Rocca* **12.50** Classification criteria for SSbD tools (OECD): *A. Sanchez*
16.00	***Keynote*** *– J. Jones*	***Keynote*** *– S. Sprio*	**13.00 Lunch**
16.20	J. Turner	G. Bertrand	**14.30** JRC Framework for SSbyD of chemicals: L. *Farcal*
16.35	E. Bernardo	C. Aubry	**14.45** OECD Early4NAdMa system: E. Swart
16.50	V. Muthuvijayan		**15.00** EU-US Nanotechnology Communities of research (CORs): present goals: B. Karn
17.05	Coffee Break / Poster Session / Exhibition		**15.15** Round Table, follow-up, conclusions **(Chair:** A. L. *Costa)*
	Chairs: C. Vitale-Brovarone & E. Bernardo	***Chairs: S. Meille & A. Barroug***	
17.25	***Keynote*** *– S. Agathopoulos*	***Keynote*** *– R. Narayan*	
17.45	G. Vecchio	N. Douard	
18.00	Q. Nawaz	J. Soulié	
18.15	M. Pagani	C. Softas	**17.05** Coffee Break
18.30	D. Angioni	R. Wang	**EC Project ASINA: 30th month General Assembly (reserved meeting)**
20.00	**Gala Dinner**		

FRIDAY, SEPTEMBER 23, 2022			
	ROOM 1	**ROOM 2**	**ROOM 3**
09.30	***Chairs: A. Boccaccini & S. Cazalbou***	**PLENARY 8** – *S. Manfredini*	
	S10_BIOCERAMICS FOR SOFT TISSUE APPLICATIONS AND SKIN CARE ***Chairs: A. Boccaccini & S. Cazalbou***	**S11_BIOGENIC CERAMICS & CIRCULAR ECONOMY** ***Chairs: M-G. Raucci & C. Ohtsuki***	**EC Project ASINA: 30th month General Assembly (reserved meeting)**
10.00	***Keynote*** – *R. Toni*	***Keynote*** – *R. Pullar*	
10.20	M. Guerin	A. Adamiano	
10.35	A. Veiga	A. Ressler	
10.50	Coffee Break / Poster Session / Exhibition		
11.10	M. Sandri	C. Piccirillo	**EC Project ASINA: 30th month General Assembly (reserved meeting)**
11.25	H. Yilmaz	A. Galotta	
11.40	A. M. Almeida Coco	A. Ruffini	
11.55	N. Mutlu		
12.10	Presentation of the EC-funded project BIOMATDB		
12.30	ECerS YCN (Young Ceramists Network) – A. Ressler		
12.45	**CLOSING CEREMONY** Best oral and best poster awards		
13.30	**End of the conference**		
VENICE EXPERIENCE: BOAT TRIPS AND TOURS			

ORAL SESSION

13

SEPTEMBER 20TH

. PLENARY LECTURE

RECENT ADVANCE OF DRUG DELIVERY SYSTEM TO ENHANCE NATURAL HEALING POTENTIALS FOR REGENERATIVE MEDICINE

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A new therapeutic trial based on the natural-healing potential of body itself to induce tissues regeneration and repairing, has been recently expected. To realize this regenerative therapy, there are two approaches of cell transplantation and tissue engineering. Tissue engineering is a biomaterial technology or methodology to artificially create a local environment which enables cells to enhance their proliferation and differentiation for tissue regeneration. If a key bio-signaling molecule is supplied to target cells at the right place and the right time period or concentration, the body system initiates to physiologically function, resulting in the natural induction of cell-based tissue regeneration. The biological functions of bio-signaling molecules can be promoted with drug delivery system (DDS) technology. Biodegradable hydrogels enabled the controlled release of various growth factors and chemokines to succeed in the healing potential -based regeneration and repairing of various tissues through the recruitment and activation of cells. This release and/or cell scaffold technologies can be combined with cell transplantation to significantly enhance the therapeutic efficacy in tissue regeneration. The DDS technology of regenerative medicine is also applicable to regulate immunological responses which play an important role in the wound healing process of tissue regeneration and repairing. For example, a positive pro-inflammation promotion of macrophages induced by a drug release technology, followed by the DDS-induced activation of stem cells recruited, further enhanced the therapeutic efficacy of cell-based tissue regeneration.In this paper, several applications of DDS technologies with or without cell scaffolds to the tissue regeneration therapy are introduced to emphasize clinical significance of biomaterials technologies in tissue regenerative therapy

PLENARY LECTURE

NOVEL ENGINEERED SYNTHETIC APPROACHES TO INNOVATIVE BIOENGINEERING

Hala Zreiqat

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The growing clinical need for synthetics that specifically enhance the repair of critical large bone defects and aged bone matched by the escalating demand for grafts, is driven largely by an ageing population whose natural regenerative responses are impaired. This presentation will describe 1) our strategies in developing a platform of patented engineered nanostructured, 3D-printed biomaterials for cell-free personalised treatment to promoting bone healing in load bearing challenging situations. 2) our approach in using biologics such as cell-secreted nanoparticles as a promising approach to replace direct stem cell transplantation for bone repair and regeneration. 3) Our anti-senescence biomaterial approach for enhanced regeneration of aged bone. The role of cellular senescence will greatly contribute to the development of novel and effective therapeutic interventions for bone tissue repair and regeneration Our technologies open avenues for skeletal and soft tissue regeneration in various clinical applications.

SYMPOSIUM 1
CaP BIOCERAMICS FOR REGENERATIVE MEDICINE

KEYNOTE LECTURE

METASTABLE BIOACTIVE CERAMICS: PROMISING NEW OPPORTUNITIES FOR BONE ENGINEERING AND BEYOND

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Metastable (biocompatible) inorganic compounds may provide various opportunities for biomedical applications, related to their high reactivity, propensity to evolve/resorb *in vivo*, and the associated release of bioactive ions. They may also be combined with bioactive molecules/drugs in view of local release.

Amorphous calcium phosphates (ACPs) are particularly appealing compounds for bone applications, as potential precursors of bone apatite *in vivo*. However, a key technological challenge is to process them into 3D scaffolds while retaining their amorphous character, which allows fast resorption and ion release. By cold sintering via SPS at low temperature, we succeeded to obtain still-amorphous cohesive ACP matrices, thus opening the way to novel applications [1]. ACPs are also cement-forming components to prepare moldable pastes for bone filling. We developed a paste with embedded apatite particles loaded with doxorubicin for the local treatment of osteosarcoma. Data obtained in a rat osteosarcoma model showed the relevance of this approach with a tendency to decrease metastatic events [2].

Bone-biomimetic apatites are also highly relevant to biomedical applications. They allow numerous ion substitutions, including with antibacterial ions. In view of tropical dentistry, we developed a *safe-by-design* antibacterial system based on peroxide-substituted biomimetic apatite to fight against periodontitis and tooth root infection. Indeed, oxygenated species are naturally used by the body to fight against pathogens including anaerobic bacteria. We demonstrated its antibacterial character while remaining non cytotoxic to osteoblast cells. We then showed the possibility to combine them to polymers to obtain composite 3D scaffolds, e.g. by freeze-casting.

Other family of metastable compounds concerns Layered Double Hydroxides (LDHs). We adapted the developed cold sintering strategy to prepare cohesive 3D monoliths of LDHs of different compositions, including Al-free. We demonstrated that the LDH interlayer spaces remained accessible to interact with molecular species.

Such metastable compounds may allow envisioning new strategies for tomorrow's medicine.

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KEYNOTE LECTURE

BIOCERAMICS FOR 21ST CENTURY：BIOACTIVE AND MULTI-FUNCTIONAL

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Bioceramics have been used for biomedical applications for many years, and biocompatibility and mechanical properties are critical factors to be considered for clinical applications. With the rapid economic development and increased aging population in 21st century, specific clinical needs are increasing, and regenerative medicine is facing new challenges such as the healing of chronic wound, tissue repair and regeneration for aging people, infection during wound healing, the reconstruction of lost tissues and trauma patients with other diseases. Some biological approaches such as growth factors and stem cell therapies have been extensively studied and advanced in recent years. However, the clinical applications of biological approaches are still facing challenge due to the complexity of biological systems. Therefore, the biological function of biomaterials for tissue regeneration has drawn more and more attention. In particular, study on bioactivity of bioceramics to stimulate bone regeneration is considered as one of the new directions of bioceramic research. In addition to bioactivity, other therapeutic functions of bioceramics such as antibacterial property, anti-tumor function and drug loading and controlled release properties are also interesting and important for clinical applications. Furthermore, traditional bioceramics are thought to be used for hard tissue repair in orthopedic and dental applications. However, recent studies have shown that some bioactive ceramics with defined chemical composition have the bioactivity to stimulate soft tissue regeneration. Therefore, studies on bioactive and multi-functional bioceramics are new trends of bioceramic development, and the clinical applications of bioceramic based materials are expanding from hard tissue repair to the regeneration of soft tissue repair. Our studies have demonstrated that bioceramics with specific chemical composition and micro/nano-structures are bioactive to stimulate tissue regeneration including bone and different type of soft tissues, and doping of different metal and rare earth elements into silicates and phosphates bioceramics endowed the materials with different functions for different biomedical applications.

PYROPHOSPHATE-STABILIZED AMORPHOUS CALCIUM CARBONATE FOR BONE SUBSTITUTION: A MULTISCALE STRUCTURAL CHARACTERIZATION FOR UNDERSTANDING ITS IN VITRO BEHAVIOR

Marion Merle (1), Jérémy Soulié (1), Christian Rey (1), Pierre Roblin (2), Capucine Sassoye (3), Christian Bonhomme (3), Shunfeng Wang (4), Werner E.G. Müller (4), Christèle Combes (1)

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Amorphous calcium carbonate (ACC) is biocompatible [1], bioactive [2], and the most reactive calcium carbonate (CC) phase in solution. To stabilize it against crystallization, polyphosphates [3] had been successfully implemented, but the use of pyrophosphate (Py) is poorly documented. The latter is known as an inhibitor of calcite crystallization and showed bioactivity in vivo [4], being cleaved by enzymes and releasing phosphate ions, fundamental for bone remodeling. The purposes of this study are to understand the influence of the pyrophosphate ratio on the composition and nanoscale organization of the pyrophosphate-stabilized ACC powders (PyACC), and to correlate it to their reactivity in aqueous media and in vitro cell behavior either for undoped or bioactive ions-doped (Sr2+, Zn2+ or Cu2+) powders. PyACC were synthetized by a co-precipitation method from a calcium solution including or not the doping cations and a pyrophosphate-containing carbonate one. They were thoroughly characterized. Then, powders were immersed into water or SBF for evolution from 6h to 28 days, and characterized after interaction as well as the liquid phase. Cytocompatibility assays were carried out on fibroblast cells, and titration of ALP activity on SaOS-2 cells. Over 3.5% of Py/CO3, the ACC crystallization was fully inhibited as shown by XRD. Indeed, pyrophosphate acts as a surfactant, reducing the particle size. SAXS, WAXS and solid-state NMR studies highlighted the fractal organization of PyACC with 5nm-clusters, composed by a 1nm-proto-calcite core [5] surrounded by a disorganized Py/water-rich layer. High amounts of doping ions could be integrated and released without any burst effect. Furthermore, strontium and zinc ion-doping enhanced PyACC biocompatibility as well as ALP activity for the latter. All together, these results highlight correlations between the nanoscale organization, the release kinetics and the in vitro properties of doped and undoped PyACC, paving the understanding and the development of such new bone substitutes.

Acknowledgements

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THE USE OF BIOMATERIALS AND TISSUE PLASTICITY FOR REGENERATION AND BONE AUGMENTATION

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Background

Regenerative medicine can provide a novel approach to treat various conditions and enhance bone regeneration. Tissues interconversion is governed not only by the interaction of the cell but also by the effect of micro-environmental changes and inductive extracellular cues.

Aim and Objectives

A preliminary study was conducted with the preparation of Muscular Extracellular Matrix (MEM) and assessment of its osteogenic potentials and colonization of blood vessels. Then the treated MEM was used to study bone regeneration and augmentation in an animal model.

Material and Methods

MEM grafts were prepared using a well-established protocol. Sub clones derived from immortalized human bone marrow stromal cells (TERT-hBMSCs) were used for graft characterization. Then Osteoblast differentiation was assessed using alkaline phosphatase (ALP), Alizarin Red S. Quantification of mineralization and histological assessment were performed for the induced MEM. Treated constructs of MEM were prepared using hBMSCs, bone cement, and Bone Morphogenic Protein 7 (BMP-7). In ex vivo 10 male nude mice were used, a calvarial defect was prepared to receive the grafts with treated MEM. 5 other mice were used as positive control samples. At 8 weeks, the regenerated tissues were assessed using microcomputed tomography (micro-CT) and histology.

Results

Clinically, cortical bone regeneration was observed bridging the defects in the study group and control group. Qualitative assessment of regenerated bone using micro-CT analysis reported thinner trabeculae, compared to normal native bone, with a high degree of anisotropy. Histologically, the study group showed one and half the thickness of the non-operated bone. Quantitative histomorphometrically assessment showed that a high median bone percentage surface area was discovered at 80.2±6.0% (26.9–90.3).

Conclusion

This study confirms the in vivo osteogenic properties of treated MEM and suggests novel strategies for bone augmentation.

KEYNOTE LECTURE

SYNTHESIS AND COLD SINTERING OF HIGHLY STABLE AMORPHOUS CALCIUM PHOSPHATES

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Amorphous calcium phosphate is a metastable calcium phosphate phase with excellent biocompatibility and better resorbability than hydroxyapatite. Despite these characteristics, metastability of amorphous calcium phosphate has restricted its use in other forms than powder or coating. Here we report multiple, simple co-precipitation and dissolution-precipitation synthesis methods that allow to obtain highly stable amorphous calcium phosphate powders with high specific surface area (>100 m2/g). The synthesis can be done at room temperature and involves the use of simple reactants (CaCl2 and H3PO4, or CaCl2, Na3PO4, and NaOH for co-precipitation synthesis methods and CaP salt (e.g. hydroxyapatite), HCl and NaOH for dissolution-precipitation synthesis method)1. The synthesized amorphous calcium phosphates can be sintered to near-full density by a simple uniaxial die pressing at 1250-1500 MPa at room temperature2,3. The relative density of the samples sintered at 1500 MPa exceeded 95 % and their specific surface area was more than 1000 times smaller than that of the starting powders. They had a grain-like microstructure with grain sizes below 100 nm. The compressive strength and Vickers hardness of the samples obtained at 1500 MPa exceeded 350 MPa and 2 GPa, respectively. The conditions used for the sintering of amorphous calcium phosphate allows to incorporate certain drugs into amorphous calcium phosphate ceramics during the sintering step and allows to produce ceramic-based drug-delivery systems where drug release is controlled by the dissolution/degradation rate of the amorphous calcium phosphate. Furthermore, the sintering conditions allows to co-sinter biopolymer/amorphous calcium phosphate composites and allows to produce porous amorphous calcium phosphate ceramics scaffolds.

IMPROVING FUNCTIONALITY OF INJECTABLE BONE SUBSTITUTES

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Injectable therapies are preferred for their minimally invasive nature, ease of use, and ability to fill complex 3D regions of low bone mass. The sol-gel synthesis approach appears to be a suitable route towards performing injectable calcium phosphates with the possibility to load bioactive and therapeutic compounds.
Here, an overview of different strategies used to prepare bioactive and osteoinductive injectable calcium phosphates (CaP) is reported. The first system concerns the loading of an osteoinductive hyperbranched nanostructures as phosphoserine-tethered poly(ε-lysine) dendrons (G3-K PS) in CaP gels with and without strontium (Sr) element used to reduce the bone resorption (G3KPS-CaP, G3KPS-SrCaP). The second system is represented by antimicrobial injectable materials based on imidazolium ionic liquids (ILs) loaded in CaP material (IL-CaP).
The injectable bone substitute materials integrating G3-K PS or ILs were successfully synthesized by sol-gel approach. For G3KPS-CaP, both *in vitro* and *in vivo* findings showed that the integration of G3-K PS would downregulate Cxcl9 gene and protein expressions to achieve an enhanced bone regeneration effect, with respect to a higher BMD and BV/TV. Immunohistological staining of Cxcl9, Runx2 and RANKL proteins were also conducted. The positive expression of Cxcl9 seemed to be less in G3KPS-CaP and G3KPS-SrCaP groups. Runx2 and RANKL positive expressions were more in G3KPS-CaP group than others. For IL-CaP system, it was observed an early pre-osteoblast differentiation in basal conditions by expression of ALP and OCN at 3 and 14 days of *in vitro* cell culture, respectively. The expression of osteogenic markers increased with an increasing IL N-alkyl chain-length. The antimicrobial studies demonstrated that IL-CaP at long alkyl chain showed antimicrobial and antifungal activity without biofilm production. The results indicated that sol-gel technology allows to synthesize therapeutic and multifunctional injectable CaP materials which can become high-performance bone substitutes for the treatment of bone defects.

Acknowledgments

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PRODUCTION OF DENSE AND PHASE-PURE A-TCP FROM NANO-CRYSTALLINE POWDER

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Introduction

Dense and pure α-tricalcium phosphate (α-TCP) samples are of interest to study the interactions between α-TCP and bone cells, in particular osteoclastic resorption. However, to the best of our knowledge, a density higher than approximately 95% has never been achieved [1]. In fact, α-TCP production is challenging due to (i) the β- to α-TCP phase transition occurring at a temperature ideal for sintering (1125°), (ii) the formation of a β-TCP - α-TCP solid solution with minute amounts of Mg, a common impurity present in calcium phosphates, and (iii) a 9% volume expansion during the β- to α-TCP phase transformation. Here, we investigated the potential of a nano-crystalline α-TCP powder to produce dense bulk materials.

Experimental

Nano-crystalline α-TCP (particle size <100 nm, <20 mg/kg Mg, <2% foreign phases) was produced through wet chemical synthesis of amorphous calcium phosphate and subsequent thermal conversion for a short period of time at 775°C (Ostwald's step rule) [2]. Pills were then compressed uniaxially and sintered by inserting them into a furnace preheated to 1200°C for 6 h (1 or 3 replicates). The density was determined from the cylinder dimensions and weight (calculated relative to the theoretical densities of the crystalline phases) and the phase composition measured by x-ray diffraction (XRD).

Results and Discussion

A higher pressure resulted in a higher relative density before as well as after sintering, corresponding to a porosity as low as 3.9% (Fig. 1A). Interestingly, the sintered pills contained approximately 4% β-TCP (Fig. 1A), independently of the green body density and of the pill height (data not shown). This result is surprising considering the β- to α-TCP phase transition temperature of 1125°C. A hypothetical pressure build-up in the microstructure during sintering could possibly favour the crystallographically denser β-TCP phase. A second surprising finding is the presence of nanopores (Fig. 1B) suggesting the release of gas during sintering.

Conclusions

Sintering of nano-crystalline α-TCP resulted in 96% dense α-TCP with a thus far unexplained presence of β-TCP. Future work will focus on the effect of sintering time and temperature on the density and phase composition.

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CARBONATE APATITE HONEYCOMB ARTIFICIAL BONE FOR CRITICAL SIZE ULNA DEFECT RECONSTRUCTION

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Both composition and structure are important for the artificial bone. Composition of the bone is carbonate apatite that contains 6-9mass% carbonate in apatitic structure. Although carbonate apatite decompose thermally at sintering temperature, it can be fabricated in an aqueous solution by compositional transformation through a dissolution–precipitation reaction using a precursor such as calcium carbonate. Therefore, calcium carbonate block fabrication leads carbonate apatite artificial bone fabrication. One of the interesting structure as artificial bone is honeycomb because it lead quick bone formation along the pores. In this study, carbonate apatite honeycomb artificial bone was fabricated and evaluated its potential using rabbits' ulna critical size bone defect.First, a mixture of calcium carbonate and binder was extruded through a honeycomb die using an extruder. The honeycomb was heat-treated for debindering, leading calcium carbonate honeycomb fabrication. Then the honeycomb was immersed in sodium phosphate solution for the compositional transformation through a dissolution–precipitation reaction.10mm bone defect was the critical size for rabbit ulna. When the defect was reconstructed with carbonate apatite honeycomb new bone was formed even at the center of the honeycomb 4 weeks after surgery. In contrast, no new bone was formed when the defect was reconstructed using commercially available beta tricalcium phosphate artificial bone (Osferion®) even after 12 weeks after surgery. The difference demonstrated clearly that carbonate apatite honeycomb artificial bone has good potential value for long bone reconstruction.

EFFECT OF SINTERING TEMPERATURE AND CALCIUM TO PHOSPHORUS RATIO ON THE OSTEOINDUCTION CAPACITY OF CALCIUM PHOSPHATE GRANULES IN MICE

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Introduction

Some bone graft substitutes (BGS) elicit the formation of bone tissue ectopically. This phenomenon is recognised as proof of osteoinduction. Currently, it can only be tested in vivo. Most in vitro attempts to measure the osteoinductive potential of calcium phosphate based BGS have been shown to correlate poorly with in vivo results. Recently, we proposed an in vitro test based on a quantitative mineralization method that allowed to predict the osteoinductive capacity of calcium phosphate bone graft substitutes. In this study we verified the osteoinductivity of calcium phosphate bone graft substitutes with different mineralization capacity by implanting them in FVB mice subcutaneously.

Materials and methods

4 types of calcium phosphate granules were synthesized in a factorial model using sintering temperature (950°C and 1100°C) and calcium to phosphorus ratio (1.50 and 1.55) as varying factors. They were characterized for specific surface area, porosity, microstructure and phase composition as well as their mineralization capacity. 100 mg of each granule type were implanted in subcutaneous pouches in 12 FVB mice (n=6) for 35 days. Decalcified histologies were prepared and stained with hematoxylin-eosin and Masson's trichrome.

Results

Specific surface area and mineralization capacity increased while pore entrance size decreased with high calcium to phosphorus ratio and low sintering temperature. Histologies revealed initiating process of osteoid formation in all 4 types of granules. Bone tissue was found in a single type of granule, which had the highest calcium to phosphorus ratio (1.55) and the lowest sintering temperature (950°C). Figure 1- Histologic cut of explants of an osteoinductive calcium phosphate granulate implanted during 35 days in the subcutis of FVB mice, Masson trichrome staining (left), Hematoxylin-Eosin staining (right) 20x magnification.

CU2+-DOPED BRUSHITE CEMENT FOR BONE SUBSTITUTION MATERIALS

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One possibility for brushite (CaHPO4·2H2O) formation is the reaction of β-tricalcium phosphate (β-TCP, β-Ca3(PO4)2) and monocalcium phosphate monohydrate (MCPM, Ca(H2PO4)2·H2O) with H2O according to (1). Cu2+ is known for its increasing effect on antibacterial activity and consequently the beneficial impact on preventing inflammation [1], as well as the positive effects on angiogenesis and wound healing [2]. These properties qualify Cu2+ as suitable doping-ion for biomaterials. One possibility to include Cu2+ in the cement system is the incorporation into the crystal structure of β-TCP. This process has been confirmed in previous studies [1,3].Ca(H2PO4)2·H2O + β-Ca3-xCux(PO4)2 + 7 H2O → 4 CaHPO4·2 H2O (1)The previous study showed the effect of Cu2+-doping in β-TCP on the hydration of a brushite cement consisting of β-TCP and MCPM with a molar ratio of β-TCP/MCPM = 1.63 and a water to solid ratio of 0.3 ml/g. Phytic acid (IP6, C6H6(OPO3H2)6) was used as a setting retarder. Heat flow calorimetry and in-situ XRD measurements showed an increasing retardation of the hydration reaction and a decreasing heat release with increasing amount of Cu2+ in β-TCP. This effect can be directly associated with the incorporated Cu2+. Brushite was detected as main phase, accompanied with neglectable amounts of monetite and an unknown secondary phase, which is assumed to be a Cu2+-containing hydrate phase. The next step is the investigation of compressive strength, injectability and initial and final setting time, as well as the effect on human cells and varies bacterial strains. The aim is to reach a cement system with a Cu2+-concentration nontoxic to human cells but providing a sufficient antibacterial activity and appropriate application relevant properties.

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THERMAL CRYSTALLIZATION OF CITRATE-STABILIZED AMORPHOUS CALCIUM PHOSPHATE INTO APATITE: AN IN SITU, REAL TIME STUDY

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Amorphous calcium phosphate (ACP) is a promising bioceramic material that in the last decade has gained high interest thanks to its unique properties, founding successful application in dentistry and orthopedics. Understanding its crystallization is of outmost importance, as ACP metastability and its transformations during crystallization are key parameters to be controlled for any ACP-related application. In this regard, ACP thermal crystallization is a highly interesting route to produce advanced bioceramics [1]. Indeed, we have discovered that the thermal treatment of a citrate-stabilized, carbonated doped ACP developed by us uniquely yields ion-doped, biomimetic hydroxyapatite as pure product [2]. Citrate-ACP thermal crystallization was markedly different from the one of classic ACP materials, which commonly yields tricalcium phosphate. We have used non-conventional characterization techniques in parallel to high-temperature thermal treatment to study ACP thermal crystallization in situ in real time. In particular, complementary Energy-Dispersive X-Ray Diffraction and Infrared Spectroscopy were used for the first time for this scope. Our in situ study evinced that several phenomena occurred before and during ACP crystallization. We corroborated previous ex situ works that proposed that before crystallization ACP undergoes a disordering process. Moreover, we confirmed that citrate-ACP uniquely crystallizes into biomimetic hydroxyapatite, on the contrary of a citrate-free control ACP. We found out that the presence of carbonate ions favors this selectivity, and an apatitic local order is created before crystallization. Finally, we discovered that citrate ions influence the crystallization kinetics and thermodynamics. Therefore, we proved that the thermal treatment of citrate-ACP represents an attractive route for producing bioactive apatite bioceramics.

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A NOVEL METHOD FOR SYNTHESIS OF EGGSHELL DERIVED APATITIC BONE CEMENT UNDER MILD CONDITIONS WITH HIGH MACROPOROSITY AND BIOACTIVITY

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Eggshell is abundant in nature and has the ability to mimic the composition of natural bone minerals because it consists of biologically beneficial ions (such as Sr2+, SiO42-, Mg2+, Na+, K+ and F- etc.) present in traces amount which are also reported to play a key role in bone remodeling process. Calcium deficient hydroxyapatite (CDHAp) based bone cement due to its similarity with natural bone minerals chemical composition is a potential bone substitute in orthopaedics. Therefore, in this study, we have prepared the apatitic (CDHAp) bone cement using eggshell as precursor with improved injectability, macroporosity, resorbability and bioactivity. Here, the solid phase is composed of nanocrystalline hydroxyapatite and eggshell derived (ESD) tricalcium phosphate and the liquid phase is composed of disodium hydrogen phosphate (well-known cement binding accelerator) with gelatin and chitosan (for improving the cement injectability) in an acidic (diluted) solution. Also, to enhance the porosity of the cement, polysorbate (Tween-80) as liquid porogen was used which was further compared with cement with mannitol as solid porogen. The desired bone cement was prepared by mixing all these in an optimized liquid-to-powder ratio. The cement paste sets within clinically acceptable setting time (≤20 minutes) and is highly injectable (>75%) with good stability at physiological pH (i.e., ~7.3-7.4). The after-set cement when immersed and incubated at physiological conditions in PBS and other similar artificial body fluids for 7 days results in pure CDHAp phased bone cement. This was confirmed by XRD and FTIR analysis. The ESD apatitic bone cement has acceptable compressive strength, in the range of trabecular bone. And, the average pore size inside it falls in between 50-250μm with interconnectivity, verified its macroporous nature confirmed through SEM, MIP and micro-CT analysis techniques. Also, the bone cement is biodegradable (degrades upto 25% within 10 weeks) in artificial body fluids at physiological conditions. The MG63 cells viability and alkaline phosphatase activity when incubated for 3 and 14 days respectively with the prepared bone cement was significantly higher when compared to their respective controls. And, the cells were adhered and spread fully over the surface of the bone cement indicates its biocompatibility nature. Through these, we can conclude that the prepared ESD apatitic bone cement may have potential to become promising material for non-load bearing defects repair in bone grafting applications.

Keywords

Calcium deficient hydroxyapatite, injectability, macroporosity, eggshell derived tricalcium phosphate, eggshell derived apatitic bone cement

SYMPOSIUM 2
ADDITIVE MANUFACTURING OF BIOCERAMICS

KEYNOTE LECTURE

TUNING NANOTEXTURE OF 3D-PRINTED CALCIUM PHOSPHATE SCAFFOLDS: INTERACTION WITH PROTEINS AND *IN VIVO* BONE FORMATION

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Extensive efforts have been made over the past decades to develop novel synthetic bone grafts with enhanced performance. The progress in 3D-printing technologies has opened the door to the design of personalized bone substitutes, mostly based on calcium phosphates (CaP). In this context, we have recently developed self-setting CaP inks based on the combination of suspensions of reactive ceramic particles with hydrogel binders, that harden at low temperature through a dissolution-precipitation process. This approach has several advantages. On the one hand it avoids the shrinkage associated to high-temperature sintering processes, and on the other the final product is a biomimetic nanostructured apatite, very close to the mineral phase of bone and more reactive than CaP ceramics. Moreover, the hardening kinetics of these self-setting inks can be adjusted by modifying the reaction conditions, using either biomimetic or hydrothermal methods. Besides impacting the reaction duration, this allows tailoring crystal morphogenesis in the hydrolysis process resulting in changes in the physicochemical and textural properties of the end product [1,2]. In this way, besides the external shape of the implant it is possible to control the internal pore architecture of the scaffold. This presentation will focus on the effect of the nanoscale textural properties of 3D-printed calcium phosphate scaffolds on their biological performance, highlighting the extent to which the differences in nanostructure, nanoporosity and nanopore size are key in both the interaction with proteins in solution and the osteogenic potential of the materials *in vivo*.

Acknowledgments

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KEYNOTE LECTURE

(BIO)MEDICAL CERAMIC COMPONENTS AND PERSONALIZED BIOMATERIALS BY HYBRID SHAPING AND ADDITIVE MANUFACTURING

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The demand for multifunctionality and the combination of components with different material and structural properties plays an increasing role in research activities dedicated to lightweight applications, automotive, aeronautics and (bio) medical components, tools or implants. Material and structural hybrids allow distinctive property combinations like electrical conductivity/ insulation, magnetism/non-magnetism, ductility/hardness or the combination of dense and porous sections. Hybrid components can be equipped with sensor or actor functions known e.g. from MEMS (Micro Electromechanical Systems). Such combinations of properties are achievable by either mixing/pairing different materials within one or more processes and/or by combining entirely different technological approaches each providing distinctive structural features. The main effort lies on the adjustment of particular material properties, on adapting shrinkage behavior as well as tuning and hybridizing co-processing and process technologies. This contribution reports about powder technological and conventional shaping methods as well as Additive Manufacturing (AM) and chosen process combinations – hybrid technologies – relevant for achieving ceramic multi-component parts and materials. Hybridization of different materials and structures usually starts with a green-state component followed by co-processing (e.g. green-in-green manufacturing and lamination of tapes) or from the sintered state followed by post-processing (e.g. post-functionalization of AM-components by aerosol and inkjet printing). Also a co-manufacturing of pre-sintered material and green state material can be applied. By combining AM with conventional and industrially well-established manufacturing processes, the advantages of each individual process were put into effect. As was shown in recent projects, functionalized and individualized components or component series can be fabricated such as, metal-ceramic surgical grippers as well as bioceramic implants with functional graded porosities and combined dense-porous sections (CerAMfacturing; EU, GA 678503). Bulky bodies produced by CIM were functionalized and individually marked with other materials (Addimat, ZIM, ZF 4076417EB6). The so-called Freeze Foaming was combined with AM to manufacture complex-shaped components for a possible use as personalized bone replacement material. Either additively manufactured shell structures were foamed-in, providing a structural hybrid with a dense, complex outer layer and a porous inside (bone-mimicking), or, topology-optimized 3D-printed support structures were foamed-in-place in order to combine strength-enhancing features and a porous lead structure for cell-ingrowth in application as artificial jawbones (Hybrid-Bone, BMBF: 03VP07633).

In order to guarantee the quality of the additively manufactured structures optical coherence tomography (OCT) was evaluated with regard to the possibility of inline implementation. Images taken on Lithography-based Ceramic Manufacturing (Vat Photopolymerization)-printed titanium oxide and zirconium oxide samples showed that OCT can be used to detect pores and other geometric defects in near-surface regions. Further optical analyses of single component samples showed that it is possible to distinguish between polymerized (illuminated) and non-polymerized (not-illuminated) areas. When multi-material and multifunctional printing approaches are implemented the risk of material contamination increases. The OCT investigations proved that contaminations, as well as different phases and mixed interphases, can be detected based on the material contrast thereby providing insights into how contaminations are incorporated into manufactured samples. This contribution gives an overview about the main challenges, opportunities and recent developments of material and process hybridization with regard to powder technological shaping technologies. Focus lies on the potential of additive manufacturing techniques for (bio)medical ceramic components and personalized biomaterials as well as measures to implement process monitoring and inline control.

Keywords: Bioceramics, Freeze Foaming, Additive manufacturing, Hybrid shaping, OCT, monitoring, Inline control

3D-PRINTED PERSONALIZED CERAMIC BONE SUBSTITUTES: FROM BIOGLASS TO HYDROXYAPATITE.

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Introduction and Objective

In the last decades, advances in bone tissue engineering mainly based on osteoinduction and on stem cell research. Only recently, new efforts by others and us focused on the micro- and nanoarchitecture needed to improve and accelerate bone regeneration and on diverse ceramic materials. By the use of additive manufacturing, diverse materials with the same microarchitecture were produced and tested for best microarchitecture for osteoconduction and bone augmentation.

Materials and Methods

For the production of scaffolds, we applied for titanium-based scaffolds selective laser melting and for ceramics the CeraFab 7500 from Lithoz, a lithography-based additive manufacturing machine. As in vivo test model, we used a calvarial defect and a bone augmentation model in rabbits.

Results

The histomorphometric analysis showed that bone formation was significantly increased with pores between 0.7-1.2 mm in diameter. In lattice microarchitectures, the optimal distance between rods is 0.8 mm. Moreover, microporosity appeared to be a strong driver of osteoconduction and influenced osteoclastic degradation. Best microarchitecture for osteoconduction and bone augmentation are different. For osteoconduction, hydroxyapatite appears to be better suited than tri-calcium phosphate.

Conclusions

In essence, additive manufacturing enabled us to generate libraries of microarchitectures to search for the best ceramic, the most osteoconductive microarchitecture and the ideal microarchitecture for bone augmentation. Moreover, additive manufacturing appears as a promising tool for the production of personalized bone tissue engineering scaffolds to be used in cranio-maxillofacial surgery, dentistry, and orthopedics.

NEW ION DELIVERY 3D PRINTED SCAFFOLDS BASED ON CERAMIC BIOCOMPOSITES

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Among several ions playing a vital role in the body Ca2+ ions are involved in the mechanism of bone formation, making them especially useful for bone tissue engineering applications. Biodegradable scaffolds, including polylactic acid (PLA) scaffolds, are commonly used in bone related clinical procedures and they can work as carrier-delivery substrate of different ions or drug to provide a constant and controlled release of bioactive factors. Over recent years, AM techniques have gained a special attention in order to process complex scaffolds and patient-customized pieces for biomedical application. Among AM techniques, the Fused Deposition Modelling (FDM/FFF) is one of the most simple and inexpensive techniques, which allows high printing speeds using thermoplastics as structurers.Following this interesting research line, a new group of bioceramics based composites, which integrates osteoinductive compounds as HA particles in a biodegradable polymeric matrix was proposed. This new family of bioceramic composite has been processed following an innovative Additive Manufacturing fabrication method based on FFF and colloidal suspension in order to prepare composite scaffolds with different loading of bioactive phases, which promote the lixiviation of ions of interest. Materials characterization shows that the new additive manufacturing processing based on colloidal chemistry allows the incorporation of a high amount of ceramic load and therefore of bioactive phase into the scaffold. The soaking in PBS promotes hydration, followed by a gradual ion or drug delivery and occasionally the precipitation of different ion species onto the scaffold surface, before the complete composite scaffold degradation. Bioceramic composites cytocompatibility have been evaluated in vitro in terms of cell adhesion but also in terms of proliferation during the scaffold degradation and the ions release process. These bioceramic based ion delivery systems and their processing paves the way to generate a new family of interesting biocomposite for bone healing applications.

KEYNOTE LECTURE

ADDITIVE MANUFACTURING OF BIOCERAMIC SCAFFOLDS BY COMBINATION OF FDM AND SLIP CASTING

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The use of bioceramics like calcium phosphates or bioactive glasses for the regeneration of critical bone defects, as they can occur for example after serious injuries or diseases, is intensively researched worldwide. The advantages of the additive manufacturing technology make it possible to process these ceramics into customized patient-specific implant devices, so called scaffolds. In this study the possibilities to produce bioceramic scaffolds by using the fused deposition modelling (FDM) technique in combination with ceramic slip casting is introduced and described. In the first step the polymer models, which represent the negative geometry of the ceramic component, are printed by a commercial FDM printer. In the next step a calcium phosphate powder slurry is casted into the polymer molds according state of the art ceramic processing technologies. After debinding and sintering the mechanical and structural properties of the scaffolds are characterized and the biocompatibility is tested via simulated body fluid.First tests indicate that the mechanical properties as well as the biocompatibility of these scaffolds are similar to scaffolds that are produced by further additive manufacturing technologies, for example binder jetting or foam casting.

PERFORMANCE OF 3D-PRINTED PATIENT-SPECIFIC CALCIUM PHOSPHATE BONE GRAFTS IN A CLINICAL CASE

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Introduction

Patient-specific bone grafts (PBG) are a novel approach, allowing for complex geometrical reconstructions. Calcium phosphate (CaP) pastes can be robocasted to achieve PBG accomplishing such demands. The dental and maxillofacial fields are two domains which benefits most from using 3D-printed PBG as the aesthetical results are very important in these areas. MimetikOss® 3D, a CaP PBG solution proposed by Mimetis Biomaterials, offers a wide variety of clinical applications where the present work shows the use of this technology in a large dental indication.

Methods

First, the bone region was segmented (Mimics Innovation Suite V24, Materialise) from computerised tomography (CT). Thereafter, a 3D model of the bone graft was designed from the defect reconstruction and the clinical inputs (Fig. 1.1). Subsequently, manufacturing by robocasting was performed, where a viscous self-setting CaP paste was extruded and consolidated using a low-temperature process, resulting in a biomimetic scaffold with similar composition and microstructure as the mineral phase of natural bone. Radiological acquisitions were used to assess the volume stability and the graft fitting accuracy (Figs. 1.4 - 1.6). Dental implants were placed 9 months post-surgery and histological analysis of two extracted trephines were performed.

Results

CT images post-surgery (Fig. 1.4 - 1.5) showed a perfect fit and fixation of the scaffold, leading to good contact with the host tissue. The soft tissues presented adequate healing and no adverse events were encountered. CT images at 6 months post-surgery (Fig. 1.6) revealed excellent osteointegration of the implant with the host tissue. The insertion torque of the dental implants was 35 Ncm proving a bone ingrowth that provided the required mechanical performance for implant placement (Fig. 1.7)

Discussion & Conclusions

This work demonstrated the suitability of robocasted CaP PBG in vestibular reconstructions, revealing the potential of this technique in dental indications requiring horizontal and vertical volume augmentations. This study is only the beginning of a multitude of possible indications for bone regeneration applications.

3D PRINTED CERAMIC MULTI-MATERIAL COMPONENTS FOR NEXT GENERATION IMPLANTS

J Homa Errore. Il segnalibro non è definito. (1), Sebastian Geier (1)

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Using multi-material combinations in 3D printing is garnering widespread attention due to the wide range of possibilities that it provides to realize parts which are more functional and have improved properties. Hence, this paper presents the combination of an established high resolution 3D printing technology for ceramics with the mentioned multi-material approach. This approach not only enables the combination of different ceramics in different layers of the printed component, but also the spatially resolved combination within the same layer and hence, paves the way to the realization of complex bi-phasic ceramic components. First successful trials that will be presented include the combination of alumina and zirconia-toughened zirconia, alumina and zirconia, as well as zirconia and hydroxyapatite, respectively. Especially the latter combination provides a promising mix of properties by bringing together strong and tough zirconia ceramic with osteoconductive and biologically active hydroxyapatite. The paper will present the actual multi-material 3D printing process, but also focus on the results and current challenges in terms of co-sintering of different ceramic materials. The initial results show that this technological approach holds great potential to path the way from classical single material structures to bi-material components and subsequently multi-material and functionally-graded ceramics that can play an integral role in next generation implants with significantly improved properties.

3D-PRINTED BIOMIMETIC HYDROXYAPATITE SCAFFOLDS WITH ENHANCED MECHANICAL PROPERTIES: KINETIC STUDY OF THE SELF-HARDENING PROCESS

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Direct Ink Writing allows to manufacture personalized bone scaffolds with a precise control of both external shape and internal porosity. Reactive ceramic inks consisting of alpha tricalcium phosphate (α-TCP) suspended in a polymeric binder are able to self-harden at low temperature through a setting reaction, resulting in biomimetic calcium deficient hydroxyapatite (CDHA). This study aims to explore the feasibility of increasing the toughness of 3D-printed CDHA scaffolds by using a polycaprolactone (PCL) organogel as binder.Two types of inks were prepared using either i) a PCL organogel or ii) a Pluronic hydrogel (control) laden with α-TCP powder. After printing, the scaffolds were hardened by immersing them in water at 37ºC for different periods of time. Bar-shaped and cylindrical-shaped scaffolds were fabricated for bending and compression tests respectively. The evolution of the physico-chemical properties, namely phase composition, microstructure, specific surface area, skeletal density, polymer content, porosity and shrinkage, was quantified over the hardening process.The presence of PCL did not hinder the hydrolysis of α-TCP to CDHA, but it slowed down slightly the reaction kinetics. The hardened scaffolds retained most of the PCL, that was homogenously distributed forming fibres that intertwined with the continuous network of CDHA nanocrystals. The mechanical strength increased progressively, in synchrony with the phase transformation and the evolution of the textural properties, reaching the maximum value after 48h. The addition of PCL, even in a low amount (< 7 wt.%), resulted in a two-fold and five-fold increase of the bending strength and the work of fracture respectively compared to the control, with no effect on the compressive strength. In conclusion, the use of PCL as binder enhanced significantly the scaffold's toughness, a critical parameter to enable the fixation of the implants during surgical procedures, while keeping the compositional and textural properties of biomimetic hydroxyapatite.

PRODUCTION, CHARACTERISATION, AND IN VITRO EVALUATION OF 3D PRINTED PCL/HANP/PEGDA SCAFFOLD FOR BONE REGENERATION

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Reconstruction of bone defects with mechanical integrity to the original surrounding bone tissues is essential for a patient's rehabilitation. In this research, a novel approach is explored to produce synthetic bone grafts mimicking the complex bone structure using additive manufacturing, comprising the construction of 3D scaffolds. For this purpose, three types of scaffolds were produced and tested: one using a thermoplastic polymer, polycaprolactone (PCL), another using a combination of PCL and hydroxyapatite nanoparticles (HANp), and the third using a combination of the two materials and polyethylene glycol diacrylate (PEGDA). After production, optimisation and characterisation of the scaffolds, an in vitro evaluation was performed with human dental pulp stem cells (hDPSCs).According to the results, the scaffolds were produced successfully presenting interconnected channel networks and good geometric accuracy. Regarding the mechanical behaviour, the results demonstrated that the addition of HANp seem to have improved the compressive rigidity of the scaffolds. After analysis of the in vitro tests, it was verified that the PCL/HANp/PEGDA-based scaffolds present superior cell proliferation when compared to the other groups.The study demonstrated PCL/HANp/PEGDA scaffolds associated with hDPSCs are a very promising therapeutic system in critical fractures treatment, to accelerate and im-prove bone regeneration. The research of this system’s performance in critical bone defects is an important step to its progression to clinical applications.

THE EFFECT OF TPMS DESIGN AND PORES SIZE ON BIOLOGICAL AND MECHANICAL PROPERTIES OF CALCIUM PHOSPHATE BONE GRAFT

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Hydroxyapatite is one of the most used ceramics for bone filling due to its strong similarity with the bone minerals. The porosity of these materials was deeply studied because of its impact on bone regeneration. Ideally bone implant needs highly porous scaffolds, high pores interconnectivity and optimal sizing. These factors allow cells migration leading to an increase of the bone regeneration. Besides these morphological properties, the mechanical properties of the implants need to be comparable to the bone's one and depend on the load applied on this implant. Since increasing porosity decreases these mechanical properties, combining high mechanical properties and sufficient porosity can be achieved by generating specific internal design of the implant. Recent developments in ceramic 3D printing allow such complex design that meets these standards.This work concerns the design and evaluation of the influence of macro-porosity and wall thickness on three different TPMS (Triply Periodic Minimal Surfaces). Compression and bending specimens of these latter were printed using Hydroxyapatite by direct light processing (DLP) stereolithography (SLA), then thermally treated for debinding and sintering. To study the effect of pore size and wall thickness on the mechanical properties of these lattices' structures, three different pores sizes and wall thicknesses were applied for each structure. The potential issue of TPMS additive manufacturing, static in vitro results will be also presented. The results allowed a better understanding of the mechanical and biological behaviours of these TPMS as well as the influence of pores sizes and wall thickness on their compression and bending resistance.

BIOPRINTING OF BIOMIMETIC NANO-STRUCTURED HYDROXYAPATITES WITHIN COMPOSITE BIOINKS FOR BONE TISSUE ENGINEERING

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In recent years, bioprinting has attracted increasing interest as potential technique to better fulfil the tissue engineering requests, and within this contest, bioinks formulation has become a new research hot topic.Bioinks components choice must be made based on factors related to biocompatibility, biochemical signals, printability, crosslinking mechanisms and degradation. In the context of bone and cartilage regeneration, gelatin (Gel) modified by methacryloyl functional groups (GelMA) has recently gained increasing attention due to its biocompatibility but also thanks to its ability of being crosslinked by the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), able to perform a crosslinking reaction within the visible light spectrum (Vis-light).GelMA as is cannot, however, provide cells with proper cues to form bone-like matrix. Instead, incorporating inorganic calcium-phosphate phases into hydrogels can provide the right stimuli and cues to the cells to differentiate and generate a biomineralized matrix.This work aimed to bioprint human bone marrow-derived mesenchymal stem cells (hBMSCs) in GelMA bioink that can be photopolymerized using Vis-light, functionalized with two different types of nano-hydroxyapatites (nHAs), physically, chemically and morphologically different, to evaluate their effects on the bioprinting process and outcomes.The nHAs synthesis were optimized to obtain two morphologically and physic-chemically different nHAs, respectively a Mg-doped hydroxyapatite more crystalline and needle-like (Mg-HA) and a Mg-CO3-doped hydroxyapatite more poorly crystalline and round-shaped (MgCO3HA). The synthetized nHAs where fully characterized to prove the desired quality were obtained, then the nHAs were independently formulated into GelMA/LAP based bioinks and these tested in all the aspects of the bioprinting process, from extrusion to scaffolds maturation.In the end, two different nHAs were successfully synthetized, obtaining the desired morphological and chemical-physical features for each of them. The synthetized nHAs were proved to be not cytotoxic, and able to promote biomineralization in 2D culture, with a better performance of Mg-HA compared to MgCO3-HA. The addition of each nHAs to GelMA/LAP based bioinks enhanced the printability and stability of the structures, and the use of LAP as photointiator, combined with Vis-light, maintained high cell viability and structural stability after 21 days, holding great promises for 3D bioprinting applied to bone tissue engineering.

SYMPOSIUM 3
CELL-MATERIALS INTERACTION AND CO-CULTURES

KEYNOTE LECTURE

LOCAL REGENERATIVE AND DRUG DELIVERY APPROACH IN CANCER

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In the field of cancer nanomedicine a multitude of nanotechnology-based platforms have been developed. In this regard smart materials could represent an attractive source for improving the therapeutic options for solid tumors. These useful tools aimed to increase drug solubility, bioavailability, extend drug half-life and to achieve a specifically tumor targeting which could offer significant advantages in improving cancer therapeutic efficacy and simultaneously reducing drug toxicity. In the panorama of implantable drug delivery devices a less explored but emerging strategy is represented by the combination of a regenerative approach in order to restore the biomechanical functions of the injured heathy tissue post-surgical treatment and a local delivery of chemotherapeutics in order to obtain a surgical radicalization of the lesion. Over the years, a wide variety of bio-inspired scaffolds have been investigated as implantable single drug delivery systems. In the last years, efforts have been also devoted to the design of scaffolds for the release of multiple compounds with distinct kinetics but limited biomedical applications have been carried out. For the above reasons, the implementation of medicated biomaterials would represent an important integrated treatment in the multidisciplinary approach of patients with solid malignancies and will establish the basis for the application of regenerative materials in cancer management. The use of nanostructured biomimetic materials with regenerative and antitumor ability could represents a new ground-breaking therapeutic strategy for tumor treatment and could revolutionize the paradigm of cancer management.

KEYNOTE LECTURE

CERAMIC MICRO- OR NANO-PARTICLES FOR CANCER TREATMENT

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Ceramic microspheres or nanoparticles with specific size, composition and structure are useful for cancer treatment. For example, yttrium oxide (Y_2O_3) microspheres 20–30 μm in size with high chemical durability can be synthesized by high-frequency induction thermal plasma melting technique. Yttrium-89 (^{89}Y) in the microspheres was radioactivated to ß-emitter ^{90}Y by neutron bombardment, and the radioactive Y_2O_3 microspheres remarkably suppressed the growth of VX2 tumor transplanted into rabbit liver.

Flake-shaped gadolinium borate ($GdBO_3$) nanoparticles, approximately 80 nm in length and 20 nm in width, can be synthesized by hydrothermal process and they don't show any serious cytotoxic effect against human umbilical vein endothelial cells. The $GdBO_3$ nanoparticles are expected to accumulate in tumors because of the enhanced permeability and retention effect and can function as boron and gadolinium nanocarriers for neutron capture therapy.

Magnetite (Fe_3O_4) is useful for hyperthermia because it can generate heat under alternating current (AC) magnetic field. Silica (SiO_2)-Fe_3O_4 core-shell microspheres 20–30 μm in size with heat-generating ability under AC magnetic field can be obtained by modified liquid phase deposition. Further, we found that Fe_3O_4 nanoparticles 24 nm in size showed excellent heat-generating ability under 300 Oe, but under 120 Oe, Fe_3O_4 nanoparticles 8 nm in size showed better heat-generating ability than those 24 nm in size.

Iron nitride ($Fe_{16}N_2$) with higher saturation magnetization than Fe_3O_4 can be candidates as next generation thermoseeds for hyperthermia. $Fe_{16}N_2$ nanoparticles can be obtained by reduction and subsequent nitriding treatment of Fe_3O_4 nanoparticles, and their heat generation estimated from an area of hysteresis loop was higher than that of Fe_3O_4 nanoparticles under AC magnetic field. Further, there is no significant difference in cytotoxicity between $Fe_{16}N_2$ nanoparticles and Fe_3O_4 nanoparticles. These results indicate that $Fe_{16}N_2$ nanoparticles have a potential as next generation thermoseeds for hyperthermia.

UNVEILING THE MAIN FACTORS TRIGGERING THE COAGULATION AT THE BIOMATERIAL-BLOOD INTERFACE

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Hemocompatibility is the most significant criterion for blood-contacting materials in a successful in vivo application. Prior to clinical applications, comprehensive in vitro analyses in accordance with ISO 10993-4 must be performed on the biomaterials. Design of a functional biomaterial requires not only a chemical investigation, but also a careful examination of the surface structure in such a way to reveal their unique properties. Indeed, even minor changes in the material's surface parameters might result in significant variances in blood reactivity.To elucidate how the coagulation pathway is triggered, we focused on specific surface terminations, crystallographic orientations, and the polymorphs of the same material (single crystalline SiC). Surface potential and the roughness of were analysed in intermittent contact-mode by Kelvin Probe Force Microscopy. Contact angle measurements were performed by the Sessile drop technique to provide information on surface wettability. Prior to in vitro tests, chemical composition analysis and phase determination were completed. To simulate physiological blood flow conditions of blood vessels, the dynamic Chandler Loop system was employed in addition to the static cell culture tests using fresh whole and isolated human blood. Cytotoxicity of the specimens were analyzed by the live-dead staining assays for human umbilical vein endothelial cells (HUVECs) and blood cells. Activated and adhered platelets were marked by P-selectin and investigated using the ELISA assay. Our results revealed that the surface potential and wettability depend strongly on the different contact surfaces of the same material, the percentage as well as the distribution of the elements on these surfaces. These changes are correlated with the results of in vitro analysis. We anticipate that the results will pave the way to reduce and even to eliminate the failures caused by materials with poor hemocompatibility. Therefore, these findings provide vital information for the improvement of ideal surfaces for cardiovascular applications.

EFFECT OF CALCIUM RELEASE ON OSTEOBLAST NUMBER AND ACTIVITY

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Introduction

There has been on going interest in the effect of calcium oxide phase impurities on the bioactivity of hydroxyapatite. When the Ca/P ratio is greater than 1.67 calcium oxide phases develop upon heat treatment ≥1100 °C. Work by Hing et al.[1] showed that the presence of calcium oxide diminished the osteogenic response both in vitro and in vivo. Work by Liu et al.[2] confirmed these results by investigating Ca/P ratios in vitro, finding that Ca/P ratios greater than 2 reduced osteoblast viability. The aim of the present work is to investigate the hypothesis that the presence of calcium oxide diminishes bioactivity through the mechanism of calcium ion release via inhibition of osteoblast activity.

Experimental

Various calcium phosphates were produced with Ca/P ratio between 1.5 and 1.8 as previously described [3]. The powders were milled to < 90 μm and pressed in a carbide die into discs, and along with paired chemistry powder samples, were heat treated at 1100 °C. The heat treated powders were analysed with XRF and XRD.The sintered discs were sterilised and primary human osteoblast cells cultured on them for 21 days. The activity of the cells was monitored through Alamar Blue and the ALP activity analysed over the period. The mineral produced and its association with the cells was visualised through labelling with tetracycline hydrochloride one day before the end of the experiment and cell nucleus/cytoskeletal counterstaining with Hoechst and Phalloidin, studied using confocal microscopy. Whenever media was changed the media was retained and analysed using ICP.

Results and Discussion

Using XRD and XRF analysis we were able to confirm the Ca/P ratios of 1.55, 1.68 and 1.77 and the presence of a phase of calcium oxide (Figure 1) where expected at Ca/P greater than 1.70. As a consequence of the cell culture experiments influence of calcium oxide on osteoblast activity was considered and correlated with calcium release.

Conclusions

Whilst previous studies have appeared to suggest a negative influence of CaO presence on osteoblast viability, this study is the first to consider directly the influence of calcium release into the media on the cell behaviour.

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KEYNOTE LECTURE

BIOMIMETIC COATINGS ON POLYMERIC SCAFFOLDS FOR BONE DEFECT REPAIR AND 3D IN VITRO MODELS

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Due to the large number of patients suffering of bone defects caused by tumor or diseases, in recent years, numerous investigations are in progress, making efforts on the development of new materials and processing techniques for bone tissue regeneration. Moreover, from decades, tumor research is based on 2D in vitro tumor models and animal models to reproduce what happens in the human body to identify and verify new anti-neoplastic therapies' effectiveness [1]. Due to the limitations of these models, only 5% of anti-neoplastic drugs, which demonstrated good results during 2D in vitro and in vivo studies, confirmed these results during clinical trials. For these reasons, during the last decade, 3D in vitro tumor models have been developed to reproduce mechanical, biochemical, and structural bone microenvironment elements, allowing cells to behave in vivo.The use of natural or synthetic polymers for the realization of 3D scaffold-based in vitro models is extremely appealing due to the possibility of processing them into 3D structures [2]. However, one of the important requirements for bone-bonding behavior is the formation of a calcium phosphate interface similar to bone apatite. The choice of appropriate characteristics is crucial to favor a firm bonding between orthopedic implants and bone cells and to permit the formation of tissue-like bone. Different approaches for the design of 3D in vitro models based on various polymeric porous scaffolds, in order to mimic the structural, physical, and mechanical properties of bone tissue will be described. In particular, to modulate the biointerface, new biomimetic coatings, having nano- and/or micro- morphological cues and a composition mimicking the mineral phase of bone will be described considering their chemico-physical and morphological properties. In vitro biological characterization will be shown providing appropriate information on the performance of the produced scaffold-based models in mimicking bone tumors.

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KEYNOTE LECTURE

BIOCERAMICS DESIGNED TO GAIN THE INFORMATIVE STATUS NECESSARY TO DRIVE PROPER CELL BEHAVIOR IN REGENERATIVE MEDICINE

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The ever increasing need of more effective and targeted therapies for the treatment of various pathologies is pushing scientists to develop new solutions associating enhanced safety with smart functionality, also permitting the establishment of personalized therapeutic approaches. Regenerative medicine often requires the use of scaffolds acting as an instructing guide for cells, so that substantial mimicry of compositional, morphological and structural features of natural tissue is highly desired. However, this target is very hard to achieve, when human tissues with high compositional and structural complexity. An emerging concept in material science is to draw inspiration from natural processes and products, which we may consider as the most advanced examples of smart nanotechnology. Ceramic-based nanomaterials represent very versatile biomaterials offering promising examples, and here two successful studies were reported. Natural structures such as woods and plants exhibit multi-scale hierarchic organization that is the source of smart and anisotropic mechanical properties associated with high porosity and lightness. Porous woods (rattan) were recently transformed into hydroxyapatite (HA) scaffolds with hierarchic organization focusing on long bone critical defect. Biomorphic 3-D hierarchically organized porous ceramics consisted in highly biomimetic, multi-substituted nanocrystalline apatite phase, directly nucleated in the 3-D state to form a scaffold exhibiting bone-mimicking structure with open and interconnected porosity and hierarchical organization at the multiscale. Such features determined very high mechanical strength and damage-tolerant mechanical behaviour, thus resulting into high mimicry of bone tissue. We have proved the cellular cross-talk between human mesenchymal stem cells and human endothelial cells in a perfusion bioreactor system as a predictive *in vitro* model for bone regeneration. These results confirm that the adoption of nature-inspired processes is an elective approach to obtain highly bioactive materials with innate cell-instruction ability, thanks to biomimetic features that are not achievable by the traditional ceramic processing. Another example of the great potential of ceramic nanomaterials is related the cell therapy. Cell therapy is one of the most exciting and promising areas for disease treatment and regenerative medicine. However the success rate of cell-based therapies, despite their great potential, is limited mainly due the ineffective delivery and retention of therapeutic cells in the specific organ. Magnetic targeting has emerged as a method to overcome these limitations. So far these attempts have used superparamagnetic iron oxide nanoparticles (SPIONs), only clinically approved metal oxide nanoparticles. Nevertheless the exposure to SPIONs has always been associated with significant toxic effects such as inflammation, apoptosis and generation of ROS. Our group, by doping HA, the mineral component of bone, with Fe^{2+}/Fe^{3+} ions, had obtained novel biocompatible and fully bioresorbable superparamagnetic nanoparticles (FeHA). This work demonstrates the opportunity of FeHA in Mesenchymal Stem Cells (MSCs) labeling. MSCs easily internalized the FeHA, and they became magnetic enough to be guided and retained to specific site by a magnet. Magnetic MSCs maintained their morphology and cell viability was not negatively affected. Due the well-known osteoinductive feature of HA, magnetic MSCs overexpress osteogenic genes. We are also investigating the possibility to combine these above-mentioned results with the contrast ability of FeHA for a real time imaging of the magnetic MSCs *in vivo* by magnetic resonance imaging. In conclusion, these new approaches promise to be a breakthrough in the synthesis of bioceramics with boosted bioactivity, potentially opening to frontier applications in regenerative medicine.

Keywords
Regenerative medicine, nanomaterial, ceramic, cell therapy, tissue engineering, 3D scaffold, co-culture.

TECHNIQUES FOR CHARACTERISATION AND BIOCOMPATIBILITY ASSESSMENT OF CERAMICS AND SCAFFOLDS FOR TISSUE REGENERATION

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The pace of development of new materials and manufacturing approaches for bioscaffolds continues to drive exciting possibilities. Characterisation of these scaffolds in terms of structure is key in developing the best set of parameters for optimal tissue regeneration results. In addition, biocompatibility of each scaffold needs to be tested and quantified in order to understand the potential of any new approach to scaffold development, manufacture or practical usage.

Microscopy plays an important and varied role in the process of characterisation and biocompatibility assessment: From structural characterisation of scaffold materials across lengthscales using X-ray and electron microscopy approaches, to fluorescence quantification and visualisation of cells within seeded scaffolds or the measurement of regenerated tissue. Selecting the optimal approach for each criteria assessment is vital in order to reach a full characterisation profile of any new bioscaffold.

This talk will provide an overview of some of the microscopy methods for scaffold assessment and tissue regeneration success and provide some insights into the latest possibilities for using these together to build a full profile of bioscaffold performance.

SCAFFOLDS BASED ON TRICALCIUM PHOSPHATE AND BACTERIA-DERIVED POLYHYDROXYOCTANOATE – CYTOCOMPATIBILITY STUDIES

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Tissue engineering faces a major challenge in providing novel, functional materials that will meet the requirements of tissue engineering. These properties can be provided by tricalcium phosphate-based bioceramics (TCP). The combination of a ceramic matrix with polymers creates great opportunities to improve the physicochemical and biological properties of the obtained composites. Polyhydroxyoctanoate (PHO) belongs to the polyhydroxyalkanoates (PHAs) family. Due to its degradation products – nourishing (R)-3-hydroxyacids[1], the application of this novel elastomeric polymer opens new routes for promoting tissue regeneration.In this study, the bioceramic/polymer based scaffolds (TCP/PHO) were prepared as previously described[2]. To evaluate TCP/PHO composites as bone tissue substitutes, their biocompatibility was tested using MC3T3-E1 mouse preosteoblastic cell line. Cells were cultured in Minimal Essential Medium Eagle medium with alpha modification - α-MEM supplemented with 10% fetal bovine serum - FBS and antibiotics: penicillin/streptomycin at 37°C in an incubator. Materials' samples were incubated in α-MEM for 24 hours at 37°C. MC3T3-E1 cells were seeded in a 96-well plate at 25·103 cells per well and incubated in 150 µl α-MEM for 24 hours. Then, medium was exchanged with extracts obtained by incubating material samples in α-MEM. Cells were cultured for 24 hours. The negative control was cells cultured in MEM. The cell viability assay (Alamar Blue) was performed.In the case of supernatants from TCP scaffolds, cell viability was 117.11 ± 5.59% - significantly higher in comparison to the negative control (100% viability). This result indicates that TCP material extract has a beneficial effect on cells during the first 24 hours. TCP/PHO scaffolds were fully biocompatible and cell viability was comparable to that of cells in α-MEM and was 105.15 ± 3.62%. The reason for these differences with TCP is the hydrophobic nature of polyhydroxyoctanoate. The obtained results suggest that both bioceramic and composite scaffolds are fully cytocompatible.

NEW APPROACH TO IDENTIFY THE PHYSIOLOGICAL STATE OF BONE CELLS AT THE SURFACE OF HYDROXYAPATITE BIOCERAMIC

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Monitoring the evolution of the biomaterial-tissue interface during tissue reparation after bioceramic scaffolds grafting in patients remains nearly impossible in a non-invasive way. Moreover, the characterization of the biological properties of bioceramics are time and sample-consuming using conventional biology methods. The aim of this work was to identify robust and reproducible signatures characterizing the different steps of bone cell differentiation, from precursors to mature bone cells. For this purpose, the features identified must be detectable using any label-free imaging method. For this reason, we focused on cell structure and morphology. This study was performed on human mesenchymal stem cells (hMSCs) grown on hydroxyapatite ceramics in comparison with cells grown on non-bioceramic supports either in growth medium (GM) or in osteogenic medium (OM). Computer-assisted image analysis was used to determine the relevant parameters for the building of signatures. Conventional cell biology methods were used as a comparison to validate this new approach. Some early morphological changes of hMSC during osteogenic differentiation were identified. Amongst these changes, cell density was lowered for cells cultured in OM. Cells cultured in OM had significantly bigger nuclei as soon as 48h after induction of differentiation, whatever the culture support. With differentiation, cells were bigger with oriented stress fibers. Their focal adhesions were reinforced. Type I collagen staining appeared more intense in OM as a common marker of osteogenic differentiation. After 7 days, this modifications were accentuated. Interestingly, the formation of type I collagen fibers was enhanced for cells cultured on bioceramics in comparison for those that have grown on glass. Used in combination these features should allow to characterize the differentiation state of hMSCs at the surface of biomaterials reducing the number of needed samples to do so. Efforts are made now to validate this approach using machine learning and unlabeled data sets.

ADVANCED THERAPY MEDICINAL BIOMATERIAL

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Oro-antral communication is an iatrogenic disease characterized by the presence of an abnormal pathological communication between the oral cavity and the maxillary sinus or nasal cavity. The proposed surgical treatments are often invasive and are accompanied by the presence of recurrent OCs[1].Considering these limitations, our work attempts to propose a solution via the elaboration of bioactive, dry, and porous foams, made from platelet lysate (PL) and type I collagen (ColI) developed according to a shaping process involving supercritical CO2 (scCO2) (figure 1).PL is a blood derivative rich in bioactive molecules (growth factors, cytokines, etc.) that can be released and ensure stimulation of cellular activity[2]. ColI is able to form a three-dimensional network that mimics the extracellular matrix and will act as a scaffold for the cells while offering mechanical properties such as tensile strength [3]. Several studies have shown that scCO2 has a sterilization potential which makes its use as a shaping process very relevant [4]. We investigate the effect of formulation and drying parameters on the physicochemical and biological properties of the foams produced. First results shows that the elaboration process allows to obtain dry foams which keep the 3D structure of the formed network. Microscopic observations show that the collagen concentration influences the density of the network and the porosity which remains above 90%.Moreover, the modifications of the three-dimensional structure make it possible to modify the rate of release of the VEGF naturally contained in the PL. It is thus possible to envisage controlling the release kinetics of the biomaterial and its duration of action by controlling its porosityFollowing these results, a formulation will be chosen and tested on a murine animal model in order to evaluate the biocompatibility, biodegradability, and in vivo efficiency of our biomaterial and, subsequently, to validate our manufacturing process.

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HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS TO EVALUATE THE BIOLOGICAL PERFORMANCES OF ION-DOPED SINTERED HYDROXYLAPATITE SCAFFOLDS

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Tissue engineering (TE) aims to repair/regenerate tissues damaged by injuries or diseases [1]. Biomaterials play a key role in these strategies, where they serve as a substrate for the incorporation and release of ions [2]. The Mg2+, Sr2+ and Zn2+ ions are active regulators of the proliferation and differentiation of osteoblasts and osteoclasts, thus modulating bone turnover [3]. Specifically, Mg2+ has attracted the attention of the scientific community in the field of TE. In the present work, an in vitro model of human adipose-derived mesenchymal stem cells (hASCs) was used to evaluate the cytocompatibility and osteoinductivity properties of four different sets of ion-doped sintered hydroxylapatite (S-HA) powders. The set includes S-HA doped with Mg2+, Sr2+ and Zn2+ ions (coded as S-MgHA, S-MgSrHA, S-MgZnHA, respectively) and non-doped S-HA, as reference material. Live/Dead dye was carried out to evaluate the percentage of living cells. The cellular metabolic activity and the cytoskeleton organisation were investigated by AlamarBlue® metabolic assay and Phalloidin-TRITC immunostaining, respectively [4]. In order to evaluate the expression of genes involved in osteogenic differentiation, a Real-Time PCR Array was performed. The Osteocalcin and Osteopontin proteins expression was assessed by performing ELISA. The cytoskeleton architecture of hASCs grown in contact with the scaffolds seems to be well organised, whereas its integrity remained uninfluenced by the scaffolds over time. Live/Dead staining and metabolic activity of hASCs grown on ion-doped materials was increased during the experiments, up to day 14. Osteogenic genes, such as BMP1 and SP7 transcription factor, were expressed on hASCs grown on different ion-doped materials with different fold-changes. Our experiments suggest that multiple-ion doping scaffolds seem to maintain a positive modulation of hASC osteogenic differentiation, compared to the control. The present work suggests that the exploration of different doping agents for scaffolds can yield new sintered materials with optimised biologic performances.

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SEPTEMBER 21ST

PLENARY LECTURE

THE ROLE OF BIOMECHANICS AND BIOLOGY ON REGENERATIVE MEDICINE

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Lesions of knee cartilage have poor self-healing capacity and can lead to persistent symptoms and impaired joint function. Moreover, if left untreated, they can further degenerate and lead to osteoarthritis. Several surgical techniques have been developed to address these lesions, although there is no agreement about the best option. In this context, there is an increased awareness of the role of subchondral bone in the pathogenetic process of articular surface lesions and, therefore, on the possibility of restoring any damaged osteochondral tissue as a single anatomic and functional unit. Several studies showed poorer results and higher failure rates when chondral restoration was performed in the presence of subchondral bone changes, supporting the need for osteochondral solutions. Nowadays, osteochondral tissue represents a significant challenge in regenerative medicine, due to its complex architecture and biomechanical properties. Therefore, the development of osteochondral scaffold faced uphill challenges due to poor tissue formation and scarce integration at the cartilage-bone interface. Different options are available to address osteochondral defects, including osteochondral autograft transplantation, fresh osteochondral allograft, and autologous chondrocyte implantation (ACI) techniques with bone augmentation. However, all these approaches present some drawbacks: those involving the transfer of autologous tissue can have a certain degree of donor-site morbidity, which mainly limits their use to small defects. Osteochondral allograft is an effective option for larger size defects, but they have limited availability in most countries. ACI/matrix-associated autologous chondrocyte transplantation (MACT) techniques require a 2-step surgery, with related higher costs and morbidity. For these reasons, cell-free off-the-shelf devices have been introduced to restore osteochondral defects through a single surgical procedure. This treatment approach led to the development of a few different devices, with variable results. Among these, an osteochondral scaffold made of type I collagen and hydroxyapatite (Maioregen; Finceramica) has been extensively investigated at short-to medium-term follow-up. Several studies showed promising clinical results in different groups of patients, although abnormal findings were reported with imaging evaluation. 10-years follow-up shows that regenerative potential of cell-free osteochondral scaffold is limited, as demonstrated by the signal alterations persisting over time on MRI scans. On the other hand, the clinical improvement was significant and stable over time both in terms of subjective and objective outcomes, including activity level, with overall good results.

PLENARY LECTURE

INNOVATIVE COATING TECHNOLOGIES AND NANOTECHNOLOGICAL APPROACHES TO REDUCE BACTERIAL BIOFILMS

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Bacterial resistance, one of the biggest threats to human health in the 21st century, is the ability of bacterial cells to resist one or more types of antibiotics. Few antibiotics have been developed in the last two decades. With limited antibiotic options and an escalating bacterial resistance there is an urgent need to explore alternative ways of meeting this global challenge. Antibiotic-resistance can be developed by bacteria using different mechanisms but one of this is related to the capacity to form biofilms. Biofilms are functional aggregates of sessile microorganisms encased within a self-generated extracellular polymeric matrix composed of polysaccharides, lipids, proteins, and DNA. Microorganisms living in biofilms are much more resistant to hostile environments than their planktonic counterparts and exhibit enhanced resistance against the microbicides. From the human perspective, biofilms can be classified into beneficial, neutral, and harmful: most of the actions are oriented to eradicate harmful biofilms. In particular biofilm infections in medical implants are a global problem presenting a significant socioeconomic impact. Infact, biofilm is considered a leading cause of medical devices failure and infection recurrence: at present, the only treatment strategy to eradicate the infection is implant removal. Despite the multifactorial causes that lead to biofilm-related medical device infections, bacterial adhesion to the implant surface is a common and essential step in all instances. The ideal implant surface would be one that minimises bacterial adhesion, inhibits biofilm formation, and confers an effective bactericidal action. Different type of coatings technologies have been developped and classified into i) passive surface modification that rely on repulsion of microbes, ii) active surface modification that attempt to kill the microorganism, and iii) approaches that affect biofilm architecture, which focus on reducing biofilm virulence factors. Whilst the strategies outlined above exist with the aim of preventing biofilm formation, there remains a need to treat biofilms that have already formed. Several studies indicate that various types of nanomaterials (both organic and inorganic) have demonstrated promising results regarding antibacterial and antibiofilm activity. It has also been claimed that the use of nanoparticles is one of the most promising strategies to overcome microbial drug resistance. The size of the nanomaterials provides a large surface-area to volume ratio, which allows the binding of a large number of high affinity ligands, equipping nanoparticles with a multivalency in eradicating bacterial cells. In particular, owing to the optical and electrical properties of gold nanoparticles (AuNPs), they have gained increasing attention. One particularly important feature is their localized surface plasmon resonance (LSPR), which plays an important role in many nanotechnological applications. Two main approaches that employ light activation in enhancing the antibacterial activity of gold nanoparticles are antibacterial photothermal therapy (APTT) and antibacterial photodynamic therapy (APDT). Although the safety and toxicity of the AuNPs is still a topic that needs to be further addressed, there are some promising studies that feature both an excellent safety profile along with potential therapeutic benefits.

In summary, utilizing multimodal prevention and therapeutic strategies that do not function through the same mechanism of action have the highest potential to terminate bacteria, without allowing for resistant strains to evolve.

SYMPOSIUM 1
CaP BIOCERAMICS FOR REGENERATIVE MEDICINE

KEYNOTE LECTURE

IN VIVO BEHAVIOR OF CALCIUM PHOSPHATES: THE IMPORTANCE OF SURFACE CHEMISTRY

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Surfaces are considered to be an essential element in determining the biological response of biomaterials. Accordingly, many studies have addressed the effect of surface chemistry and topography on cells in vitro and on biological tissues in vivo. Even though some biomaterials, in particular calcium phosphates, may present very large surface areas and trigger surface mineralization or protein adsorption, few efforts have been invested in trying to determine the effect of local changes of chemical composition on the biological response of biomaterials. Recent studies have shown that surfaces can induce very significant changes of the local chemical composition, in particular in the pores of scaffolds. These changes can be due to the uptake of ions by surface mineralization (the so-called "bioactivity"), to an uptake and release of ions during apatite maturation, or due to the release of chemical impurities present on the surfaces. Calcium-deficient hydroxyapatite tends to take up calcium ions and release acidic phosphate species. Typical impurities of sintered calcium phosphates include β-tricalcium phosphate, Ca oxide, and Mg oxide. The aim of this presentation is to review our current knowledge on the link between calcium phosphate surface chemistry and biological response. The focus is set on material aspects, more specifically on ways to modify calcium phosphate surfaces to trigger interesting biological reactions such as osteoinduction.

KEYNOTE LECTURE

MANUFACTURING METHODS OF BIOCERAMIC SCAFFOLDS

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For many years, bone substitutes have been made from calcium phosphate powders because of their excellent biological properties. Different methods are used to manufacture scaffolds with a porous architecture that aims to approximate the natural bone skeleton.

Several fabrication methods for preparation of porous ceramic have been developed in the laboratory such as the addition of sacrificial PMMA porogens, freeze casting and additive manufacturing methods. The physical characteristics of the porous scaffolds obtained by these different techniques and the biological responses will be presented and compared.

Finally, an original process combining microwave-assisted thermal treatment and an additive manufacturing technique allowing the rapid fabrication of customized bioceramics will be presented.

DESIGN OF ORGANIC-INORGANIC HYBRIDS WITH OSTEOCONDUCTIVITY THROUGH UNDERSTANDING CHEMICAL REACTION OF CALCIUM SILICATE-BASED GLASS IN BODY ENVIRONMENT

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Osteoconductive glasses and glass-ceramics are classified as bioactive materials that make direct bonding to living bone after implantation in bony defects. Bioactive glasses and glass-ceramics have been reported to show osteoconduction by formation of hydroxyapatite layer on their surface through the surface reaction with surrounding body fluid. The hydroxyapatite layer formed on the surface has similar characteristics to inorganic substances in bone mineral, and thus called as bone-like apatite layer. Capability of formation of the bone-like apatite layer can be evaluated by using a type of simulated body fluid (abbreviated as SBF) that mimics human blood plasma, proposed by Kokubo and his colleagues. SBF is an acellular solution, buffered with trishyroxyethylaminomethane-HCl, without proteins. Previous studies of in vitro investigation on the mechanism of hydroxyapatite layer formation in SBF have shown that calcium silicate glasses are likely to exhibit hydroxyapatite layer formation, i.e., osteoconductivity, after being implanted in vivo. It is also known that this formation of hydroxyapatite is attributed to the increased supersaturation to hydroxyapatite caused by the dissolution of calcium ions and the silica hydrogel formed on the material surface, which induces heterogeneous nucleation of hydroxyapatite. Therefore, it is possible to develop new osteoconductive materials by controlling the release of calcium ions from the material and the formation of silica hydrogel on the material surface. In this study, investigation of the organic modification of calcium silicate is reviewed to develop an organic-inorganic hybrid material with osteoconductivity. Modification of organic polymer with Si–OH groups and calcium salts successfully produced bioactive organic-inorganic hybrids from polyhydroxyethylmethacrylate(HEMA)-methacryloxyproply trimethoxysilane (MPS), poly(tetramethylene oxide), polysaccharides, polyamino acid, from the same concept. This design of biomaterials leads novel artificial bone that has unique functions derived from organic substances.

EFFECTS OF INCORPORATION OF DICARBOXYLATE IONS INTO OCTACALCIUM PHOSPHATE ON A TOPOTACTIC TRANSFORMATION INTO HYDROXYAPATITE: REACTIVITY AND MORPHOLOGY

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Octacalcium phosphate (OCP) has been received much attention as a novel biodegradable bone repairing material. The OCP crystal is composed of apatitic and hydrated layers. The hydrogen phosphate ions in the hydrated layers can be substituted by dicarboxylate ions. Although the syntheses of OCP with incorporated dicarboxylate ions have been previously studied, its reactivity and transformation to HAp is less understood. In this study, we investigated the transformation of OCP with incorporated suberate ions (Sub-OCP) into HAp under hydrothermal conditions, and characterized the resultant HAp.Plain OCP and Sub-OCP were synthesized by the wet-chemical process. These OCPs were added to a Teflon® vessel with ultrapure water. The samples were hydrothermally treated at 120, 150, and 180 °C. The treated samples were collected by vacuum filtration and dried overnight. The crystalline phases of the products were characterized by powder X-ray diffraction (XRD). The morphology of the samples was observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The XRD patterns of the samples before and after the hydrothermal treatment indicated that the crystalline phase of these samples was changed to HAp by the hydrothermal treatment. The HAp was obtained from plain OCP and Sub-OCP treated at 180 and 150 °C, respectively. The crystal morphology of samples was almost retained after the hydrothermal treatment under SEM observation, although the crystalline phase changed from OCP phase to HAp. According to the TEM observation, the HAp particle synthesized from Sub-OCP has a mesocrystal structure, while that synthesized from plain OCP was single crystal.These findings indicate that the incorporation of dicarboxylate ions into OCP could be one of factors for controlling degradability of OCPs in vivo environment, and Sub-OCP can be used as a precursor phase of HAp mesocrystals for biomedical applications.

SYMPOSIUM 4
ANTIBACTERIAL BIOCERAMICS FOR SMART PROSTHETIC APPLICATIONS – *SPONSORED BY ACERS*

KEYNOTE LECTURE

ADDITIVE MANUFACTURING OF CUSTOMIZED SIC ARTIFICIAL BONE GRAFT

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Synthetic bone implants have been made especially viable with the rise of the 3D bioprinting industry. According to *Grand View Research*, the 3D bioprinting market is forecasted to grow at a compound annual growth rate of 17.4% and reach $4.2 Billion by 2027. The market drivers for the 3D bioprinting market are the technological advancements of bioprinters and biomaterials for manufacturing customized products. The current FDA approved porous scaffolds used for bone grafting in trauma and vertebrae surgeries are made of titanium alloy and poly[aryl-ether-ether-ketone] (PEEK). These orthopedic implant materials are not bioactive, in the sense that they do not bond to bone and do not support bone cell function. Moreover, the release of toxic metal ions and polymer degradation products is a typical problem associated with systemic effects and implant failure. Hydroxyapatite and tricalcium phosphate ceramics are bioactive materials however they lack the mechanical strength necessary for load bearing applications. Moreover, they have limited stimulatory effects on bone cell function due to inappropriate dissolution in physiological solution. Therefore, there is a need for a new design for a bioactive graft that can be processed by additive manufacturing, mechanically compatible to bone and able to stimulate tissue formation and vascularization.

Silicon carbide (SiC) is an inert material with excellent biocompatibility and mechanical properties due to the strong Si-C covalent bond. For the same reason SiC is difficult to machine and its processing into 3D objects requires sintering temperature close to 2000 C at 100-1000 atmospheric pressure. As a biomaterial, SiC has been relatively unexplored, despite its good wear characteristics and resistance to corrosion. We activated the surface of SiC by NaOH solution to generate 0.1-2 micron thick silica gel layer rich in silanol groups. Hydration of the surface activated SiC particles during 3D printing in a powder bed printer promoted bonding of the particles via the polymerization of the silanol groups at room temperature. Subsequent thermal treatment of the 3D printed porous SiC objects in the temperature range (550 – 1000 ^{O}C) in air or oxygen environment controlled the porosity characteristics, strength, toughness and modulus of elasticity. The surface modified SiC scaffold acquired negative zeta potential (- 40 mV) that attracted calcium ions from physiological solution and enhance the deposition of a biomimetic carbonate-containing calcium-deficient hydroxyapatite layer. The porous SiC scaffold facilitated cell invasion through its entire thickness and stimulated osteoblast differentiation and mineralized bone matrix formation. The bioactivity mechanism of the SiC porous scaffold is twofold including the surface bioactivity created by the silica gel layer and the electric charge created by the piezoelectric properties of SiC. The porous bioactive SiC bone implant can be used as a delivery system for cells and biological molecules in elder patients with systemic diseases or in patients with significant bone losses.

Keywords
SiC scaffold, 3D printing, Surface charge, bone graft, bioactivity

NANOSTRUCTURED TCP-BASED COATINGS WITH DIFFERENT ION DOPING AND THEIR CAPABILITY TO INHIBIT BACTERIAL COLONIZATION

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Biomaterials in orthopaedics are required to promote osseointegration and discourage infection. To meet these two aims, the composition and morphology of the biointerfaces are crucial to determine the interactions with bacteria and with healthy cells.Here, we propose new nanostructured coatings, made by Ionized Jet Deposition of beta-tricalcium phosphate (ß-TCP) and deposited onto titanium-aluminum-vanadium alloy disks (surface roughness - Ra: 5 μm). Different ion-doping (Ag, Cu, Zn, Fe) of the TCP is tested to address different bacterial contamination. Coatings morphology and topography (FEG-SEM, AFM), composition (FT-IR, EDS), adhesion to substrate (micro-scratch), were investigated. The absence of cytotoxicity was verified against host cells (mesenchymal stem cells derived from adipose tissue). Finally, efficacy against Gram + (S. aureus, P. aeruginosa) and Gram – (E. coli, E. faecalis) is investigated in terms of inhibition of bacterial viability and growth and in terms of reduction of bacterial capability to adhere to the substrate. Statistical analysis was performed on at least three replicates, using one-way Anova test. The coatings have submicrometric thickness and nanostructured surface morphology. The composition is preserved from the coating to the target both in terms of main CaP phase and of ion doping. All films are non-cytotoxic. High adhesion is found to titanium substrates.Coatings show high antibacterial efficacy, as they inhibit bacterial viability for both Gram + and Gram – and also reduce the capability of all bacterial strains to adhere to the substrate, which is the first step leading to biofilm formation. The efficacy towards the different strains is bacteria-specific, indeed compound with silver and iron show higher efficacy towards the Gram – strains, while zinc and copper are more efficient against the Gram +. The developed coatings appear promising for applications in orthopaedic surgery.

FABRICATION OF BIOACTIVE GLASS - HYDROXYAPATITE COMPOSITES BY ELECTROSTATIC SPRAY DEPOSITION FOR TI-BASED IMPLANTS

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Replacement of missing or diseased hard tissues has become a common procedure in medicine and dentistry. In this field, metallic implants are often coated to improve their biological performance. Herein, the novel electrostatic spray deposition (ESD) technique was employed to fabricate a stacking of bioactive glass (BG) and hydroxyapatite (HAP) deposited onto Ti6Al4V ELI substrates. ESD is an innovative, low-cost method based on electrohydrodynamics' laws that allows the deposition of films with a large variety of original morphologies and phases, including crystalline and amorphous ones [1, 2]. In this work, a highly porous coral-like BG coating with an S58 (58 SiO2 – 37 CaO – 5 P2O5 mol. %) formulation was deposited onto a thin and dense HAP layer starting from homogeneous liquid precursor solutions [2, 3]. The constituent phases were selected to combine the high bone-bonding ability of BG with the long-term stability and protection of HAP. Two composites samples (C1 and C2, Fig 1) were fabricated by varying the deposition time of the S58 topcoat to analyze the influence of BG coating thickness on the composite bioactivity i.e., calcium phosphate (CaP) forming ability. In vitro studies were carried out by immersion in simulated body fluid (SBF) solution. Single-layer samples were used as a control. The SBF test revealed that the presence of the BG topcoat layer significantly improves the reactivity, in terms of mineralization response, compared to a single-layer HAP coating. A complete conversion into CaP precipitated layer was produced within 7 days soaking in SBF for both composite samples. Furthermore, the S58 coating thickness was found to influence the bioactivity response. The thinner C1 composite is proposed over the thicker one to promote osteointegration of Ti6Al4V-based implants while maintaining the stability of the device.

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CERAMIC FINGER JOINT IMPLANTS: AI DESIGNED AND MANUFACTURED BY DIRECT SHAPING METHODS

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Patient-specific implants promise a high degree of fit accuracy and thus better functionality and durability. Individualization is also a great opportunity for those fields in which the possibilities of implant restoration and remobilization are still limited. For small joints, especially in the area of finger joints, therapy consists mainly of stiffening joints, which reduces mobility. In Germany alone, this affects 5 million people suffering from symptomatic arthrosis and 1.5 million people suffering from rheumatic diseases. In the Fraunhofer-internal project "Remobilization of Finger Joints by AI-based Reconstruction and Development of Patient-Specific Ceramic Implants – FingerKit" for the first time, a continuous, automatable process chain in the manufacture of patient-specific implants, from design to manufacture, to certification-compliant testing.The project focuses on the material, surface design and manufacturing of ceramic implants. The long-term stability of custom-made finger joint implants is to be significantly increased compared with commercially available standard variants by customizing the mechanical strength, osseointegration (growth of bone cells onto the implant) and implant design. The slip casting process for the oxide ceramic material alumina-toughened zirconia (ATZ) enables direct shaping in a porous, structured mold. This requires the production of complex shaped casting molds with an integrated structure. The molding behavior was investigated during the casting process and material characteristics were determined. The goal is to manufacture finger joint prototypes with a macro-/microstructure of the outer surfaces in a single process step.In a parallel development track, silicon nitride (Si3N4) is used as bioceramic material. Implant production will be realized with the CerAM VPP process (Lithoz LCM technology), a 3D printing technology. This process can be used to create, for example, the so-called TPMS structures (triply periodic minimal surfaces), which have many advantages in terms of mechanical properties and osseointegration.

INCREASING THE RELIABILITY OF BIOCERAMICS PROCESSED THROUGH STEREOLITHOGRAPHY USING TOUGH ZIRCONIA-BASED COMPOSITES

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Ceramic laser stereolithography (SLA) offers a high degree of design freedom and resolution required in the development of complex and dense ceramic pieces, such as structural biomedical implants. Our study aims to understand and limit the creation of defects at each step of the additive manufacturing process. In this context, we will discuss here: I) the paste rheology and ageing II) the effect of laser parameters in the photopolymerization III) the creation of defects during printing and post-printing steps and IV) the mechanical properties of Ce-TZP-based ceramics prepared by SLA.SLA pastes have a high content of ceramic particles (ca. 50 vol.%) making their mechanical response to scraping quite difficult to rationalize without a fine structural analysis. To address this question, we conducted rheological investigations in order to: 1) provide quantitative parameters describing the "printability" of the paste 2) characterize the paste ageing behaviour and its possible reuse over a year and 3) understand the restructuring of ceramic particles under shear stresses.Besides, the laser energy received per volume element was estimated in order to better understand the effects of printing parameters and laser patterning on the photopolymerization of the paste. The monomer conversion and solidification was investigated by FT-IR, NMR and compared to Jacob's equation results.SLA printed samples have been submitted to DSC & ATG tests in order to understand phenomena involved during debinding. Laser parameters and heating cycles were optimized to fully densify zirconia-based ceramics.Finally, dense ceramics prepared from a specially developed Ce-TZP-composite paste showed promising mechanical properties despite the classical printing defects still caused by this technology. Zirconia phase transformation behaviour demonstrated its ability to increase the resistance of composites to the presence of microstructural inhomogeneities, layer decohesions and other defects.We finally close this discussion by showing some previous results on zirconia bone integration and projected work on femtosecond laser surface architecturation for enhancing bone-cells adhesion to SLA manufactured Ce-TZP based biomedical implants.

SYMPOSIUM 1
CaP BIOCERAMICS FOR REGENERATIVE MEDICINE

KEYNOTE LECTURE

BIOACTIVE CERAMIC, GLASS-CERAMIC AND COMPOSITE PLATFORMS FOR BONE TISSUE ENGINEERING: FROM THE FORMULATION TO THE POTENTIAL APPLICATIONS

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Bone tissue engineering is gaining a progressively increasing attention, due to the population ageing, associated to the quality of life improvement, and consequent life expectancy increase. Thus, a significant bone injuries and diseases incidence increment has been recorded, with substantial socioeconomic and healthy impacts [1]. Calcium phosphates (CaPs) have shown a good in vitro and in vivo cellular/tissue responsiveness, but present many criticisms in terms of mechanical properties and bioactivity, preventing their clinical applicability. Thus, there is an urgent need of new strategies aimed at overcoming these drawbacks. The proposed approaches consist in the production of biomimetic CaPs, characterised by a nanosized structure and by the presence of vicarious ions (e.g. Si4+, F-, Mg2+, Sr2+, K+...) within their lattice, in order to mimic the bone structure and composition [2-3], and of composite systems, based on a biopolymer matrix. Moreover, bioactive glasses (BGs) can be considered potential alternative materials, due to their improved osteoinductive behaviour, bioactivity, ability to bond to both soft and hard tissues. Moreover, their ionic dissolution products promote the osteoblastic cell genes expression, in vitro and in vivo angiogenesis, and antibacterial/anti-inflammatory actions [4,5].Both CaPs and BGs are employed to obtain: porous scaffolds [6]; hybrid fibrous membranes based on CaPs/BGs and biopolymers [7-9]; ceramic composites [10,11]; bioactive coatings on metallic supports [3] and polymeric scaffolds [10]. The effect of the selected synthesis process (wet precipitation for CaPs, sol-gel process for BGs), thermal treatment conditions (temperature, dwelling time), and dopant ions incorporation on the composition, thermal behaviour, phase evolution, sinterability, and bioactivity is discussed. The appropriateness of the obtained scaffolds as bone regeneration templates is investigated by the physicochemical, thermal, mechanical and biological points of view. In vitro cell tests are performed for the biological responsiveness assessment, using different stem cell lines, preosteoblasts and preosteoclasts [6,8-10].

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KEYNOTE LECTURE

BIPHASIC CALCIUM PHOSPHATE SCAFFOLDS USED AS PLATFORMS FOR BONE TISSUE ENGINEERING STRATEGIES

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Background

During the last decades Biphasic Calcium Phosphate (BCP) material became one of the synthetic Bone Graft Substitute (BGS) among the most used in clinical situations to replace the gold standard treatment (autograft). BCP based medical devices are recognized osteoconductive, bioactive, and even possessing osteoinduction/osteo-immunomodulation properties[1]. In critical clinical cases, a synthetic BGS alone is not sufficient for bone regeneration due to the lack of osteogenicity. Thus, in these situations bone tissue engineering strategies demonstrated promising results in different clinical trials. A challenging step for the future of bone tissue engineering strategy will be the usability improvement of the combined medical device for the surgeons. A special focus will be done on the MBCP® technologies used in bone tissue engineering strategies (granules and dried powder to be rehydrated for a moldable and cohesive putty).

Results

MBCP®+ granules are micro macroporous ceramics composed of 20% Hydroxyapatite and 80% beta-Tricalcium Phosphate. These granules were selected as scaffold for combination with B2A molecule which is a bioactive synthetic peptide that augments the osteodifferentiation via increasing BMP2 at local site of the implantation. This concept were evaluated in two different clinical indications with Prefix[2] study in lumbar spine fusion and with Amplex[3,4] study in foot and ankle fusion. Both kinds of clinical trials demonstrated safety and efficiency. The same BCP granules were used associated with autologous mesenchymal stromal cells (MSCs) in 3 different European projects (Reborne[5], Maxibone[6], and OrthoUnion[7]) demonstrating promising results with bone regeneration in non-union long bone and in atrophied mandibular bone regenerations.

A new generation of BGS called FDBS (Freeze Dried Bone Scaffold) was recently designed as a dried powder to be rehydrated with any solution chosen by the surgeon to get a highly moldable and cohesive putty. The improvement of the usability properties was particularly appreciated by the end users (by Voice of Customers survey evaluation compared to previous generation of BGS). Saline solution used to rehydrate FDBS showed efficient bone regeneration (*in vivo* implantation study), with comparable results with both granule shape and ready-to-use putty already cleared on the market. Moreover, FDBS rehydrated with MSCs suspension demonstrated the new product as a viable moldable and cohesive platform with similar cell behavior compared to granule shape (proliferation and differentiation of the MSCs into the putty compared to MBCP®+ scaffold by seeding the MSCs onto the granules). Preliminary data FDBS also demonstrated the capability of the putty to be used with PRP preparation for a controlled release of growth factors.

Conclusion

Recent studies (bench tests, *in vitro*, and *in vivo*) with Biphasic Calcium Phosphate based products have clearly demonstrated that several kinds of tissue engineering strategies could be used in the future for highly critical bone defects. A special focus seems important for medical device companies in the next years to design ATMP products easy to use with standardized clinical protocols.

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MULTI-PHYSICS AND MULTI-SCALE INVESTIGATION OF THE SETTING PROCESS OF GYPSUM AS A MODEL MATERIAL FOR BONE CEMENTS

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More than 2 million bone grafting surgeries are performed every year worldwide. Compared with conventional surgeries, minimally invasive procedures enable the implantation of biomaterials through a small incision, offering several benefits (lower pain, fewer complications ...). Because of their setting ability, hydraulic binding materials such as calcium phosphate (CaP) cements satisfy these surgery specifications.The setting process is continuous and always initiated by mixing one or several fine powders with an aqueous solution. The dissolution of the initial reactive powders results in the formation of a viscous and moldable paste, which properties evolve with time to form a porous monolithic ceramic through the nucleation and precipitation of more stable phases.The need to control the rate of reaction and final properties requires the precise understanding of the setting process in a multiphysic and multiscale approach. This issue has already been addressed in studies that were somehow limited regarding one or several aspects:– only one aspect of the setting process was studied (e.g. chemical reaction, evolution of the mechanicalproperties or of the microstructure).– only one length scale was investigated.– the setting process was stopped at discrete terms, with methods that risk to modify the pores sizes and shapes prior to characterization.In this study, gypsum plaster is studied in standard conditions (e.g. liquid to solid mass ratio), to develop in-situ and ex-situ multiphysic and multiscale characterization techniques to monitor the evolution of the:– phase composition (rate of dissolution and precipitation) using XRD and FT-IR spectroscopy,– microstructure using SEM (evolutions of crystals and porosities) and μ-CT,– mechanical properties using DMA, rheology and compression tests.This panel of techniques enables to monitor and to correlate the different physical transitions occurring during the setting process and to draw a global picture of the on-going phenomena.

SETTING PERFORMANCE OF AN OSTEOGENIC LITHIUM DOPED BRUSHITE CEMENT FOR BONE REGENERATION

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Due to their excellent biocompatibility and fast resorption in the human body, enabling replacement by newly formed bone, brushite cements are well suitable for bone regeneration. Their regenerative potential can be further improved by doping with bioactive ions like lithium, which promotes osteogenic differentiation-related gene expression, essential for osteogenesis.Hence, this study investigated the setting performance of brushite cements fabricated from Li+-doped β-tricalcium phosphate (Li+ contents of 0, 2.5 and 5 mol%), monocalcium phosphate monohydrate and an aqueous solution of the setting retarder phytic acid (IP6) in different concentrations. Isothermal calorimetry showed an accelerating effect of Li+ on the hydration kinetics, especially for low IP6 concentrations. Accordingly, a decrease of initial and final setting times with increasing Li+ content was detected with Gillmore needle apparatus. Setting times could be adjusted for clinical needs by varying the IP6 concentration. The cements presented compressive strengths within the ranges reported for cancellous bone.In-situ X-ray diffraction (XRD) and in-situ 1H-nuclear magnetic resonance (1H-NMR) proved precipitation of brushite as main hydration product, accompanied by monetite. There were indications that the fraction of monetite increased with increasing Li+ content. Within these studies, 1H-NMR turned out as versatile tool for the analysis of brushite cements. Its combination with in-situ XRD is highly valuable for the detailed characterization of the setting reaction. Li+ was detected in the remaining pore solution after completion of hydration, instead of being incorporated in the crystalline hydration products. Accordingly, the major fraction of Li+ was released in PBS already after 1 d, indicating excellent availability in the human body. Immunodetection and gene expression profiles of osteogenic-related markers highlighted the potential of Li+ incorporation for increasing bone density in vivo.In conclusion, the physicochemical properties of the developed Li+-doped brushite cements and their osteogenic potential render these materials very promising for bone regeneration.

ZINC-DOPED HYDROXYAPATITE NANOPARTICLES FOR THE TREATMENT OF BONE INFECTIONS.

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Bone infections can be caused by bacteria like Staphylococcus aureus (S. aureus) hiding intracellularly in osteocytes or macrophages. These bone infections are notoriously difficult to treat, because most commonly used antibiotics are not efficient to penetrate cell membranes and reach such hidden bacteria. We propose the use of hydroxyapatite nanoparticles (HA NPs) to treat intracellular bone infections, due to their ability to cross the membrane. Doping HA NPs with ions like zinc (Zn-HA NPs) can endow these nanoparticles with antibacterial properties. However, the optimal physicochemical properties of such antibacterial Zn-HA NPs remain unclear. For instance, altering the crystallinity of the Zn-HA NPs might influence the release of zinc and therefore the antibacterial property. In this study, Zn-HA NPs were synthesized using a precipitation reaction between calcium acetate, sodium phosphate and zinc nitrate under three conditions to establish highly crystalline (HC), low crystalline (LC), and amorphous particles (AP). Undoped HA NPs were also prepared as a control group. The degradation and release kinetics of zinc from the different Zn-HA NPs, were investigated at pH 5.5 and 7.4 representing physiological and inflammatory pH values, respectively. Afterwards, the antibacterial effect of the particles against S. aureus was investigated at different concentrations. Amorphous particles degraded more rapidly than crystalline particles, while all particles dissolved faster in acidic pH compared to more neutral conditions. Generally, faster nanoparticle degradation resulted in faster release of zinc ions. Similarly, amorphous Zn-HA NPs exhibited the most pronounced antibacterial effect against S. aureus (Figure 1). Concluding, a lower crystallinity or lower environmental pH led to increased degradation of the Zn-HA NPs. This in turn enhanced the antibacterial effect of the nanoparticles.Future studies will include cell culture to investigate the cytotoxicity of the particles, as well as mechanistic investigation on cellular uptake of the particles.

MECHANICAL CHARACTERIZATION AND DAMAGE BEHAVIOR UNDER UNIAXIAL COMPRESSION OF 3D PRINTED HYDROXYAPATITE SCAFFOLDS DESIGNED FOR BIOMEDICAL APPLICATIONS

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The objective of the present study was to characterize the mechanical response and the fracture process occurring during compressive loading of additive-manufactured hydroxyapatite (HA) scaffolds designed for biomedical applications. Cylindrical samples fabricated by Lithography-based Ceramic Manufacturing (LCM) were provided by Lithoz (GmbH, Vienna). The compressive strength was evaluated by uniaxial testing method and the Young's modulus was calculated from the slope of load–displacement curves during unloading.In order to describe the damage mechanisms occurring during compressive loading, an experimental investigation was performed using in-situ X-ray Computed Tomography (X-CT) and acoustic emission monitoring, as well as scanning electron microscopy observations at different loading stages. To analyze the stress distribution in the studied structure, a finite element analysis was conducted using both a modeled structure and a numerical model generated from CT scans. The studied scaffolds showed a high compressive strength of 60 MPa and a Weibull modulus of 6, higher to the one generally obtained on porous scaffolds fabricated by other techniques with the same level of total porosity, which is about 32 %. The Young's modulus initially of 85 GPa, decreased very slightly during the loading/unloading tests. In situ X-ray tomography scans showed a progressive damage evolution starting at a load value superior to 25 MPa. The cracks responsible of the damage were initiated during the compressive test from the edge of the sample and propagated parallel to the applied force, which explains the small variation of the Young's modulus. Moreover, the pre-existing microcracks formed during the manufacturing process have no influence on the compressive strength. Finite element analysis confirmed that the stress is non-uniform and maximum at the edge of the sample, which confirms the scenario of damage evolution.

SYMPOSIUM 5
SMART NANOSYSTEMS & ANTIBACTERIAL DEVICES

KEYNOTE LECTURE

SURGICAL INFECTION PROPHYLAXIS, ANTIBIOTIC LIMITATIONS AND SMART BIOCERAMICS

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Recently, it has been reported that that poor antibiotic delivery with a PMMA spacer is independently associated with acute kidney injury (1). Other studies have shown that the five year mortality rate for patients with an infected joint replacement is higher than for many lethal cancers (2). Thus, methods for much better administration of the potent antibiotics used in surgical infection prophylaxis than currently exist are needed.

Sol gel processing methods can be used to produce nanoporous silica materials that simultaneously exhibit synthetic bone graft behavior, i.e. bone bioactive behavior. Herein, the correlation is addressed between *in vitro* release and *in vivo* efficacy for a variety of sol gel processed methodologies and physical forms (thin films, composite hybrid films and microparticles), a variety of nanoporous characteristics, a variety of delivery mechanisms, and a variety of therapeutic molecules. The presentation will also review the concept of versatility of combining bone graft properties with the ability to add drugs and biological molecules to address specific surgery-dependent needs.

Even though new antibiotics are needed to address raising antibiotic microbial resistance, optimized delivery methods may create much greater treatment efficacy for existing antibiotics, even against resistant infections.

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KEYNOTE LECTURE

SUSTAINABLE ANTIMICROBIAL AND BIOMIMETIC STRATEGIES FOR BIOMEDICAL CEMENTS AND COATED IMPLANTS

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According to the World Health Organization, between 2015 and 2050, the world's population over 60 will be almost doubled from 12 to 22%. Among the challenges in responding to the increasing number of orthopaedic surgeries, the development of novel materials related to biomedical implants plays an important role. Coated metals and bone cements are very promising to furnish a tailorable drug release for patient-specific treatment. The objective of this work is to provide a variety of bioactive materials developed for cements [1] and coated multifunctional surfaces on metal implants, with the focus on biomimetic and antimicrobial properties. Antibiotic drugs used for clinical therapies are not always effective, partially substituting them with easily affordable antimicrobial ionic species may provide a better treatment option. The developed materials are mainly composed of calcium phosphates [2-4] mono- and di- substituted with antimicrobial and bioactive ions, such as Cu, Zn, Mn, Sr, Fe, Gd, Ag, and bioactive glass materials [5,6], containing Mn and other ions, involved in the natural bone and connective tissue formation. In this research, they are also used for coatings deposition on biodegradable metal implants [7], to control their degradation rate and impart them with bioactivity characteristics. The dependence of antimicrobial properties on substitution ion position in the calcium phosphate crystal structure and on its concentration is shown on the example of Cu. In vitro bioactivity and microbiology tests data focused on the specific trace ion influence are presented. One of the scopes of the present research is to combine and connect structural analysis and phase composition of the mono- and di- ion substituted calcium phosphates with their biological properties, i.e. the influence on microorganisms. The obtained results suggest that the developed sustainable biomimetic nanostructured materials are particularly relevant for novel strategies for biomedical implant design and bone tissue replacement and regeneration.

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INVESTIGATIONS ON THE RELEASE BEHAVIOR AND ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN-LOADED A BONE COMPOSITE CEMENT

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Calcium phosphate cements (CPC) have proven to be efficient bone substitutes in different applications thanks to their interesting biological properties, and similarity to the bone mineral [1]. Due to their low-setting temperature, these materials can incorporate various drugs, without denaturation and losing their therapeutic activity [2]. This property makes CPC-based materials very appealing candidates for the administration of various drugs for the treatment of bone diseases [3].This research described an in-vitro study of the release kinetics and antibacterial activity of ciprofloxacin-loaded-cement (CPC) composed of CaCO3-DCPD associated with bioactive glass and sodium alginate. The in vitro drug release study investigated on dried and fresh cements revealed prolonged release profiles lasting 18 days, and the elution rate was dependent on the cement composition and the amount of Cip incorporated. However, a massive release phenomenon during the first two days was observed in the case of dried cements. The fitting and modeling of release data following Korsmeyer - Peppas indicated that the release process is governed by a Fickian diffusion mechanism. The amounts of released ciprofloxacin per day were at a therapeutic level and were close to the minimum inhibitory concentration of the antibiotic, consequently sufficient for the treatment of orthopedic implant-associated infections.The antibacterial activity of the cement composites loaded with ciprofloxacin (3 and 9%) was assessed qualitatively via the diffusion method. The results demonstrated the effectiveness of these composites to inhibit the growth of Staphylococcus aureus and Escherichia coli, pathogens responsible for bone infection.The findings of the formulated composites indicated that they are promising materials as a local antibiotic delivery bone-substitute material, particularly for the prevention of post-operative bone infections.

MICROSPHERES-LOADED INJECTABLE BONE CEMENT FOR SILVER DELIVERY ABLE TO REDUCE IMPLANT-ASSOCIATED INFECTION RISK

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In the challenging quest for reducing the risk of implant-associated infections in bone surgery, the use of silver ions is promising regarding its broad spectrum on planktonic as well as multiresistant bacteria [1-3]. In view of controlling its early delivery in situ at the desired dose, we investigated its encapsulation in carboxymethyl cellulose (CMC) microparticles (micro-CMC-Ag) by spray-drying and included them in an apatitic bone cement whose Ag+ load/release could be adapted to reduce the risk of infections by the main pathogens introduced during the surgery [4-5]. The micro-CMC-Ag microspheres must combine: 1) A high silver encapsulation efficiency; 2) Controlled release properties, especially regarding “race to the surface”, of efficient Ag+ quantities for the early control of adhesion and biofilm formation; 3) Size distribution permitting to improve cement paste injectability and self-setting ability. We implemented a step-by-step methodology starting from the in vitro study of the antibacterial properties and cytotoxicity of AgNO3 and Ag3PO4 salts presenting different solubilities. Then we were able to design cements containing efficient concentrations in silver regarding biofilm formation inhibition for S. aureus CIP 4.83 and S. epidermidis CIP 6821T. We showed that the silver release (37°C, in Tris buffer) occurred over two weeks without burst effect and at a dose range conferring anti-biofilm efficiency without cytotoxicity from 3 hours of release. Modelization of the silver release data supported a higher release rate attributed to lower silver/cement interactions and to the presence of hydrophilic reservoir zones, leading more rapidly to silver active doses. In addition, the introduction of micro-CMC-Ag in the cement formulation led to a fully injectable and highly porous (77%) cement, showing a compressive strength analogous to cancellous bone. This injectable composite cement formulation constitutes a versatile bone substitute with tunable drug delivery properties, able to fight against bone implant-associated infection.

ANTIBACTERIAL CU-BASED VITREOUS ENAMELS FOR METAL COATING

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We have developed a copper-based enamel for coatings of metal surfaces based on the antibacterial effect of water released copper ions. The amount of released Cu ions was found to be correlated to CuO present in the composition of glass, giving the possibility to test bactericide effects of the treated surfaces through microbiological and chemical analysis.

Release experiments were carried out by contact of distilled water or suitable solution with the surface of the treated sample and the solutions were then sampled and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) making possible to describe a model of the extraction process from the glass phase.

Microbiological tests were then carried out according to ISO 22196:2011. Specifically, the antibacterial effect of Cu ions released from different types of enamel was evaluated by placing an aliquot of bacterial suspension on the surface of specimens, collecting it after 24 h of incubation (under appropriate conditions for the inoculated bacteria survival - 35±1°C - RH>90%) and evaluating the number of viable bacteria recovered from each specimen. The results obtained show that there is a correlation between the amount of copper released by an extraction method using water or a suitable surface solution and the composition of the enamel, and that a correlation can also be established between the presence of free copper ions at the surface and antibacterial behaviour.

PRODUCTION OF COMPOSITE DRUG DELIVERY SYSTEM CONTAINING ANTIMICROBIAL PEPTIDE FUNCTIONALISED CORALLINE HYDROXYAPATITE FOR ORAL AND MAXILLOFACIAL APPLICATIONS

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Electrospining can be used for the production of next generation antimicrobial drug delivery systems1. Immunologically inert antimicrobial peptides (AMPs) can be synthesised based on natural sequences of host ◎- and◎◎- human defensins2. It has been suggested that, the change of a single amino acid in the peptide sequence can affect the antimicrobial function of the peptides3. Furthermore, the peptide delivery system and release kinetics have also a significant impact on the antimicrobial activity4. Even though there are several versatile encapsulation methods available, oral infections cannot be cured in a short time and need to be treated within the first three weeks5. Therefore, appropriate delivery systems need to be designed that could be used for longer time periods and retain a sustainable antimicrobial activity. It has been suggested that hydroxyapatite based electrospun drug delivery polymer systems may have the appropriate characteristics to be used for long term antimicrobial activity 6-8. In this study, our aim was to design and produce a unique fructan based biocompatible antimicrobial peptide delivery system using electrospining based on our previous work9, 10. The main component in this system was coralline hydroxyapatite converted from coral by an established hydrothermal method6. Coraline hydroxyapatite was then coated with fructan-based AMPs containing fibers by electrospinning7. The AMPs used for this system were peptides (HBD-3) that are active against oral pathogens. Release of the AMPs from the system was followed by UV-vis spectrometry and the cytotoxicity of the system was evaluated using an MTT assay at 12, 24 and 72 h.

FIRST BACTERICIDAL NANOPILLARED TOPOGRAPHIES IN ANTIBIOTIC-FREE CALCIUM PHOSPHATE BIOMATERIALS

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The alarming increase in antibiotic resistance worldwide raises the urgent need to develop novel strategies to combat infections without antibiotics. The recent discovery of the mechanobactericidal properties of nanostructured surfaces is attracting interest as a non-antibiotic strategy. In this study, calcium phosphates (CaPs) with nanostructured bactericidal topographies have been obtained for the first time without the addition of antimicrobial ions. In addition, the reactivity of the different materials has been analysed, to rule out any effect on cell behaviour.For this study, the bactericidal potential of two nanopillared CaPs prepared by hydrolysis of two different sizes of α-tricalcium phosphate powders under biomimetic (B: 37ºC, 7 days, 1 atm) or hydrothermal conditions (H: 121ºC, 30 min, 2 atm) was explored. The hydrothermally reacted CaP presented a more irregular topography in terms of pillar tip (radius: 20-60 nm), spacing (100-1500 nm) and distribution (pillar groups forming bouquets) than the biomimetically treated one (radius: 20-40 nm and spacing: 50-200 nm with a homogeneous pillar distribution). Bacterial assessment was made with Pseudomonas aeruginosa incubated in nutrient-rich/depleted media. In both media, hydrothermally treated CaP presented higher lethality (~75% nutrient-free, ~56% nutrient-rich) than the biomimetically treated (~55% nutrient-free, ~40% nutrient-rich). The reactivity of the material was influenced by the type of medium and the presence or not of bacteria. Lower reactivity and superior bacterial attachment were observed in the nutrient-free medium, while lower attachment was observed for the nutrient rich medium. This was attributed to the superior reactivity of the material and to the lower tendency of bacteria to adhere to surfaces in the presence of nutrients. Nevertheless, the ionic exchanges were not toxic to planktonic cells. Thus, we can conclude that topography was the main contributor to mortality.

COPPER-DOPED MESOPOROUS BIOACTIVE GLASS NANOPARTICLES FOR THERAPEUTIC APPLICATION IN BONE REGENERATION

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Successful treatment of bone infection has become a major clinical challenge due to the emergence of antibiotic-resistant bacteria. These hard-to-treat pathogens can induce severe bone infection and even intracellular persistence of these pathogens upon their invasion into host cells such as macrophages and osteoblasts. Besides the limited therapeutic efficacy of currently available antibiotics, their low penetration into host cell plasma membranes increases the chance of recurrence of infection, which imposes an ever-increasing challenge for various surgical application areas [1]. Therefore, advanced biomaterials possessing inherent antimicrobial properties along with internalization ability are urgently required to combat resistant bacteria. Mesoporous bioactive glass (MBG) nanoparticles have received increasing attention in bone-related applications due to their excellent functional properties including cytocompatibility, apatite-inducing capacity, and antimicrobial properties [2]. In this study, we synthesized a library of MBG nanoparticles enriched with copper to enhance their inherent antibacterial properties and then assessed their apatite forming ability, antibacterial properties against resistant bacteria involved in bone infection, and internalization ability .Our results revealed a successful synthesis of copper-containing MBG nanoparticles possessing meso/nanoscale morphological characteristics with a favorable apatite-forming ability. In addition, Si and Ca ions were released through the particles in a sustained manner confirming the degradability of particles, whereas Cu ions were released within 48 h. Notably, nanoparticles containing 5 mol% of Cu exhibited remarkable antibacterial performance as evidenced by complete eradication of Methicillin-resistant Staphylococcus aureus bacteria. We further confirmed those MBG nanoparticles could be internalized into preosteoblast and macrophages, highlighting their capacity as an intracellular carrier to combat intracellular bacteria. Collectively, the developed MBG nanoparticles with favorable physicochemical properties along with their antimicrobial capability and internalization ability could be used as building blocks to construct new antibiotic-free antimicrobial biomaterials to effectively treat infected bone defects.

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3D PRINTING OF AN ANTIMICROBIAL HYDROGEL WITH A DUAL-CROSSLINKING MECHANISM

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In recent years, multidrug resistant pathogens made their way to become one of the top public health hazards (1). This results in the rise of implant failures due to prosthetic joint infections (PJIs) and justifies the growing need to develop new antimicrobial biomaterials (2). In this framework, we developed a 3D printable hydrogel made of GelMA, alginate and human platelets lysate – which is a reserve for angiogenic factors and fibrin, known for its bioactive potential (3). While GelMA and platelets lysate provide a bioactive support for eukaryotic cells, thanks to their RGD motif and high angiogenic factors content respectively, alginate has been functionalized with antimicrobial peptides (AMPs) to prevent antimicrobial adhesion. The gels were dual crosslinked after printing: first, GelMA was crosslinked via UV-light and then, alginate via ionic crosslinking. The employment of platelets lysate allows to produce a tailored biomaterial, since it can be directly harvested from the patient.To obtain the AMP-functionalized alginate, we allowed alginate to react with NHS/EDC and the AMPs. Then, we characterized the blend from a rheological perspective, determining the printability of the blend and the mechanical stability of crosslinked hydrogels. Following rheological analysis, we successfully 3D printed the blend and performed an ELISA test in order to study the release kinetics of the VEGF naturally contained in the gel (released from the LP). Finally, we determined the biological and antimicrobial responses to the gel. The functionalised hydrogels were tested on S. aureus and E. coli. Also, we assessed the gels bioavailability on eukaryotic cell lines.Finally, future work will be aimed at improving the biocompatibility on eukaryotic cell lines. Also, antibiofilm properties of the gels will be determined while also assessing the hydrogels activity on different bacteria strains.

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KEYNOTE LECTURE

ANTIMICROBIAL PEPTIDES LOADED INHALABLE CALCIUM PHOSPHATES NANOPARTICLES FOR THE COUNTERACTION OF ANTIBIOTIC RESISTANCE: TOWARDS A NEW THERAPY FOR RESPIRATORY INFECTIONS

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Antibiotic resistance (AMR) is one of the top 10 global public health threats facing humanity. In the last two decades, respiratory bacteria have increasingly becoming resistant to several antibiotics, and the prevalence of resistant strains is growing rapidly [1]. Hospital-acquired pneumonia, ventilator-acquired pneumonia and chronic respiratory diseases such as cystic fibrosis are some examples of respiratory diseases complicated by infections with multidrug resistant pathogens [2]. The search for new antibiotic formulations for these infections is therefore a primary objective in the current health context. Antimicrobial peptides (AMPs) are emerging category of therapeutic agents with a huge potential application [3]. These molecules have a broad spectrum of action and differently from the usually employed drugs, act by different mechanisms against which bacteria hardly develop resistance mechanisms. However, one of the main concerns is related to stability of AMPs after administration, which dramatically may reduce their power. Additionally, the efficacy of providing a sustained localization at specific site might not be properly achieved. In this view, the use of nanoparticles (NPs) for the AMPs delivery provides different advantages: (i) release of molecule in a more specific time and spatial window, and (ii) peptide protection against early degradation [4]. Therefore, a multifunctional NP-based therapeutic formulation certainly represents an effective alternative to traditional therapies. In this talk I will present the most recent approaches about the use of a new therapeutic formulation based on inhalable and biodegradable calcium phosphate (CaPs) NPs, functionalized with AMPs. CaP-NPs are mainly used because are inhalable and fully biocompatible. Preparations of CaP-NPs, functionalization strategies, and in vitro data will be presented, highlighting the excellent features of CaP-NPs to be used as nanocarries in the treatment of pulmonary diseases to overcame antibiotics resistance.

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UPTAKE, RELEASE, AND ANTIBACTERIAL ACTIVITY OF CIPROFLOXACIN LOADED-HYDROXYAPATITE-CHITOSAN COMPOSITE FOR DRUG DELIVERY APPLICATIONS

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In recent years, synthetic calcium phosphate-based materials have attracted great attention in the biomedical field as bone defect repair and as drug delivery systems, owing to their biocompatibility and reactivity 1. However, their low biodegradability and mechanical properties as well as poor knowledge of their surface behavior have paved the way for the use of inorganic-organic biocomposites as promising materials for mimicking the natural bone. Thus, several investigations focused on the development of hydroxyapatite (HA)-polymer composites with targeted properties 2. In this context, the present study aims to investigate the uptake and release ability of HA-chitosan composites toward an antibacterial agent, ciprofloxacin (CIP).The hydroxyapatite-chitosan (HA-CS) composites were first synthesized via in situ precipitation method and used as a starting material. The interaction between HA-CS particles and CIP antibiotic was evaluated through batch adsorption essays. A fast adsorption process was noticed, and the kinetic data were fitted to the pseudo-second-order. Adsorption data collected from various conditions are well described by the Freundlich isotherms, indicating a very weak loading capacity of the adsorbents. Alternatively, CIP loaded-three dimensional HA-CS composites were produced through a solid-liquid mixing coupled with the freeze-drying process. The physicochemical characterization of the specimens confirmed the effectiveness of the formulation process used and the homogeneity of the composites. The in vitro release results showed a sustained and controlled antibiotic release over 10 days. The CIP amounts eluted per day were at a therapeutic level (0.1 - 3 mg/L) and close to the antibiotic minimum inhibitory concentration. On the other hand, the developed composite revealed an antibacterial activity against Escherichia coli and Staphylococcus aureus bacteria. The correlation between the intrinsic properties of the composites components and their interfacial processes may provide fundamental tools for the development of potential formulations to deliver drugs to the living systems.

HIGHLY BIOACTIVE AND MAGNETIC HETEROSTRUCTURES FOR HYPERTHERMIA CANCER TREATMENT AND BONE REGENERATION

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Primary bone cancers commonly involve surgery to remove the malignant tumor, complemented with a postoperative treatment. In this respect, the design of a multifunctional bone substitute, would be of great interest to repair critical bone defects resulting from tumor resection and help preventing cancer resurgence.Bioactive glass nanoparticles (BG NPs) are a promising material for bone tissue regeneration because when implanted, a carbonated hydroxyapatite (HAC) layer is quickly formed onto their surface, bonding them to natural bone tissue.[1] By coupling the high bioactivity of large specific surface area bioactive glass particles with the heating ability of superparamagnetic iron oxide nanoparticles (SPIONs) under an alternating magnetic field (AMF), this material could allow hyperthermia treatment before promoting bone regeneration.[2,3]In order to obtain a large specific surface area, we synthesized mesoporous BG NPs by a soft chemistry route. Calcium ions being known to disturb the surfactant assembly and thus the porosity,[4] we systematically studied the impact of different synthesis parameters on the NPs characteristics, and bioactivity was assessed after immersion in Simulated Body Fluid at 37°C. A decrease of specific surface area was observed with increasing quantity of calcium, highlighting an optimal %Ca that provides best HAC growth kinetics. In addition, BG NPs were doped with copper ions, known to promote angiogenesis and antibacterial properties.[5]Based on these results, magnetic and highly bioactive core-shell NPs were successfully synthesized (Fig.1) and magnetic hyperthermia was assessed by calorimetric measurements. An aqueous suspension of these γ-Fe2O3@SiO2-CaO particles (30 mg/mL) initially thermalized at 37 °C was able to heat the media up to 46 °C after 15 min under clinically relevant AMF parameters. This maximum temperature can easily be tuned by controlling the NPs concentration or AMF parameters (Fig.1). Cytotoxicity assessments with MC3T3-E1 cells were finally performed after different passivation treatments at different concentration of nanoparticles.[Figure 1: TEM picture of SPIONs@MBG core shell NPs and heating curve under clinically relevant AMF parameters]

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LUMINESCENT APATITE NANOPARTICLES AS PLATFORMS FOR DICLOFENAC DELIVERY IN INFLAMMATORY ENVIROMENTS

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Important bone tissue pathologies, such as osteoporosis, osteoarthritis and rheumatoid arthritis, which today represent important health problems with considerable socio-economic burden, due to the general population aging, are characterized by a clinical condition of inflammation. For all these disorders the available therapeutic strategies are still unsatisfactory, namely because of the associated side effects. Sodium diclofenac (DF) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory and anti-pyretic activities, exerting its activity by competitively blocking the enzyme cyclooxygenase-2 (COX-2) responsible for the synthesis of the inflammatory mediator prostaglandin E2 (PGE2). As all the NSAIDs, DF has multiple adverse systemic side effects. Local delivery of this drug via luminescent nanoparticles (NPs) could bypass these inconveniences, while allowing their localization by luminescence emission. Herein, apatite (Ap) NPs, consisting of calcium phosphate and closely mimiking bone apatite nanocrystals, were doped with the luminescence-emitting Tb3+ ion (Tb3+:Ap). We explored the loading/release ability of diclofenac in both undoped and Tb3+-doped citrate-coated carbonated apatite NPs at different temperatures (25, 37, 40 ºC) and pHs (7.4, 5.2). Adsorption isotherms fitted the Langmuir-Freundlich model. The maximum adsorbed amounts at 37 ºC were higher than at 25 ºC, and particularly when using Tb3+:Ap NPs. DF-release efficiencies were higher at pH 5.2, a condition simulating a local inflammation. The luminescence properties of DF-loaded Tb3+:Ap NPs were affected by pH, being the relative luminescence intensity higher at pH 5.2, but not influenced either by the temperature or by the DF-loaded amount. Both Ap and Tb3+:Ap NPs were cytocompatible on two osteosarcoma cell lines and primary human osteoblasts. In addition, DF release increased COX-2 mRNA expression and decreased PGE2 production in an in vitro osteoblast's cytokine–induced inflammation model. These findings evidence the potential of these NPs for osteo-localized delivery of NSAIDs and the possibility to localize the inflammation by changes in luminescence.

SMART BIOMIMETIC APATITES: TOWARDS THE FOURTH GENERATION OF BIOMATERIALS

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Antimicrobial Resistance (AMR) related diseases are becoming a major concern for the medical community and the society. Their general impact is especially worrying in orthopaedics, leading to the rising threat of nosocomial infections, complexified by the porous structure of bone. It is estimated that, by 2050, AMR will cause more deaths than cancers and will have a financial impact comparable to the 2008 economical crisis[1]. This is the background of the AIMed project (Antimicrobial Integrated Methodologies for orthopaedic applications; H2020, www.aimed-itn.eu; Grant Number: 861138), a European Innovative Training Network. The main focus is to develop innovative smart biomaterials with antibacterial properties, suitable to orthopaedics and developing alternative strategies to antibiotics.One of the strategies pursued in the present work is to elaborate engineered bioactive compounds based on heterogeneously-substituted biomimetic apatites with intrinsic antimicrobial properties to be used as precursors to produce small autonomous bone implants or coat osteoarticular prostheses. The aforementioned properties are introduced through ionic substitution with known bioactive ions (i.e. Ag+, Zn2+, Cu2+). This approach has the advantage to confer additional features such as neo-angiogenesis (i.e. Cu2+ ions[2]) or pro-osteogenesis (i.e. Zn2+ ions[3]). A key objective is to distribute the ions in layered apatite-based structures (Fig.1a), typically via core-shell(s) particles with different compositions, to ultimately predict/control the biological outcome of the biomaterial in terms of infection control and bone regrowth after implantation. In this contribution, the preparation of the apatitic precursors was investigated following two paths, aiming to produce such multi-layered systems with controlled spatial ion distribution, with the view to lead to sequential releases of the bioactive agents:i) a room-temperature sequential precipitation method[4]ii) a specific 3-fluids nozzle spray drying approach (Fig.1b).Preliminary studies were then initiated, aiming the realisation of 3D scaffolds that include these engineered apatite precursors, or else the coating of TA6V substrates.

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DUAL FUNCTIONALIZATION OF CALCIUM PHOSPHATE NANOCARRIERS AS A PROMISING THERAPY FOR PANCREATIC CANCER

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Pancreatic cancer (PC) is one of the major causes of cancer deaths due to its late diagnosis and an ineffective therapy. Some combination of chemotherapeutic agents such as 5-fluorouracil, leucovorin, irinotecan, gemcitabine and oxaliplatin among others achieved better responses but still being unsatisfactory.[1] In recent years, Poly ADP-ribose polymerase (PARP) inhibitors have shown efficacy in breast and ovarian cancer cells since they are involved in DNA-repairing mechanisms.[2] Furthermore, the effectiveness of the selected drug can be enhanced in combinatorial therapy with a selective damaging tumoral-DNA agent.[3] This advances give lights to PC patients but new alternatives that ensures a higher life expectancy and improve patient's quality of life are needed.In this scenario, nanotechnology has paved the way for new efficient and less toxic alternatives in biomedicine, especially in the oncological field.[4] The possibility of tuning nanoparticles properties and their active surface have opened the door in their use for cancer treatments.[4] Specifically, calcium phosphate nanoparticles (CaP NPs) have attracted scientific attention due to its biocompatibility and biodegradability.[5] Moreover, its ability to accept foreign ions and a high specific surface area where adsorb active biomolecules, make this material an ideal nanoplatform to carry drugs with recent encouraging results against PC.[6] Considering those precedents, we have carried out a dual functionalization (combining PARP inhibitors with damaging tumoral-DNA agents) of the surface CaP NPs. The structure and composition of the resulting nanocomposite have been in-depth characterised. In addition, in vitro and in vivo experiments on the tumour lines of PC and animal models have been carried out to evaluate the effectiveness of the nanocomposites. Results demonstrated that inhibitory effects are enhanced with the treatment of nanocomposites containing the two active molecules, showing a clear synergistic effect.

Acknowledgments

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SYMPOSIUM 9
BIOCERAMICS FOR DENTAL APPLICATIONS

KEYNOTE LECTURE

BACKGROUNDS AND CLINICAL APPLICATION OF DENTAL CERAMIC IMPLANTS

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European Society for Ceramic Implantology, Switzerland **Switzerland**

Titanium dental implants have provided a safe and evidence-based standard care for the replacement of missing teeth for more than 50 years.

But also ceramic dental implants have long since left behind their former rather marginal status as the exotics of implantology and are now increasingly being adopted in modern dental practice.

The health awareness of the general public and hence the increased demand for metal-free treatment options are also certainly factors in this perceptible trend

However, the first available preclinical, as well as the short- and medium-term clinical data of the biomaterial "zirconium dioxide" show promising results and thus offer concrete practical advantages over titanium implants. These include healthier soft tissue, reduced peri-implant inflammation (peri-implantitis), no corrosion and improved aesthetics. Numerous clinical and preclinical studies have been able to prove these success factors of the new generation of ceramic dental implants.

In the past ceramic dental implants were still subject to functional complications such as fractures and unacceptable failure rates. However, in recent years there has been a massive technological development in terms of material properties and design of the implant surfaces. Even in these aspects, modern implants made of zirconium dioxide already meet titanium implants at nearly the same level.

Today the developments are mostly dealing with the practicable and stable connection of the implant as the invisible part in the bone with the dental crown as visible part. Various concepts are currently available on the market: one-piece and two-piece implants, cemented, glued or screwed restorations. The challenges are the material properties: titanium as metal is elastic, flexible and therefore easy to connect. On the other side zirconium dioxide as ceramic is not elastic, hard, prone to bending forces and therefore difficult to connect. New approaches are being pursued with carbon fibre reinforced connecting bolts.

A further development of the biomechanical properties of the biomaterial "ceramics" and the development of long-term reliable connection concepts is desirable.

The prerequisites for this are basic knowledge of the clinical indications, the requirements of dental implant systems and the way of their application … as presented in this lecture.

KEYNOTE LECTURE

INCORPORATION OF BIOCERAMIC NANOPARTICLES INTO COLLOIDAL COMPOSITE BIOMATERIALS FOR REGENERATION OF DISEASED BONE

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Bone diseases such as infection, osteoporosis and bone cancer cause severe bone destruction, thereby reducing a person's health and quality of life dramatically. To treat these degenerative diseases, long-term systemic delivery of therapeutic biomolecules is still the gold standard. Unfortunately, systemic drug delivery causes severe side effects, whereas the effective drug concentration in diseased bone is often too low to reach an optimal effect. Therefore, biomaterials are increasingly used as carriers for local drug delivery. However, their therapeutic efficacy is poor due to their very invasive clinical application, their poor spatiotemporal control over drug delivery and their inability to deliver drugs directly into the interior of cells.

Colloidal composite gels have the potential to overcome the above-mentioned shortcomings. These materials are both injectable, porous and self-healing due to their tunable self-assembly from submicron particles. This presentation will focus on the incorporation of several types of bioceramic nanoparticles (calcium phosphate, (mesoporous) silica/bioglass) into colloidal composite gels for treatment of defects in diseased bone. Specific attention will be paid to their viscoelastic properties as well as their capacity to facilitate local drug delivery. The presented data open up new avenues of research on the application of novel colloidal biomaterials for regeneration of diseased bone.

3D-PRINTED LITHIUM DISILICATE DENTAL RESTORATIONS: ALREADY SUITABLE FOR CLINICAL USAGE?

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Healthy teeth are mandatory for chewing ability, adequate speech, aesthetic and an overall healthy appearance. Hence, it is the aim of dental professionals to preserve the function of all structures of the oral cavity against the daily wear. State of the art tooth replacement is conducted more and more in a minimal invasive way to preserve as much of the healthy tooth substance as possible. Materials of choice are partially stabilized zirconia and lithium disilicate due to their outstanding mechanical and aesthetical properties.In the last decades CAD/CAM (milling) became the dominant manufacturing method for dental restorations due to it's easy and inexpensive availability paired with numerous optimizations over the years. However, 3D printing (additive manufacturing) gets more and more attention as a cost-efficient, sustainable and scalable alternative. The biggest motivation for using 3D printing for dental restorations usually arises from significantly lower material waste and the possibility to overcome restrictions or disadvantages of CAD/CAM. The latter are frequently consequences of the high mechanical forces during milling and limited resolution due to rotating tool diameter. With 3D printing unprecedented details in fissure geometries and significant thinner veneers can be manufactured. Lithography-based Ceramic Manufacturing (LCM) is a technology based on liquid raw materials (ceramic suspension in photocurable binder) cured layer wise with visible light by means of photopolymerization similar to digital light processing (DLP). The parts subsequently undergo a cleaning step followed by thermal debinding and sintering resulting in fully dense ceramic dental restorations. The technology allows the production of highly accurate ceramic restorations with comparable physical properties. Within our study we will present the production workflow of 3D printed lithium disilicate dental restorations based on a clinical case. The fit of the prosthesis and aesthetic result will be evaluated. Furthermore, the 3D printed material is analyzed regarding its leachability, cytotoxicity and residual carbon content and compared with CAD/CAM manufactured lithium disilicate.

LASER MACHINING OF ALUMINA TOUGHENED ZIRCONIA COMPOSITE DENTAL CERAMIC IMPLANTS FORMED VIA 3D-PRINTED DENTAL PROSTHESES CASTS

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This study focuses on an in-vitro study to evaluate the 3D-printed prostheses casts fabricated by 3D-printing and pulsed laser machining for manufacturing Alumina toughened Zirconia composite (ATZ) as dental implants. Also, an approach for machining costumed hard green materials with arbitrary designed shapes is developed. The 3D-printed epoxy resin casts were fabricated using a digital virtual cast by digital 360-degree photogrammetric scans. The master model data was superimposed to FDPs and turned into 3D analysis data. An ATZ composite with special formulation (74% ZrO2, 23% Al2O3 and 3% Y2O3, sintered at heating rate of 50∘C/h up to 700∘C, dwelling for 2 h at 700∘C, and heating of 100∘C/h up to temperature sintering of 1500∘C and dwelling for 2 h) and dimensions was manufactured with laser ablation of green formed parts in above mentioned casts, via tangential and radial ablation of single and multi-pulse arrays on formed green parts. Samples were formed with an ablation rate of 2.5 mm3 min−1 and a surface roughness Ra of 0.1 μm. The tetragonal composite ceramic was assessed via X-ray diffraction and Raman spectroscopy. Mechanical properties of final ATZ implants was compressive strength (GPa) of 3.0 ± 0.3, vickers hardness (GPa) of 19.1 ± 0.3, and fracture toughness (MPa·m1/2) of 8.8 ± 0.1.

KEYNOTE LECTURE

USE OF A CERAMIC-BASED SCAFFOLD FOR ALVEOLAR RIDGE PRESERVATION. RESULTS OF A PROSPECTIVE COMPARATIVE STUDY

Silvio Taschieri

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Purpose

This prospective study aims to clinically and histologically investigate the efficacy of a magnesium-hydroxyapatite (MgHA) / collagen-based biomimetic bone substitute for socket preservation.

Materials

Patients scheduled for single posterior tooth extraction were included. The socket was filled with MgHA or deproteinized bovine bone matrix (DBBM). In the DBBM group, a mucosal punch was taken from the palate and used to cover the graft. Vertical and horizontal dimensional changes of the alveolar process were clinically assessed with a periodontal probe and three-dimensional (3D) analysis of a cast model. The postoperative quality of life was assessed through a questionnaire. After 6 months of healing, a biopsy of the alveolar tissue was taken for histological and histomorphometric analysis of the newly formed tissue. After checking for normality of the distributions, parametric or non-parametric tests were used for statistical comparisons.

Results

20 patients were treated (12 males, 8 females, mean age 42.8 ± 5.1 years, range 33-50 years). After 6 months, the vertical and horizontal resorption of the alveolar ridge was similar in the two groups. 3D analysis of the models showed significantly more resorption on the buccal side than on the palatal / lingual side. Histomorphometric analysis showed similar new bone formation for the MgHA group (23.07 ± 10.3%) and DBBM (22.77 ± 6.95%), and a significantly higher percentage of residual material for the DBBM (15.77 ± 1.95%) compared to MgHA (5.01 ± 1.04%). In the first 3 days after surgery, patients in the MgHA group reported significantly less pain.

Conclusions

MGHA was found to be as safe and effective as DBBM and may represent a feasible bone substitute for socket preservation.

KEYNOTE LECTURE

FIFTEEN-YEAR PROSPECTIVE CLINICAL STUDY ON ZIRCONIA BASED PROSTHESIS APPLICATIONS IN DENTISTRY

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University of Milan, Italy

A convergence of professionals is now growing around ceramic materials regarding the use of materials of choice for indirect restorations in dentistry. Moreover, data from pre-clinical trials are strong enough to convince researchers to plan clinical trials in order to utilize them also as dental root replacement materials. The decreased induced inflammatory response and bone resorption when compared to metals are two main improvement clinicians will expect alongside their use. Preliminary data shows they match better than others the improvements the dental field is necessitating towards a more bio-inspired dentistry. On the other hand, the enthusiasm must embrace the knowledge because feldspathic ceramics, glass ceramics and many types of zirconia, have variations in composition, microstructure and processing which affect the mechanical and esthetic properties and consequently their clinical use. Moreover, the digital dentistry era accompanied by the transition to more automataized restoration fabrication using these materials implies we must carefully follow some rules. We have learned from the past about ceramics and fused metal porcelain use in dentistry from our best dental craftsmen, to avoid failure and complications in their long terms clinical use. The rules to correctly manage these materials are fundamental for clinical purposes. The way to get the best out of their characteristics is narrower than when using traditional materials and techniques and it requires and advanced and specific expertise. After this presentation, the attendant should be able to: explain the types of ceramics used in dentistry; understand clinical selection based on properties; discuss the differences in zirconia-based ceramics and their long term use, based on the evidence from long-term clinical trials.

KEYNOTE LECTURE

THE IMPACT OF BIOACTIVE SPHENE CERAMICS ON BONE TISSUE REGENERATION.

Giulia Brunello

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The aging of the population and the high patients' expectations relating to aesthetics and function have led to a rapidly growing request for implant-supported restorations. In order to improve the biological properties of titanium dental implants, several surface modifications have been proposed, including the application of bioactive silicate-based ceramic coatings. Sphene-based coatings ($CaTiSiO_5$) were obtained using a preceramic polymer containing nano-sized active fillers as precursors, and were deposited by airbrushing. After the encouraging preliminary *in vitro* findings, an *in vivo* study in rat femurs confirmed the biocompatibility of the investigated coating material.

In presence of bone deficiencies, alveolar bone augmentation procedures are often necessary in order to achieve a correct implant placement. Among several strategies, synthetic customized bioceramic scaffolds produced by additive manufacturing technologies are attracting growing attention. Sphene ceramic scaffolds were produced by means of direct ink writing and tested both *in vitro* and *in vivo* in a rat calvaria bone defect model. The so produced scaffolds seem to be promising candidates for bone regeneration.

DENTAL ZIRCONIA CERAMICS: CHALLENGES AND OPPORTUNITIES

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There have been profound advances in the application of zirconia dental ceramics (3Y-TZP) in restorative dentistry establishing it as material of choice for tooth-colored, all-ceramic systems suitable for crowns and bridges, root posts and implants. The success of the first generation 3Y-TZP lies in the ability to exert stress-induced (t-m) transformation toughening mechanism. However, the 3Y-TZP`s tetragonal phase metastability has a detrimental side in spontaneous low-temperature degradation (LTD) or ageing. Recently, new generations of high-translucent zirconia grades with superior aesthetics were launched to comply with the full-contour monolithic paradigm cancelling out the veneer overlaying. The increase in yttria content (i.e. 4 to 5 mol.%) leads to partially stabilized tetragonal crystal lattice, which in turn increases translucency and resistance towards LTD at the expense of decreased mechanical strength since the t-m transformation is restrained. Here we will report on some translational research progress showing how this diverse microstructure-property relationship of the yttrium-doped dental zirconia family can govern its performance in vitro as well as in vivo, determining suitable processing methods and laboratory procedures, such as airborne-particle abrasion (APA) and regeneration firing, which all can have pronounced effect on the mechanical strength and reliability. Finally, the prospects and challenges of entering the field of additive manufacturing workflow for dental zirconia will be discussed with the ultimate goal of enabling the fabrication of custom-made prosthetic options with improved functional (aesthetics) and structural (strength, toughness) properties. In order to succeed, an alternative manufacturing workflow enabling fabrication of multimaterial defect- and stress-free nanoscale microstructures needs to be established.

DEVELOPMENT OF A DAMAGE-TOLERANT, ZIRCONIA-BASED COMPOSITE FOR ORAL IMPLANTOLOGY

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High aesthetic demand and the increasing sensitivity to titanium has driven the growing acceptance of zirconia as effective alternative material to titanium in oral implantology. One-piece reconstructions have been initially employed, two- piece implant systems are being progressively introduced into the market and the current trend is towards all-ceramic modular implants with screw-retained abutments. Moreover, zirconia surface modifications allow comparable bone in-growth to titanium implants without impacting the implant structural integrity. In this scenario, a material with enhanced biomechanical characteristics allowing advanced surface modifications for even more ambitious implant designs may be desirable. A novel pure white zirconia-based material for dental and medical applications is herewith presented. The material has been designed to enhance the tolerance with regard to surface flaws. The goal has been achieved with the addition of several stabilizing agents and strontium aluminate as secondary reinforcing phase into yttria stabilized zirconia matrix. The fine tuning of the chemical composition, microstructure and manufacturing process enables new benchmarks to be set: flexural strength of 1800MPa with a Weibull Modulus of 15 and an indentation toughness of 15 MPa m1/2. The robustness of the material to damage (e.g. machining), as evaluated by indentation strength in bending method, i.e. residual strength of bending bars Vickers' indented at the load of 50kg on ground surface is 3-fold or more that of an yttria-stabilized zirconia material, whereas the resistance to hydrothermal aging has been maintained.

KEYNOTE LECTURE

BIOINSPIRED MINERALIZATION OF NATURAL POLYMERS FOR BIOMEDICAL APPLICATIONS

Conrado Aparicio

Division of Basic Sciences Faculty of Odontology, UIC Barcelona – Universitat Internacional de Catalunya

We use natural organic matrices as structural templates for bottom-up fabrication of hybrid nanocomposites to build advanced biomaterials for tissue engineering. By controlling mineral deposition in the organic matrices, predictable morphology of the mineralized nanocomposites can be obtained. We have designed and used elastin-like recombinamers (ELRs) and different forms of nanocellulose to template mineralization of hydroxyapatite nanocrystals using biomimetic processes. The minerals are deposited within the framework/fibril of the polymeric template, attaining high mineral density, bioactive response, and mechanical properties similar to those of natural hard tissues. ELRs are recombinant polypeptides that can self-assemble into twisted filaments. Cellulose nanofibers can be aligned so that intrafibrillar nanocompartments can be achieved. Amorphous mineral precursors infiltrate into the nanocompartments between the polymer's ordered structures and then coalesce and crystallize. Diverse hybrid nanocomposites with optimized mechanical and biological properties can be constructed, suited for the treatment of hard tissue defects using regenerative medicine approaches.

KEYNOTE LECTURE

BIOACTIVE CALCIUM ALKALI ORTHOPHOSPHATE BONE GRAFT MATERIALS AND 3D PRINTED SCAFFOLDS FOR BONE TISSUE ENGINEERING ENHANCE OSTEOGENESIS AND FACILITATE BONE REPAIR IN VIVO – TRANSLATIONAL RESEARCH IN MAXILLOFACIAL SURGERY AND IMPLANT DENTISTRY

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For alveolar ridge augmentation bone substitute materials are extensively studied in order to avoid harvesting autogenous bone. Given the clinical findings with current bone grafting materials there continues to be interest in bone substitute materials which degrade more rapidly, but still stimulate osteogenesis at the same time. This has led to the synthesis of rapidly resorbable calcium alkali orthophosphate (CAOP) materials with the main crystalline phase $Ca_2KNa(PO_4)_2$ which exhibit a greater biodegradability than β-tricalcium phosphate (TCP).

In order to evaluate the osteogenic potential *in vitro*, we examined the effect of various CAOPs on osteoblastic differentiation *in vitro* as compared to the currently clinically used materials TCP and bioactive glass 45S5.

In order to correlate the *in vitro* results with *in vivo* performance, we subsequently examined the effect of the same selection of bioactive ceramics (previously studied *in vitro*) on osteogenic marker expression and bone formation after implantation in in the sheep mandible and sinus floor *in vivo* using a clinically relevant large animal model.

Among the various test materials the silica containing CAOP (Si-CAOP) material GB9 displayed the greatest stimulatory effect on osteoblast differentiation *in vitro* and *in vivo* as well as on bone formation *in vivo.* Subsequently, studies to characterize the cell adhesion and intracellular signaling mechanism leading to this stimulatory effect on osteogenesis followed. Collectively, the data generated in these various studies led to FDA approval of this Si-CAOP in 2013.

Moreover, we also correlated the findings from the preclinical *in vivo* animal studies with in vivo data from clinical studies, in which the effect of various calcium phosphate particulate bone grafting materials with varying porosity on bone formation and on osteogenic marker expression in biopsies sampled six months after sinus floor augmentation (SFA) was studied, thereby rendering valuable insight in the performance of these materials in the human case and establishing a clinical study model for controlled clinical studies, which are required for taking novel bone grafting materials to the clinical arena in an evidence-based fashion. A first clinical study which evaluated the effect of this Si-CAOP (commercial name: Osseolive®) 6 months after SFA on bone formation as compared to TCP demonstrated significantly greater bone formation and resorption of the Si-CAOP in the apical area of the biopsies, i.e. at the greatest distance from the native bone, when compared to TCP. We furthermore used this clinical study model for elucidating the role of patient-specific host factors on craniofacial bone generation with bioactive TCP bone grafts.

A bone tissue engineering project employed 3D printed Si-CAOP scaffolds and a perfusion flow cell seeding and culture technique, and dealt with generating a 3D printed tissue engineered synthetic bone graft with homogenously distributed osteoblasts and mineralizing bone matrix *in vitro*, which thereby mimics the advantageous properties of autogenous bone grafts rendering it an excellent candidate for subsequent *in vivo* implantation for reconstruction of segmental discontinuity bone defects. These constructs were then used in combination with a microvascular technique for repair of critical-size segmental discontinuity defects in a femoral rat model. Histomorphometric, immunohistochemical and angio-μCT analyses rendered this concept an excellent tissue engineering approach for achieving excellent vascularization and repair of critical-size segmental discontinuity defects i*n vivo*.

MECHANISM OF BONE REGENERATION IN EXTRACTION SOCKETS GRAFTED WITH SCPC RESORBABLE BIOACTIVE CERAMIC

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Introduction

Bone graft materials are widely used in orthopedic and maxillofacial surgeries. The controlled resorbability of the graft material is essential for bone regeneration. Histology analyses showed absence of osteoclasts and macrophages during the resorption of bioactive silica-calcium phosphate composite (SCPC) granules grafted in mandibular defects in human. The hypothesis of the present is that bioactive SCPC inhibits osteoclast activity due to the presence of resorbable silica phase in the material. The objective of the present study is to analyze the effect of SCPC dissolution products on the resorption activity of osteoclasts.

Materials and Method

The conditioned medium was prepared by immersion of SCPC resorbable bioactive porous granules (Shefabone, Inc, USA) in cell culture medium at various ratios at 37ºC for 3 days. The concentration of Si ions released from SCPC granules into cell culture medium was measured using ICP-OES. Osteoclast precursors derived from human bone marrow were seeded on bone slices at a density of 1x106 cells/cm2 and cultured in the conditioned medium containing 10% FBS and osteoclast induction factors. The cells were evaluated by the staining for TRAP and measurement of resorption pits.

Results

Human osteoclasts cultured for 14 days with the osteoclast induction factors were positively stained for TRAP on the bone slices. Multinuclear TRAP-positive cells are assumed to be mature osteoclasts. In conjunction with the increased silica concentration, the number of multinuclear TRAP-positive cells was significantly decreased with the higher concentrations of SCPC whereas was insignificant with the lower concentration of SCPC.

Conclusion

Results of the study demonstrated that SCPC down regulated osteoclast differentiation and activities. The dissolution of silica from SCPC into the culture medium correlates well with downregulation of osteoclast differentiation. The results of the study suggest that SCPC resorption is mainly mediated by osteoblasts.

Keywords—bone graft, bone regeneration, osteoclast, silica calcium phosphate

LITHIUM-DOPED BIPHASIC CALCIUM PHOSPHATE; SYNTHESIS, STRUCTURAL ANALYSIS, AND BIOLOGICAL PROPERTIES TOWARD DENTAL PULP STEM CELLS

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The element-doped calcium phosphate is expected to develop more bioactive properties for bone tissue engineering. In this work, biphasic calcium phosphate (BCP) was used as the doping matrix and lithium was chosen as the doping element for improving the osteogenic properties. The lithium-doped BCP with different contents (0, 5, 10, 20 mol%) were synthesized by a high throughput chemical precipitation method. To identify the effects of Li-ion content, structural changes, and biological responses of Li-doped BCP were investigated comparatively. The results showed that BCPs with similar composition while different lithium doping content was successfully prepared, and lithium tended to enter the β-TCP structure by substituting calcium at the Ca (5) and Ca (4) sites. After the powder was degraded for 1 day in a cell culture medium, the lithium concentration in powder extracts rose steadily with the doping content of lithium. However, burst release of calcium in the BCPs into the extracts appeared when the lithium doping content was higher than 10 mol%. In this study, Lithium doping could effectively promote the osteogenic differentiation of dental pulp stem cells (DPSCs) and the optimal doping contents was 10 mol%. This work provides a reference for figuring out the effective doping content range of lithium in BCP for enhancing the osteogenic properties.

APPLICATION TO OPEN WOUND EXTRACTION SOCKET OF NEW BONE REGENERATIVE MATERIAL

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New bone regenerative material of octacalcium phosphate and collagen composite (OCPcol) has exhibited excellent operability with osteogenic cell invasion and angiogenesis (1, 2). And, the bone regenerative properties of OCPcol surpassed other bone substitutes, and it was reported the synergetic effects with teriparatide (3). Through multicenter clinical trial, it has been commercialized in Japan for treating bone defects (4). However, it has never been to elucidate the application of OCPcol for open wound extraction socket, although the previous studies suggested that OCPcol inhibited bone resorption if implanted into the closed wound extraction sockets. Therefore, the present study investigated whether OCPcol would be applicable for open wound extraction sockets. The prepared OCPcol disks or nothing was implanted into the canine mandibular extraction sockets, and each group was closed watertight or left as an open wound. That is, the experimental groups were divided into four groups; OCPcol closed wound group (OCPcol-C), OCPcol open wound group (OCPcol-O), Untreated closed wound group (Unt-C), Untreated open wound group (Unt-O). After 3 months of operation, radiomorphometric analyses was conducted by measuring alveolar cross-sectional areas of the 1/4 (Sq) and 1/2 (Sh)of the mandibular height near the alveolar apex. During the experimental periods, all animals were generally well and no eating disorders nor postoperative infections were observed including OCPcol-O. At 3 months after operation, it was shown that the radiographic findings of prepared extraction sockets in each group were similarly occupied by uniform radiopaque images. However, the Sq of OCPcol-O was significantly more than other three groups, and the Sh of OCPcol-O was significantly more than those of Unt-C and Unt-O groups. These results suggest that it would be applicable to OCPcol for extraction sockets with an open wound.

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POLYDIMETHYLSILOXANE-BASED CEMENTS CONTAINING CALCIUM BIOCERAMICS AND BIOGLASSES WITH ENHANCED BIOACTIVITY

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Vibrational IR and Raman spectroscopies were successfully used to characterize at a molecular level a calcium bioglass containing commercial cement based on polydimethylsiloxane (PDMS), i.e., GuttaFlow Bioseal, used as root canal sealer in endodontics. Its bioactivity was compared to GuttaFlow 2 (a similar commercial PDMS-based cement, but without the calcium bioglass) and its doped variants containing 20% w/w dicalcium phosphate dihydrate (DCPD) or hydroxyapatite or a tricalcium silicate-based cement.Ageing tests in Hank's Balanced Salt Solution (28 d, 37 °C) showed that GuttaFlow Bioseal was the most bioactive cement, thanks to the formation of a silica-rich layer with nucleation sites for the deposition of a B-type carbonated apatite. This phase appeared thicker when the material disk was aged in the presence of a guttapercha cone, i.e., under conditions more similar to those clinically available, with the two materials influencing each other.The doped types of cement showed higher bioactivity than simple Guttaflow 2, suggesting that the particles of the mineralizing agents are spontaneously exposed on the cement surface. However, the hydrophobicity of the PDMS matrix slowed down apatite deposition.

SEPTEMBER 22ND

PLENARY LECTURE

ZIRCONIA CERAMICS FOR DENTAL APPLICATIONS: CURRENT STATE AND NEW OPTIONS

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40 years ago, Garvie and his Australian co-workers reported that the stress induced transformation of metastable tetragonal zirconia grains to the monoclinic symmetry could give rise to a powerful toughening mechanism. Their results even led them to consider zirconia systems as analogues of certain steels in terms of mechanical performances. This seminal paper generated extraordinary excitement in the ceramic community and led to a large variety of new applications, with a special interest in orthopedics first then in dentistry today. Zirconia in dentistry was first developed for dental restorations, for which translucency and optical properties must be associated to a high mechanical resistance and stability in vivo, then for implantology where biological integrations are also emphasized.

Here we show that 'zirconia' is not one, but a family of materials, with many different (mechanical/optical) properties. Playing with the microstructure (grain size), alloying (choice of dopant and content) and phase content through processing, it is possible to develop zirconia ceramics with a high degree of translucency and/or high strength and/or even a certain transformation-induced plasticity before failure. Thus, 40 years after their inception for structural applications, zirconia ceramics can answer different needs as a function of the targeted application/product. We will thus review the current choice of zirconia ceramics available for dental use and show current trends both for restoration and implantology. In particular, we present our recent work on ultrafine yttria-doped zirconia with an excellent balance between translucency-aging resistance-strength and on specific compositions that exhibit some transformation-induced plasticity before failure and strain-accommodation. These new developments may create new opportunities for clinicians in their practice.

PLENARY LECTURE

BIOACTIVE GLASSES RELEASING MULTIPLE BIOLOGICALLY ACTIVE IONS FOR REGENERATIVE MEDICINE

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Bioactive materials, specially surface reactive materials such as bioactive glasses (BGs), are being increasingly considered in the tissue engineering (TE) field, for both hard (bone) TE and soft tissue repair and wound healing. Such applications rely on the biochemical reactions occurring at the interface between the material surface and the biological environment, which involve the (controlled) release of biologically active ions as dissolution products to stimulate specific cellular responses involved in new tissue growth [1]. In addition, immunomodulatory effects of BGs in the framework of bone regeneration and wound healing are being increasingly investigated [2].

Selected metal ions released from BGs have been shown to induce an angiogenic effect, e.g. in specific concentrations they enhance the secretion of vascular endothelial growth factor from stem cells, a very important property for tissue regeneration. Such angiogenic effects of BGs will be discussed showing results on different scaffold types and BG compositions. Moreover, the result of cell culture studies characterizing the variation of ion concentration (BG dissolution) in the medium and resultant time dependent effects on stem cells will be presented. In addition, strategies employed to affect immune cell response for enhancing tissue repair and regeneration based on BGs will be discussed, including surface functionalization, morphological optimization and controlled release of immunomodulatory ions.

In this context, applications of ion releasing BGs (e.g. as mesoporous nanoparticles) in the field of 3D bioprinting (biofabrication) have emerged in the last few years expanding the application potential of BGs in TE. In the second part of the presentation, the progress in the development and characterization of TE scaffolds made purely from BGs or by combining BGs and biopolymers, including their application in the field of 3D bioprinting, will be discussed. Examples of such applications will be presented highlighting the latest developments of multimaterial bioinks based on hydrogel-bioactive glass composites for cell encapsulation and for biofabrication of cell laden scaffolds of increasing complexity [3]. The author's views on the challenges and opportunities for further research in the field will be presented.

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PLENARY LECTURE

FROM NANOTOXICOLOGY TO NANOMEDICINE: LESSONS LEARNED FROM EU-FUNDED NANOSAFETY PROJECTS

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Nanomaterials possess unique properties that make them attractive for a variety of applications, not least in medicine (Fadeel B, Alexiou C. *Biochem Biophys Res Commun*. 2020;533(1):36-49; Fadeel B. *J Intern Med*. 2021;290(3):746-8). The successful exploitation of nanomaterials in the clinic requires the concerted efforts of those who understand the materials and those who understand the biology of human disease. The biocompatibility of any novel (nano)material must also be critically evaluated.

Nanomaterials have been widely scrutinized with respect to their potential toxicity using *in vitro* and *in vivo* models. However, a detailed understanding of the mechanism(s) of toxicity is still lacking. We have been engaged in several pan-European nanosafety projects and screened a large number of nanomaterials using primary cells or cell lines. Specifically, we aimed at understanding toxicity mechanism(s) focusing on regulated cell death. We also found that some nanomaterials elicit selective effects on normal and cancerous cells. In the present lecture some lessons learned in these projects are discussed.

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PLENARY TALK: **H. OONISHI AWARD**

CALCIUM PHOSPHATE BIOCERAMICS EVOLUTION IN BONE REGENERATION: FROM BONE SUBSTITUTES TO ORTHOBIOLOGY

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From several decades, there was numerous clinical used of synthetic bone grafts, particularly Calcium Phosphate Bioceramics. The ideal synthetic graft material should favor bone apposition and growth at the expense of the bone substitute. The Bone Substitute is simultaneously: a) dissolved by body fluids interactions and by cells degradation and replaced by b) bone ingrowth by osteogenic cells. Ultimately, the material should be replaced by mature physiological bone tissue within a healing period of several weeks or months.

Because autologous and allogenic bone grafts fulfill some of these requirements, clinicians in spite of intrinsic limitations, routinely use these biological materials. In addition, these bone grafts are not the best templates for the new technologies developed for Tissue Engineering and Delivery Scaffolds with osteoinductive bioactive factors. For these reasons during the last 15 years, researchers and clinicians have developed Orthobiology with osteogenic Bioceramics to support the challenge of bone regeneration, particularly required for patient at risk (aging population, bone pathology, low trophic bone situation, revision surgical complications, large reconstruction, etc.).

This key note lecture will present the evolution from the "generic" CaP bioceramics, developed in the 80s, to the recent "smart " bioceramics bone graft, for Tissue Engineering; and how these new generations of osteogenic/osteoinductive synthetic bone grafts, (granules, blocks, putties) support Orthobiology research and development; and justify the evolution of the classification of Medical Device (MD) to Combined Medical Device (CMD).

In addition, we must to demonstrate how these biomaterials and technologies must to be in accordance with the new MDR for CE approval or FDA 510K approval. The evolution of the biomaterials, the technologies, and the regulatory are more and more complicated and difficult to perform, requiring large Tissue Engineering Clinical trials, both in orthopedic and in maxillofacial indications.

SYMPOSIUM 4
ANTIBACTERIAL BIOCERAMICS FOR SMART PROSTHETIC APPLICATIONS – *SPONSORED BY ACERS*

KEYNOTE LECTURE

BIOMATERIALS AND BIOENEGINEERING-BASED STRATEGIES TO COMBAT IMPLANT INFECTIONS: NEW PERSPECTIVES TO OLD CHALLENGES

Bikramjit Basu
Materials Research Center & Center for Biosystems Science and Engineering, Indian Institute of Science, Bangalore, INDIA.

Considering the high prevalence rates and the lack of a current gold-standard treatment, peri-implant infection is rapidly becoming an – if not the most – important clinical challenge for indwelling medical devices. As an alternative to current perioperative antibiotic prophylactic treatments, a plethora of biomaterial/bioengineering based antimicrobial strategies are emerging to restrict or ideally to eliminate microbial adhesion and biofilm formation on the implant surfaces. Yet, the development of such approaches faces specific challenges, such as biocompatibility concerns, reduced antimicrobial effectiveness, long-term stability issues and antibiotic resistance development, which limit translation into the clinical setting. In this lecture, I will present two generic approaches, which demonstrate labscale success to induce bactericidal or bacteriostatic effects, in vitro. The biomaterials-based approaches will include the gold nanoparticles and HA-based antibacterial composites. The bioengineering approach will be discussed in reference to the intermittent delivery of electric or magnetic pulses to the bacterial growth medium, *in vitro*.

The first part of the presentation will dwell on the bacteriotoxic effects of the ultrasmall GNPs with median sizes of 0.8 nm and 1.4 nm and stabilized by monosulphonated triphenylphosphine ligands. A near 5 log reduction in viable staphylococcal strains (*S.aureus* and *S.epidermidis*) was observed in the first 5 h of 0.8 nm and 1.4 nm GNP treatment. Apart from exhibiting bactericidal effect in planktonic cultures accompanied by membrane blebbing and cell wall thinning, a 2x MIC dosage of the ultrasmall GNPs caused around 80-90% reduction in the viability of staphylococcal biofilms, with marked biofilm destruction. The toxicity dosages of such GNPs provide a therapeutic dosage window for the utilization of ultrasmall GNPs as a treatment option against prosthetic infection.

In the second part, three main antimicrobial strategies will be discussed – i) exposure of bacteria cultured on HA or HA-Fe_3O_4 composites, to moderate intensity static magnetic fields (SMF) of 100 mT; ii) exposure of pathogenic strains to high strength pulse magnetic field (PMF) of 1 – 4 Tesla and iii) electric field stimulation (1-2.5 V/cm) of pathogenic strains, when grown on conductive carbon or HA-ZnO composites. In all the instances, the possible mechanisms, including the synergistic effects of biomaterial properties with external stimulation parameters, for the observed bactericidal effect via the generation of reactive oxygen species and membrane damage will be discussed.

Towards the end, it will be emphasized as how the integration of computational tools and experimental databases using artificial intelligence (AI) based approaches would spur the development of next generation technologies for accelerated discovery of antimicrobial strategies.

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KEYNOTE LECTURE

DLP-BASED STEREOLITHOGRAPHY OF COMPOSITES IN THE ZIRCONIA-ALUMINA SYSTEM: DESIGN, PROCESSING AND PROPERTIES

Barbara Inserra, Bartolomeo Coppola, Laura Montanaro, Jean-Marc Tulliani, **Paola Palmero**

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Stereolithography is a new 3D printing technology which enables the fabrication of technical ceramics, providing advantages of high resolution and good surface finishing of the parts. The technique implies the layer-by-layer photopolymerization under a UV light of liquid monomer filled with ceramic particles, to build the 3D construct.
Today, several studies have already demonstrated the feasibility to fabricate alumina and zirconia ceramics, with properties comparable to materials produced by traditional techniques. On the opposite, only few works investigated the fabrication of their composites, despite their increasingly wide applications in the biomedical field, including orthopaedic and dental areas.
Aim of this research was to set-up a process to develop different types of composites in the zirconia-alumina system when using a DLP-based stereolithography equipment. Some key parameters were investigated, starting from the preparation of the slurries, to the optimization of the printing parameters, to the set-up of proper debinding and sintering cycles.
An additional challenge was related to the choice of ceria-stabilized zirconia as the matrix of high-strength/high-toughness composite ceramics[1,2] due to the absorbance of the UV light by cerium oxide, thus requiring an even more rigorous tailoring of all the processing steps.
Powders were dispersed in a commercial UV curable resin, at solid loadings ranging from 50 to 78wt%, while the addition of commercial dispersants was optimized as well, with the aim to maximize powder content while providing the slurry the correct rheological behaviour. The debinding and sintering cycles were optimized on the ground of DTA-TG and dilatometry studies, respectively, allowing to achieve final densities close to the theoretical ones. Microstructural observations showed highly homogeneous structures, made by an optimal distribution of all the phases in the composite materials. Preliminary results on the mechanical behaviour showed that the materials were able to achieve properties comparable to those fabricated by conventional manufacturing technologies.

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QUANTIFYING SURFACE LATTICE DISTORTIONS IN Y-TZP

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Introduction

XRD peaks of tetragonal zirconia (Y-TZP) may show a strong shoulder-like distortion of varying width and intensity on the left side. The phenomenon has been described as rhombohedral zirconia [1] or lattice distortions in the tetragonal phase [2]. In contrast to the formation of monoclinic phase, the effects of distortions in the tetragonal phase on the mechanical properties have not yet been investigated. In the present study, we developed a crystallographic model to quantitatively describe lattice distortions at the surface of Y-TZP samples based on XRD data as a first step towards understanding their formation, crystallographic nature, and implications on the mechanical properties.

Materials and Methods

A crystallographic model was developed describing a gradient of increasing lattice stress and micro-strain towards the surface of Y-TZP samples. The gradient was approximated by up to 6 Y-TZP structures, for which the following parameters were refined: Unit cell distortions due to lattice stress, peak broadening due to micro-strain, a scale factor, and a function correcting the peak intensity of transparent layers along 2θ. Peak broadening was coupled to the peak displacement using a linear function with a refined slope parameter.

Results and Discussion

Our model was able to fit shoulders of Y-TZP peaks with high accuracy (Fig.). A correlation between lattice stress and micro-strain was confirmed. The intensity correction function of the layers showed a drop of intensity towards higher diffraction angles as expected for transparent layers, whereas the one for the substrate showed the expected inverse characteristic.

Conclusion

Our results corroborated the hypothesis that the shoulder is related to lattice distortions in the tetragonal phase, and that the distortions are concentrated at the sample surface. Efforts to relate the refined model parameters to the layer thickness and to investigate the effect of Y-TZP distortions on the mechanical properties are ongoing.

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FUNCTIONALIZATION OF SILICON NITRIDE WITH BIOACTIVE AND ANTIPATHOGENIC PROPERTIES

Wei Xia (1), Ioannis Katsaros (1), Huasi Zhou (1), Le Fu (2), Håkan Engqvist (1)

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Silicon nitride ceramic is well-known as an engineering ceramic because of its good mechanical properties, especially in the high-loading area, and has been introduced for biomedical applications, i.e. joint replacement, spinal fusion, and the latest anti-pathogenic and bioactive applications. Generally, sintering additives have been used for silicon nitride sintering, therefore we could consider the final body is a ceramic-glass composite. It makes us easy to modify and/or functionalize ceramic itself by different methods. Our strategy is (1) to use biocompatible and bioactive additives as the glass phases to replace toxic ones in silicon nitride ceramics and (2) oxidization of silicon nitride ceramic and its surfaces. The aim was to study their bioactive and antipathogenic properties while keeping good mechanical strength. SPS, oxidization furnace, and CVD have been used to prepare samples. The pre-treated powders, sintering ceramics, and coatings were analysed by XRD, SEM/EDS, TEM, XPS, and ICP-OES for their composition, phases, structure, solubility, and morphologies. Mouse osteoblast cell line was used for the biocompatibility testing. Several bacteria and viruses have been tested for antibacterial and antivirus properties. The results show by adding the bioactive oxides can improve the in vitro bioactivity of modified silicon nitride ceramic. Both the powder, bulk and coating forms of modified silicon nitride had good antibacterial effects and the inactivation of viruses. The powder form shows the best performance because of the higher specific surface area. The surface hydrophilicity and content of the functionalization influenced the efficiency of the bacteriostatic and antipathogenic effects. The additives did not significantly decrease the mechanical properties of bulk silicon nitrides. All results indicate that we could move forward with the potential applications of silicon nitride based ceramics, not just for bone and dental but also for antipathogenic applications.

INFLUENCE OF LASER ENGRAVING ON ALUMINA-ZIRCONIA COMPOSITES

Laurent Gremillard (1), Luis Cardenas (2), Helen Reveron (1), Thierry Douillard (1), Amélie Vogl (1), Audrey Delos (1), **Karen Hans** (3), Thomas Oberbach (3)

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Laser technology is used for different applications such as joining and cutting as well as structuring and marking of surfaces.Orthopedic implants are marked by laser engraving to ensure product traceability according to ISO13485. In this study the influence of a solid-state laser on surface properties of two alumina-zirconia composites (ATZ; ZTA) is analyzed. The laser was used to mark the ceramic. A good visibility and readability as well as no negative influence on the properties and application performance of the ceramic is indispensable.Both ceramics were lasered in two different states: as green and sintered body. Different laser parameters were used. The laser engraving was done by two commercially available marking stations with solid-state lasers made of Nd:YAG and Nd:YVO4.The laser engravings were analyzed regarding the macrostructure (LSM), phase content (XRD), and microstructure (SEM, FIB-Tomography, EDS, TEM) and chemistry (XPS, EDS).In the lasered zone the color changed from white to brown-black. The higher the zirconia content and the higher the energy density the darker the color. Higher energy density also led to deeper engravings. Phase analysis showed a change in XRD pattern at the (101) tetragonal peak of the zirconia which might be linked to a lattice deformation or presence of a solid solution layer. SEM and FIB-tomography analyses showed a formation of slight surface cracks. Just below the surface a kind of solid solution layer of Al-Zr-O was detected. XPS revealed probably changes in oxidation state and formation of vacancies.In the study the influence of the changes at the surface is discussed regarding the mechanical properties and ageing resistance.

SURFACE ENGINEERING BY FEMTOSECOND LASER OF 3D TCP/ZRO2 CERAMIC MATRICES FOR IMPROVING BIOCOMPATIBILITY PROPERTIES

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Traditional chemical processing techniques used to alter ceramic surface properties possess drawbacks expressed in the formation of cracks and leaving additional chemical toxicity from the used solvents. As an alternative, ultra-short pulsed laser texturing is a non-contact method that enables a unique route to manipulate hard ceramic materials without triggering severe thermal damage. The combination of freeze foaming with laser-based texturing of 3D scaffolds allows the creation of multifunctional geometries with the potential to affect the biomimetic properties of the scaffolds. In this study, specialized laser processing has been applied to 3D TCP/ZrO2 samples synthesized by the method of Freeze foaming in order to enhance the surface properties of the ceramic material. Freeze foaming is a novel method that has been applied for the synthesis of well-controlled biomimetic porous materials based on ceramics. By tuning the parameters of the freeze foaming method, the morphology of obtained structures can be changed, simultaneously having the functionality of biological systems. This research is particularly focused towards the optimization of femtosecond laser processing parameters (scanning velocity (v), laser fluence (F), and a number of applied laser pulses (N)) applied to 3D support composite ceramics structures, in order to obtain textures with diverse dimensions from micro- to nano- scale. The surface roughness was altered in order to achieve increased biocompatibility of the support ceramic structures. It was discovered that the obtained patterns improved the osteoconductivity of the sample simultaneously providing improved surfaces for osteoblasts adhesion and proliferation. The conducted experiments have demonstrated that surface topography has a great influence on the biological behavior of bioinert ceramics.

Acknowledgments

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CELL ADHESION AND VIABILITY ON SUPERHYDROPHOBIC COATINGS

Alessandro Corozzi (1), Giada Bassi (1), Maria Caruso (1), Elena Giusto (1), Monica Montesi (1), Silvia Panseri (1), Mariarosa Raimondo (1), Arianna Rossi (1)

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Surface wettability might be a promising way to regulate biological adhesion on material surfaces in order to have: i) an improvement of biocompatibility, ii) an increase of tissue ingrowth and iii) a de-crease of bacterial adhesion and inflammatory response [1] [2]. Super-hydrophobic surfaces (SHS) have high potential for wide functionality in biomedical and bio-logical sciences due to their availability, easy fabrication, and versatility [3] [4]. We present the design of super-hydrophobic hybrid coatings via Lotus leaf-like and Slippery Liquid Infused Porous Surfaces (SLIPS) with an almost negligible wettability (surface free energy 5-50mN/m^2) and a low contact angle hysteresis (less than 20°). The inorganic, porous layer is based on ceramic nanoparticles obtained via sol-gel, while the organic layer consists of grafted fluoroalkyl Silanes molecules and trapped polymeric media in the nano-cavities of the solid interface.The surface morphology and chemistry were investigated both by secondary electrons imaging, EDS analysis, and Atomic Force Microscopy. To assess cell toxicity and biocompatibility, Murine fibroblasts culture was used as in-vitro model. MTT as well as PrestoBlue™ assay together with morphological analyses were used to assess cell viability and proliferation on day 1, 2, 3 and 7. We were able to generate bioinspired superhydrophobic surfaces where samples' hydrophobicity significantly affects the cell adhesion and viability.

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Keywords

Cell adhesive spectra; nanotopographic interaction; surface wettability; slippery surfaces; biofilm prevention; nonfouling materials. //

KEYNOTE LECTURE

BIOCERAMIC-BASED COMPOSITES AND SCAFFOLDS WITH ANTIBACTERIAL FUNCTION FOR BONE REPAIR AND REGENERATION

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Bone defects caused by trauma, tumor, and other causes have been substantially impacted the lives and health of human.

Bioceramic-based composites and scaffolds provide significant benefits over biological counterparts and are critical for different clinical applications in orthopedic surgery related to the bone repair and regeneration, from bone substitutes and cements to scaffolds.

Clinical practice demonstrate that is high probability to occur a bacterial infection during the surgical procedures or on bioceramic-based composites and scaffolds. Therefore, it is of great significance to obtain bioceramic-based composites and scaffolds with integrative antibacterial and osteogenic functions for treating bone implant-associated infection and promoting bone repair. In order to fight against infection problems, various antibacterial strategies are developed and tested for bone repair and regeneration.

Recent progresses in bioceramic-based composites and scaffolds with antibacterial function will be described.

Some new materials like PMMA bone cements with antimicrobial additives like silver nanoparticles incorporated in a ceramic glass matrix, and composite scaffolds based on cellulose acetate (CA) and hydroxyapatite (HAP) entrapped silver ions will be presented not just in the term of obtaining and characterization by various techniques (FT-IR, SEM, XRD, thermal properties) but also in the term of biocompatibility and antimicrobial effect evaluation.

Finally, the challenges and opportunities of antibacterial bioceramic-based composites and scaffolds will be discussed.

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KEYNOTE LECTURE

CARBON NANOSTRUCTURES: GOOD FRIENDS FOR BIOCERAMICS?

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The amazing chemical and physical properties of carbon nanostructures render this family of materials an extraordinary tool for applications in many different fields. From the discovery of fullerene to the most recent reports on graphene and carbon quantum dots, they have been proposed as drug delivery systems, active species in sensors, useful material in tissue engineering.

Following the research in this fields, we will examine the most relevant developments, from the struggle in obtaining suitable functionalization of the materials, through their applications, to end with the exploration of the possible interaction between carbon nanostructures and bioceramics.

2D MATERIALS FOR INNOVATIVE MEDICAL APPLICATIONS - GRAPHENES AND MXENES

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Since graphene has been developed in 2004, many research studies have been focused on discovering and synthesis of innovative layered two-dimensional (2D) materials which can efficaciously find their application in such fields as drug-delivery systems, protective or multifunctional coatings, biochemical sensing, tumour therapies and many more. Therefore, our working group, besides graphene, focuses on newly developed MXenes, having a huge potential for above mentioned applications. In our comprehensive study, we compared graphene oxides and MXenes in regard to their physico-chemical as well as biological properties. The graphene oxide (GO) was prepared according to the modified Marcano method (Fig. 1a), [1]. MXenes (MX) were manufactured with chemical etching method of Ti3AlC2-MAX-phase as precursor, by using LiF/HCl solution (Fig. 1b), [2]. Such obtained graphene oxides and Ti3C2-MXenes were characterized by applying X-ray diffraction (XRD), scanning electron microscope (SEM/EDS), as well as X-ray photoelectron spectroscopy (XPS) methods. Subsequently the immobilization of 2D-nanofilms have been performed on the inert ceramic to modify the surface. For the coating, the inert ceramics by the example of zirconia were functionalized with aminopropydiisopropylethoxysilane (APDS) to covalently attach 2D-nanofilms to the surfaces. The obtained GO and MX coatings were evaluated in regard to their morphology, coupling behaviour, covering properties, wettability, and stability in aqueous environment. In our study we could show that the immobilization of 2D-nanomaterials (GO and MX) on the inert ceramic surfaces has been successfully performed. The properties of 2D-coatings and inert ceramic could be merged to create a versatile biomaterial. This method could be applied for creating adjusted surfaces for medical use. This opens up completely new possibilities for designing the surface properties depending on the area of application.

MECHANICAL BEHAVIOR OF A NEWLY DEVELOPED YTTRIA-DOPED ZIRCONIA WITH OPTIMIZED STRENGTH AND TOUGHNESS

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Due to their excellent mechanical properties, wear resistance and biocompatibility, zirconia-based ceramics are increasingly used in several engineering fields and in biomedical applications. One of the main challenges in the development of these materials is to combine both good strength, high toughness and hydrothermal stability. This work deals with an ultrafine yttria-doped zirconia with an yttria content lower than usual (i.e. 1.5 mol% yttria instead of 3 mol.%). An experimental investigation of the mechanical behavior of this material allowed to determine the flexural strength, the fracture toughness, the crack growth resistance curve (R-curve) and the slow crack growth resistance. The results show that this material offers a very attractive combination of high strength (1 GPa) and toughness (8.5 MPa.m1/2), near the optimum between a brittle and ductile behavior. Moreover, a relatively high Weibull modulus of 16 and a significant crack growth resistance was observed, demonstrating a high flaw tolerance in these ceramics. This is attributed to the crack shielding effect due to phase transformation toughening mechanism, which is here more efficient compared to conventional 3Y-TZP zirconia bioceramics. It was also observed that the transformation crack shielding shifts the slow crack growth curve (i.e. crack growth rate vs stress intensity factor) towards higher stress intensity factor values and increases the threshold of crack propagation, compared to 3Y-TZP. Last, hydrothermal stability was assessed and was considered as acceptable in view of a biomedical use. This novel zirconia grade thus appears promising when a high toughness and crack resistance are necessary in demanding structural applications.

DEVELOPMENT AND OPTIMIZATION OF PLASMA ELECTROLYTIC OXIDATION BIOACTIVE COATINGS ON AZ31 MG FOR BIOCORROSION CONTROL

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Magnesium alloys represent promising bone substitute candidates due to their biodegradability, combined with their mechanical properties almost matching those of natural bone [1]. However, Mg-based biomaterials present some strong limitations, specifically a low corrosion resistance which can impair their load-bearing abilities and affect the healing processes by causing an adverse inflammatory response in the surrounding tissues [2]. To overcome these major challenges, electrochemical surface modifications such as Plasma Electrolytic Oxidation (PEO) can be exploited to produce thick ceramic conversion coatings on the magnesium surface in order to reduce its susceptibility to corrosion while supporting bone healing at the implantation site [3].In our work, we focused on the development and optimization of novel silicate-based PEO coatings on AZ31B Mg alloy, capable of improving bone cell activity while increasing corrosion resistance, for prospective application in bone defect repair. The produced coatings have a thickness of about 15 microns and feature the microporous surface with evenly distributed pores typical of PEO treatments conducted in the macro-sparking regime (Fig. 1), and are mainly composed of forsterite (Mg_2SiO_4), which possesses superior mechanical, bioactive and potentially antibacterial properties compared to periclase (MgO) [4]. Early degradation studies (potentiodynamic polarization tests) of coated samples in simulated body fluid (SBF) highlighted an about 45 times lower corrosion rate (from 7.43 to 0.16 mm/year) compared to commercial AZ31B alloy, as well as a more positive Ecorr (Fig. 2), both functional conditions to a longer-term retention of its mechanical properties. Ongoing studies are focusing on evaluating the coatings composition (through FTIR spectroscopy), as well as their performance in biological-like environments and mesenchymal stem cells response. Moreover, further investigations are being conducted to assess the promising antibacterial features and improved corrosion resistance linked to the inclusion of cerium ions into the oxide film [5].

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WEAR BEHAVIOUR OF ZIRCONIA/ ALUMINA HIP IMPLANTS IN COMBINATION WITH ARTIFICIAL AGEING

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Introduction

In this study, a methodology was devised to assess the in vitro wear behaviour of Zirconia Toughened Alumina Ceramics (ZTA) and Alumina Toughened Zirconia Ceramics (ATZ) under adverse edge loading conditions in hip simulator combined with accelerated ageing in an autoclave under hydrothermal conditions.

Experimental Methods

Femoral heads and acetabular liners of hip prostheses were made of two materials: Alumina Toughened Zirconia (ATZ) and Zirconia Toughened Alumina Ceramic (ZTA). Two material combinations were tested in this study: ATZ-on-ATZ and ZTA-on-ZTA.A total of six bearing couples were studied. The study was run for a total of eight million cycles. The first two million cycles were run using standard gait conditions and the subsequent 6 million cycles were run under edge loading conditions due to dynamic separation between the femoral head and the acetabular cup. All femoral heads and acetabular cups were hydrothermally aged during the wear study after every million cycles of testing. It lasted 2 hours at 134°C after each million cycles. The volume monoclinic fraction was determined. Scanning Electron Microscopy observations were conducted on pristine, worn and aged surfaces on the heads after various testing times. The wear was measured gravimetrically at an interval of one million cycles. A coordinate measuring machine was used to reconstruct the surface of the femoral head and acetabular cup. RedLux software was used to visualise the size, shape and penetration depth of the wear areas.

Results

The wear rates of both aged materials, ATZ-on-ATZ and ZTA-on-ZTA, under standard conditions were very low, i.e. <0.01 mm3/million cycles. There was no measureable change in wear rate due to ageing under standard conditions.The wear rates increased when edge loading conditions driven by separation was introduced. The mean wear rate of aged ZTA-on-ZTA after six million cycles of testing under edge loading conditions was 0.19 ± 0.47 mm3/million cycles. The mean wear rate of aged ATZ-on-ATZ was 0.07 ± 0.05 mm3/million cycles.There was no visible damage on the surfaces of the femoral head and acetabular cup after testing under standard conditions. In contrast, under edge loading conditions, a stripe-like wear area was observed on the femoral head with corresponding wear on the rim of the acetabular liner. The penetration depths on the femoral heads and acetabular liners of the ZTA-on-ZTA bearings were higher than that of the ATZ-on-ATZ bearings. No significant ageing occurred in the ZTA material. The monoclinic fractions remained very low over both the wear stripe and the unworn surface. Ageing of ATZ heads was significant. On the wear stripe, each autoclave step increased the monoclinic fraction. However, each one million cycles of wear simulation decreased the monoclinic fraction significantly.SEM observations of the worn ATZ and ZTA surfaces showed that the first 2 million cycles (without edge loading) did not significantly damage the surfaces. Microstructural damage was first observed after edge loading. In ATZ damage was located in a small wear stripe 15 μm wide. In ZTA, damage was first located in a much more diffuse area around 50 μm wide.

Conclusion

In this study, the performance of composite ceramic materials was assessed under a combination of edge loading gait conditions and hydrothermal ageing.The damage of ceramic components increased by the symbiotic effect of ageing, wear and shocks but remained at a very low level for both ceramic materials. It was shown that the performance of ATZ-on-ATZ materials in vitro may be superior to ZTA-on-ZTA materials despite the higher zirconia content in the ATZ materials.

SYMPOSIUM 7
COMMEMORATIVE SESSION: BIOCERAMICS AND GLASS TECHNOLOGY

KEYNOTE LECTURE

3D PRINTABLE BOUNCY BIOGLASS FOR ARTICULAR CARTILAGE REGENERATION AND LOAD SHARING BONE DEFECTS

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There is unmet clinical need for materials that can regenerate torn cartilage and also an unmet need for scaffolds that can regeneration non-union bone defects. In both cases, mechanical properties are key. Sol-gel inorganic/organic hybrids can give control over mechanical properties due to their co-networks of inorganic and organic components. Our new Bouncy Bioglass hybrid materials can be made to have a coefficient of friction matching that of articular cartilage (in media). This gives unprecedented combination of mechanical properties and control of biodegradation. Hybrid inks were 3D printed in log-pile-like architectures and responded well to cyclic loads. We found that guide bone marrow stem cells differentiated down a chondrogenic route and produced articular cartilage-like matrix, rich in Collagen II, Aggregan and GAG, but only when the pore channel size was 250 μm. When the pore size was larger, Collagen I was prevalent. In vivo sheep studies showed similar results, wherein scaffolds with 250 μm pores showed excellent cartilage regeneration after 3 months, while defects remained when scaffolds with 500 μm pores were used. For bone regeneration, the challenge is introducing osteostimulation by incorporating calcium. We achieved this through use of calcium alkoxide precursors. I will show mechanical data and cell studies.

A Quantitative Review of the Relationship Between Si Released from Bioactive Glasses and Their Cellular Interactions.

Joel Turner (1), Gavin Jell (1), Julian Jones (2), Aldo Boccaccini (3)

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Silicate bioactive glasses (SiBG) have been in use clinically for over 20 years. Despite the clinical success and high number of scientific investigations, a lack of understanding of how soluble silicate (Si) species influence cellular responses remains. Using a systematic approach, this investigation quantitatively compares the in-vitro responses to BG dissolution products reported in literature and specifically determine if there is a relationship between the concentration of Si released from SiBGs and cellular behaviour.Web of science databases were used to search for articles involving the use of SiBG-dissolution products on cells in-vitro. Following the exclusion of studies that did not perform 2D cultures or quantify silicate ion concentrations ([Si]) within the media, 90 articles (from a total of 665) were analysed. The concentration of [Si] and whether they caused a significant increase (positive), decrease (negative) or no significant difference, compared to the control, were recorded. The median [Si] that most papers reported positive (desirable) cellular responses was 30.2ppm, with concentrations below this producing no significant differences. [Si] above 50ppm were, however, almost 3 times more likely to cause unfavourable cellular responses. There was, however, no statistical difference between the [Si] that caused altered expression of ALP, osteocalcin, collagen, VEGF in the reported literature. Commonality in the literature in cell-type and species-specific differences in responses to [Si] were also found, where for example, an increase in cell proliferation was observed in response to [Si] in cells derived from human species ($P<0.001$), but not other species. This review has, for the first time, attempted to quantitatively compare cellular responses to [Si] released from BGs, in order to provide insight and evidence for the appropriate ion ranges for BG design and Si ionic therapy. Whilst evidence is presented for the likely range of [Si] to cause a positive cellular response (<50ppm), the review demonstrates the need for greater standardisation of methodological approaches and reporting methods for in vitro studies. The systematic and quantitative approach used here can be adopted when studying other biomaterials to improve material-cellular modelling, develop improved regulatory standards and to increase translation.

SILICONE-ASSISTED ADVANCED ADDITIVE MANUFACTURING OF GLASS-CERAMIC SCAFFOLDS

Enrico Bernardo (1), Hamada Elsayed (1), Franco Matias Stabile (2)

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Silicone resins are attractive both as precursors of silicate bioceramics and as feedstock for additive manufacturing technologies, including masked stereolithography. The two aspects may be successfully combined when using, as binders for ceramic powders, engineered blends consisting of a silicone polymer mixed with photocurable acrylates. A first case study concerns scaffolds with a composition resembling well-established Biosilicate® glass-ceramics, from direct thermal transformation of silicone into silica, reacting with sodium and calcium salts (carbonates and phosphates). The technology enables the obtainment of novel composites, with the silicone yielding also pyrolytic carbon, by firing in nitrogen. The latter phase provides extra functionalities, such as intensive heating by absorption of IR light, useful for disinfection purposes. A second case study regards wollastonite-diopside glass-ceramics, in which the final phase assemblage relies on the chemical interaction, upon firing, between binder-derived silica and softened glass. Compared to glass-ceramic scaffolds from stereolithography with fully sacrificial acrylate binders, with the same overall oxide formulation, the new methodology enables a distinctive topological control.

MESOPOROUS SILICA NANOPARTICLES (MSNS)-BASED NANOCOMPOSITE SCAFFOLDS FOR BONE TISSUE ENGINEERING – AN IN VIVO STUDY

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For the past few decades, research on bone implants has gained enormous attention in the scientific community to develop suitable implants for bone repair or regeneration. As the bone ECM is a nanocomposite material, researchers have explored the potential of using nanoparticles as fillers in a continuous matrix. In our earlier study, we have demonstrated that the interpenetrating polymer network (IPN) scaffold prepared using a carbohydrate polymer (Konjac glucomannan) and synthetic polymers (PVA & PCL) possess desirable physicochemical and biological properties for bone tissue engineering. Mesoporous silica nanoparticles (MSNs) have shown tremendous potential for drug delivery and tissue engineering owing to their unique properties such as orderly mesoporous structure, large surface area, and pore volume. In this study, we have developed a nanocomposite scaffold by dispersing MSNs in the IPN scaffolds. In vitro cell culture assays proved the cytocompatibility and enhanced osteogenic properties of the nanocomposite scaffolds. We also found that the MSNs stimulated new blood vessel formation, which was confirmed using CAM assay. In vivo studies were performed to evaluate the bone regenerative property of the fabricated bioactive nanocomposite scaffolds. The in vivo studies demonstrated that the nanocomposite scaffold resulted in better bone regeneration in the critical-size rat tibial bone defect compared to IPN scaffolds and untreated control. Also, the results of micro-CT, H&E staining, and Masson's trichrome staining confirmed that the combination of MSNs with the IPN scaffolds enhances osteogenesis significantly than the normal scaffold without MSNs and the control. Furthermore, the immunostaining images corroborated the same, depicting angiogenesis near the newly formed bone cells, and the presence of early-stage connective tissues, fibroblasts, and osteoblasts at the defect site 8 weeks after surgery. These in vivo results of enhanced angiogenic and osteogenic activity are due to the presence of MSNs. Hence, these advantageous biological properties, combined with the suitable physicochemical properties, confirm that the fabricated nanocomposite IPN scaffolds are ideal for treating bone defects.

KEYNOTE LECTURE

GLASS-CERAMICS FOR DENTAL APPLICATIONS: THE CASE OF CAO-MGO-SIO2 SYSTEM

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This work reports on the synthesis and characterization (sintering, crystallization, microstructure, mechanical properties, and in vitro and in vivo biological performance) of novel alumina-free and alumina-containing (1 - 8 mol%) glass-ceramics (GCs) in the CaO-MgO-SiO2 system with additives of K2O, Na2O, P2O5, CaF2, and Al2O3 [1]. Glass frit is prepared via glass melting and then quenching. Thorough thermal analysis (DSC) is conducted to set the conditions for the optimal crystallization process and determine the Tg, the crystallization temperature (Tc), the sintering window (Tc - Tg), as well as the activation energy (Ea) of glass crystallization and the crystal growth dimensionality represented by the Avrami exponent (nA). In the investigated systems, fully dense and well-sintered GCs with excellent aesthetics can be obtained. More specifically:The alumina-free and 1 mol% Al2O3-containing GCs are bioactive, since they favor the spontaneous formation of hydroxyapatite (HA) on their surface after immersion in SBF at 37 oC. In vitro tests with cell cultures, in vivo implantation in experimental animals as well as clinical trials also provide evidence of biocompatibility and bioactivity. The K-free GCs consist of diopside, wollastonite, and fluorapatite, while, in the K-containing GCs, α-PMS is developed instead of wollastonite, as a result of the structural silica-units of the parent glasses (mainly Q2 and Q3). Their mechanical properties are better than those of titanium and zirconia dental implant materials, and their modulus of elasticity (27 - 34 GPa), microhardness (5.2 - 6.7 GPa), and fracture toughness (1.4 - 2.6 MPa.m0.5) are a good match to those of human jaw bone and dentine.The addition of 8 mol% Al2O3 totally suppresses the bioactivity of the produced GCs (i.e. there is no evidence of HA formation on the surface of the GCs). The crystalline phases formed in the K-free GCs are melilite and diopside, and melilite and gehlenite in the K-containing GCs. The produced bioinert GCs satisfied the criteria described in the ISO 6872 “Dentistry-Ceramic Materials [2]”, which refers to the mechanical properties of the dental restorative materials. More specifically, the mechanical properties of the produced bioinert GCs are a good match to the corresponding properties of enamel and dentine, i.e. flexural strength 120 - 171 MPa, modulus of elasticity 28 - 42 GPa, Vickers microhardness 6.3 - 7.0 GPa and fracture toughness 2.6 - 2.8 MPa.m0.5.

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BIOACTIVE GLASS NANOPARTICLES DECORATED WITH CATECHOL-FUNCTIONALIZED POLYESTERS: TOWARDS MACROPOROUS NANOCOMPOSITE SCAFFOLDS

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In the recent years several solutions adopting bioactive glass nanoparticles-based composites were proposed in the field of bone repair, especially concerning the maxillofacial area. In fact, bone substitutes require characteristics such as adequate mechanical properties, biodegradability, osteoconductivity, osteoinductivity, that are mostly satisfied by the development of organic/inorganic nanocomposite[1]. Very recently, new systems were developed associating bioactive glass nanoparticles covalently grafted with poly(D,L-lactic acid) in order to improve spatial homogeneity and mechanical properties of the resulting freeze-cast scaffolds[2]. In this work we aim at reporting an innovative strategy to prepare nanocomposite macroporous scaffolds for bone repair based on the hydrogen-bond interaction that is established between catechol-functionalized polyesters and the silanol groups on the surface of binary bioactive glass nanoparticles. Catechol-functionalized polymers, inspired by the ability of marine mussels to adhere to any hydroxylated surface, have attracted a great interest in materials science[3]. Indeed, the molecular mechanism of mussels' adhesion is related to proteins bearing several catechol groups (1,2-dihydroxybenzene), able to form strong hydrogen bonds with hydroxylated surface[4]. Here, for the first time, poly(D,L-lactic acid) bearing a catechol end-chain moiety was synthesized by ring-opening polymerization (ROP) of D,L-lactide initiated by dopamine (3,4-dihydroxy-phenethylamine, DOPA). A series of polyesters were targeted with molecular weights ranging from 3000 to 100000 g·mol^{-1}, and their physico-chemical properties were fully investigated. Then, model silica nanoparticles were synthesized by sol-gel reaction. Finally, in order to prove the interaction between the two components, DOPA-PDLLA and silica nanoparticles were put together as a proof of concept and the resulting non-covalent nanocomposite was characterized at the nanoscale by DLS, TEM, Solid-State NMR and SAXS. It was found that, through a single mixing step, the functionalized polymer chains are uniformly distributed around the nanoparticles, with the catechol moieties oriented towards their surfaces. Such nanocomposites, finally led to the freeze-cast scaffolds[5].

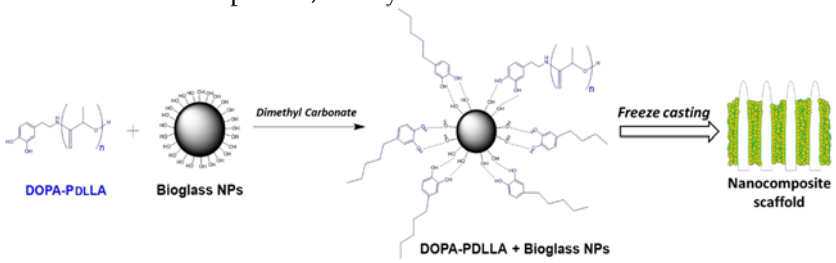

Figure 1 *– (a) PDLLA-BG NPs prepared by self-assembly of DOPA-PDLLA and Bioglass NPs*

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3D Printed Bioactive Glass Scaffolds: Effect of Structural Design and Composition on in vitro Cytocompatibility

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45S5 bioactive glass has a strong tendency to crystallize, which significantly limits its sinterability and processability into complex geometry. The development of bioactive glasses with an optimally adjusted tendency to crystallize with regard to a well-balanced relationship between solubility and sinterability (or crystallization tendency) is necessary. A well-adjusted tendency to crystallization can also be used specifically for the production of complex and large sintered structures in order to limit viscous flow of the component during sintering. 13-93 and fluorine-containing glasses have solubility comparable to 45S5 glass and better sinterability, thus allowing the sintering of glassy complex shapes. In this regard, 13-93 (54.6 SiO_2, 1.7P_2O_5, 22.1CaO, 7.7MgO, 6Na_2O, 7.9K_2O (mole %)) and F3 (44.8SiO_2, 2.5P_2O_5, 36.5CaO, 6.6Na_2O, 6.6K_2O, 3CaF_2 (mole %)) bioactive glasses were used to fabricate 3D scaffolds using Binder jet 3D printing. Scaffolds of different structures, designs, and porosity were made. The in vitro cytocompatibility of these structures was investigated via a direct approach (cells were seeded on the surface of printed scaffolds) using osteoblast cell line (MC3T3-E1 cells). Cellular response (cell attachment and proliferation) depending upon the open porosity and pore size of scaffolds was investigated. All the printed 3D structures exhibited bioactivity upon immersion in simulated body fluid. Results revealed that the structural properties of the printed scaffolds influence the attachment and proliferation. Preliminary results showed non-toxicity of printed structures, thus indicating possible application in bone tissue engineering applications.

SR-CONTAINING MESOPOROUS BIOACTIVE GLASSES BIO-FUNCTIONALIZED WITH RECOMBINANT ICOS-FC: A POWERFUL TOOL TO STIMULATE COMPROMISED BONE REMODELLING.

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Osteoporosis disease is due to an imbalance between osteoclast bone resorption and osteoblast bone formation and exposes the affected people to a high increased risk of fracture resulting in a large number of unsuccessful bone healing cases[1].This critical issue needs personalized and specific treatments in order to stimulate osteoporotic bone tissue regeneration.Consequently,the design of multifunctional nano-biomaterials able to combine both chemical and biological cues in order to exert multiple therapeutic effects is an attractive strategy.With this perspective,mesoporous bioactive glasses(MBGs)have gained increasing attention for their enhanced bioactivity and their ability to release therapeutic ions[2],i.e.,Sr ions already proven in the literature to exert an in vitro pro-osteogenic effect[3].More recently,in the frame of the H2020GIOTTOproject[4]Sr-containing MBGs(nano-and micro-particles)were synthesised and bio-functionalised with ICOS-Fc,a recombinant molecule able to reversibly inhibit osteoclast activity[5],in order to combine in a single device the intrinsic properties of MBGs with the anti-osteoclastogenic properties of the biomolecule.The successful grafting of ICOS-Fc on the surface of MBGs was assessed using different analyses.Moreover,the peculiar ability to release pro-osteogenic Sr ions and the retention of the excellent bioactivity after functionalization of theMBGs were also proved.An ELISA-like performed on ICOS-Fc-grafted MBGs confirmed the maintained ability of the biomolecule to bind its ligand ICOS-L and evidenced that the covalent binding was stable in an aqueous environment up to21days.The inhibitory effect of the migratory activity of ICOS-Fc grafted MBGs was demonstrated by using ICOSL positive cell lines as much as its strong inhibitory effect on osteoclast differentiation and function was confirmed by monitoring the differentiation of monocyte-derived osteoclasts(MDOCs)and by the downregulation of osteoclast differentiation genes.The overall results showed that the combination of ICOS-Fc with the intrinsic properties of Sr-containing MBGs represents a promising perspective to design personalized solutions for patients affected by compromised bone remodelling,such as osteoporotic fractures.

NOVEL BIOACTIVE GLASS-HYDROXYAPATITE COMPOSITES FOR BONE TISSUE ENGINEERING: PROCESSING, MECHANICAL, AND IN VITRO BIOLOGICAL PROPERTIES

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The recently developed BGMS10 bioactive glass (47.2 SiO2, 25.6 CaO, 10.0 MgO, 10.0 SrO, 2.3 Na2O, 2.3 K2O, and 2.6 P2O5, mol.%), which exhibits low tendency to crystallize, is combined in this work with commercial hydroxyapatite powders (50:50 wt.%). The obtained mixture is processed for the first time by Spark plasma sintering (SPS) for the fabrication of bulk composites. The influence of the SPS temperature (750-900°C), applied pressure (16-70 MPa), and ball milling treatment of the powder mixture (0-120 min), on the densification behavior and glass crystallization is systematically investigated. The amorphous character of the glassy phase is preserved up to 800°C, regardless the applied pressure, which slightly promotes powder consolidation. The occurrence of glass crystallization is observed for dwell temperatures equal and exceeding 850°C. Mechanical properties of the resulting bulk products, with fully amorphous or partially-crystallized glass phase and relative densities in the range 92-96%, are compared. Biological tests in SBF are also conducted to assess the effect of the processing parameters above on HCA formation.

SYMPOSIUM 6
HYBRID AND COMPOSITE BIOCERAMICS FOR BONE & OSTEOCHONDRAL REGENERATION

KEYNOTE LECTURE

TYPE I COLLAGEN-APATITE FIBRILLAR NANOCOMPOSITES: MINERALIZATION, TERBIUM DOPING, CROSSLINKING AND ANTIINFLAMMATORY COCRYSTAL IMPREGNATION FOR BONE TISSUE ENGINEERING

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We have studied in detail a base-acid neutralization method to simultaneously self-assemble and mineralize type I collagen (Col) with nanocrystalline apatites (nAp). Two variants of the method were tested: base-acid titration (BAT), a solution of Ca(OH)2 was added dropwise to a stirred Col-H3PO4 solution, and direct mixing (DM), the Ca(OH)2 was added by fast dripping. BAT experiments yielded fibrils mineralized with either nAp or amorphous calcium phosphate plus a precipitate composed of nAp, brushite, and calcite, while DM led to Col/nAp fibrils plus nAp powder. The adding of Tb3+ in DM experiments produced luminescent Tb3+-doped Col/nAp biocomposites useful for imaging the first stage of bone regeneration. Samples did not present cytotoxicity on human mesenchymal stem cells (hMSCs) and they were capable by themselves of inducing osteogenic differentiation in hMSCs, particularly those samples fabricated by DM, and more notably the Tb3+-doped biocomposites [1]. In a second step, we have studied the crosslinking of fibbers by different reagents, namely glutaraldehyde, tannic acid, EDC/NHS, and genipin, with the aim of producing a biopolymeric Ap-Col based drug delivery scaffold and, in parallel, different pathways to impregnate the scaffold with the cocrystal diclofenac-metformin, a non-steroidal anti-inflammatory drug. The result showed the sequence of fibbers impregnation followed by crosslinking led to the maximum cocrystal loading. The impregnated material is expected to be useful in settings with excessive and prolonged inflammation, since they affect negatively the fracture healing/bone repair processes, especially during the early stages of healing [2].

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KEYNOTE LECTURE

BIORESORBABLE DEVICES TO TREAT OSTEOPOROTIC FRACTURES: THE GIOTTO PROJECT

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Osteoporosis is a worldwide disease and the number of osteoporotic fractures is steadily increasing worldwide due to population ageing. Appropriate anti-osteoporotic drugs are available but have several side effects and they do not promote fracture healing. As osteoporotic fractures occur in several body parts, specific devices should be developed for their treatment. In this context, the GIOTTO project aims to target three different types of osteoporotic fractures through innovative nanomaterial-based, composite solutions. The first one consists of a 3D printed bioresorbable scaffold based on an *ad hoc* developed polymeric blend to treat, in combination with standard fixation plates periprosthetic fractures, with specific reference to hip fractures. The second device will target confined pelvic fractures that, due to the invasive surgery that would be required and to patient frailty, are usually not treated (e.g. bed resting). It consists of a bioactive and bioresorbable fibrous scaffold produced by electrospinning that will be injected into the fracture site through a cannulated instrument. The third device is an injectable, bioresorbable, radiopaque ceramic cement to stabilise vertebral compression fractures and promote fracture healing. All three devices contain ceramic materials in the form of strontium-containing mesoporous glass in the form of both nano and micro-particles, and strontium substituted nano-hydroxyapatites in order to stimulate bone regeneration while reducing bone loss. Moreover, in the three devices an active soluble recombinant molecule (ICOS-Fc) able to inhibit osteoclast activity has been incorporated by surface grafting or encapsulation in a resorbable carrier, in order to regulate bone resorption. As an additional value, the device functionalisation with superparamagnetic nanoparticles able to boost bone regeneration by the activation of cellular mechanotransduction has been explored. The efficacy of the three devices is currently under evaluation in an *in vivo* osteoporotic mouse model after which, on the best performing prototypes, a larger animal model will be used (New Zealand White Rabbits and sheep).

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TUNING THE VISCOELASTICITY OF LIVING CELLULOSE THROUGH BACTERIAL PROLIFERATION

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Materials known as switching materials are characterised by changing their properties in response to an external stimulus, activating or deactivating an "on-off" state. Examples are those materials that can change from dielectric to conductive, from colourless to coloured, from paramagnetic to ferromagnetic, etc. On the other hand, the external stimulus enabling such a change can be pressure for piezoelectrics, light for photochromics, magnetic fields, heat, etc. In biomedicine, the best known example is the use of a family of resins that harden under UV light irradiation, but there are also internal stimuli such as, for example, the pH change between blood and lysosome, which allows the specific release of drugs from certain biomolecules. In our laboratory, we have developed a new hybrid living material (HLM [1]), called probiotic cellulose, which combines an inert bacterial cellulose (BC) matrix with live and active probiotic bacteria by co-culturing Acetobacter xylinum and Lactobacillus fermentum [2]. We observed that the proliferation of probiotics in the cellulose matrix modifies its viscoelasticity. While BC (without probiotics) shows the typical viscoelastic moduli of pure BC, the proliferation of probiotics within the probiotic cellulose leads to a decrease in fluidity in the first hours of incubation, and an increase when the bacterial density is very high until it reaches properties similar to an elastic solid. This biomaterial is the first example where life (bacterial growth) is, in itself, the stimulus to modify the fluidity of a material, moving from highly viscoelastic materials to elastic solids.

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PROCESSING AND MECHANICAL PROPERTIES OF UNIDIRECTIONAL, POROUS CERAMIC-POLYMER COMPOSITES FOR BIOMEDICAL APPLICATIONS

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Macroporous ceramics are extensively used in biomedical applications, as bone substitutes or coatings. However, their overall performance is usually maximized increasing the total pore volume, at expenses of their mechanical stability. One strategy to mitigate this issue is to optimize the pore architecture in terms of pore directionality, size, and shape. In the last decade, ice-templating (or freeze casting) has emerged as a powerful technique to process unidirectional macroporous materials in a relatively simple and versatile way.This results in unidirectional porous ceramics with an increased strength when loaded in the direction of the porosity, while keeping the interconnected porosity needed for fluid flow and/or for cell access. However, such materials are still very brittle and it is difficult to produce samples with a controlled geometry.In this work, we focus on the reinforcement of freeze-cast porous ceramics with a polymer, to limit brittleness and to ease sample preparation and manipulation. Different polymers and addition processes are tested, while keeping an interconnected macroporosity. We study the microstructure of the porous composites as well as the impact of polymer addition on mechanical properties (strength, toughness).

COMPOSITE SCAFFOLDS BASED ON B TRICALCIUM PHOSPHATE AND POLYHYDROXYALKANOATE BLENDS FOR BONE TISSUE REGENERATION

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β-tricalcium phosphate (βTCP) scaffolds have been commonly applied in bone tissue engineering due to their excellent biological properties, which promote bone tissue regeneration. However, porous βTCP materials exhibit high brittleness and low compressive strength, limiting their range of applications. The combination of brittle ceramics with degradable polymers may result in improved mechanical properties and confer additional functions as carriers of bioactive substances. Polyhydroxyalkanoates (PHAs) are a promising group of biocompatible and biodegradable, bacterial-origin polymers. PHAs have various physicochemical properties, depending on their chemical structure. Thus, the blending of PHAs may expand their range of applications. In our study, we used blends of poly(3-hydroxybutyrate) (P(3HB)) and medium chain length PHA (mclPHA) to coat βTCP scaffolds. The influence of polymeric coatings on physicochemical properties of materials has been investigated.Ceramic βTCP scaffolds were prepared by a polyurethane sponge replica method. P(3HB) and mclPHA were synthesized through bacterial fermentation. Two blends containing 80:20 (B1) and 70:30 (B2) by weight of P(3HB):mclPHA ratio were obtained by mixing appropriate amounts of polymers solutions in chloroform. Next, βTCP scaffolds were treated with 5% citric acid and infiltrated with 5% blends solutions, dried and subjected to further studies. SEM observations revealed that the macroporous βTCP scaffolds were uniformly covered with blends. The polymeric layer did not significantly influence the pore size (with a mean of about 420 ± 140 μm) or the open porosity (~ 65 vol.%). Composites possessed higher comprehensive strength (4.4 ± 0.6MPa (B1) and 4.9 ± 0.9 MPa (B2)) in contrast to βTCP scaffolds (3.7 ± 0.9 MPa). Moreover, composite samples after mechanical tests remained their integrity. Blends of brittle P(3HB) with mclPHA may serve as coatings on bioceramic scaffolds, improving their mechanical properties. Further in vitro cytocompatibility studies are necessary.

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ZINC-DOPED BIOACTIVE GLASS/ POLYCAPROLACTONE HYBRID SCAFFOLDS MANUFACTURED BY DIRECT AND INDIRECT 3D PRINTING METHODS

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Bioactive glasses are known for bone regenerating ability, but also as platform materials in which it is rather easy to introduce other properties, e.g., antibacterial activity. We propose a novel organic-inorganic hybrid material, based on SiO2-CaO-ZnO (75/15/10 Si/Ca/Zn relative atomic percent) bioactive glass (BG) and polycaprolactone (PCL) for its organic part. Hybrids consisting of 30 wt. % BG – 70 wt.% PCL were produced by partially conducting the silica sol-gel process inside a PCL solution, mixing a solution of PCL with the BG sol right before its gelation [1]. The hybrid sol was then processed into scaffolds in two manners: one based on the direct printing of dried hybrid granules, the other one based on a templating method involving 3D printed paraffin templates infilled with the hybrid sol. In vitro apatite-forming ability tests in Simulated Body Fluid (SBF) confirm the ion release along with the hybrid's bioactivity. However, the kinetics differ significantly between directly and indirectly 3D-printed scaffolds, the former requiring longer soaking periods to degrade, while the latter demonstrate faster calcium phosphate (CaP) formation. These distinct behaviours can be attributed to the denser structure obtained with 3D direct printing using FDM (Fused Deposition Modelling), which results from the coarse (several hundred μm) printing resolution and hot extrusion, while the 3D indirect templated method leads to finer pore sizes, thinner struts with internal porosity therefore enhancing apatite nucleation. Remarkably, diffusion and accumulation of zinc are observed at the surface of both kind of hybrids, within the newly-formed active CaP layer. Zn release was found to be dependent on the scaffold printing method but also on the medium, with Zn2+ concentrations released being 2 orders of magnitude higher in Muller-Hinton Broth bacterial culture medium compared to a simple saline solution such as SBF, reinforcing previous observations about proteins' ability to enhance the dissolution of ZnO by binding their peptides to zinc and creating highly soluble complexes [2-4].

APATITE-GRAPHENE AND APATITE-GRAPHENE OXIDE NANOCOMPOSITES: HYBRID MATERIALS WITH TAILORED BIOLOGICAL AND LUMINESCENT PROPERTIES

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Apatite nanocomposites with graphene (G) or graphene oxide (GO) nanoflakes, as well as with related carbonaceous materials, present promising applications in hard tissue engineering, biomedicine, or drug delivery [1]. Different methodologies have been explored in the last years to prepare apatite-based nanocomposites. Specifically, sitting drop vapour diffusion (SDVD) methodology induces the heterogeneous nucleation of biomimetic apatite on the reinforcement material, improving biological properties of the nanocomposites such as bioactivity and osteoinduction. In this work SDVD was used to prepare apatite-G and apatite-GO nanocomposites. Prior the SDVD experiments, G flakes were obtained by sonication-assisted liquid-phase exfoliation (LPE) using L-Lysine, L-Arginine, L-Aspartic Acid, and Citrate as dispersing biomolecules, while a commercial aqueous Graphene Oxide (GO) dispersion was used for the nucleation essays in presence of the same biomolecules. A parallel set of nucleation experiments was performed in presence of Tb^{3+} ions, to endow the nanocomposites of luminescent properties. A whole characterization by XRD, FTIR, Raman, FESEM, TEM demonstrated the heterogeneous nucleation of needle-shaped apatite nanocrystals on the surfaces of G and GO flakes. We also observed an increment of amorphous calcium phosphate in citrate and Tb-doped experiments. Fluorescence spectroscopy certified the presence of Tb^{3+} ions in the nanocomposites resulting in luminescent materials which can be used in imaging or theragnostic. Finally, in vitro tests with mesenchymal stem cells revealed excellent cytocompatibility and cell proliferation in presence of all the nanocomposites.

AN INJECTABLE, RESORBABLE AND PRO-OSTEOGENIC CEMENT TO TREAT OSTEOPOROTIC VERTEBRAL COMPRESSION FRACTURES

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Approximately 200 million people worldwide are suffering from osteoporosis (OP)[1], a metabolic bone disease caused by excessive osteoclasts (OCs) resorption activity that increases the risk of fracture. Particularly, vertebral compression fractures are one of the most frequent[2]. In the frame of the H2020-GIOTTO project[3], an injectable composite cement was developed to stabilize these fractures alongside stimulating bone regeneration. The cement was prepared by mixing a dry phase consisting of a mixture of powders with an aqueous phase to obtain a paste-like material, directly injectable into the fractured site.The powder component consists of α-calcium sulphate hemihydrate as matrix, strontium-containing mesoporous bioactive glasses(Sr-MBG)and zirconia particles to impart resorbability, pro-osteogenic effect and radiopacity, respectively. Furthermore, ICOS-Fc, a recombinant protein recently patented by NOVAICOS and able to decrease OC activity[4], was incorporated into the formulation to confer anti-osteoclastogenic properties exploiting two routes:biomolecule encapsulation into resorbable polymeric nanoparticles or covalent immobilisation onto Sr-MBG surface. The cement setting times were evaluated in accordance with the ASTM-C266 indicating timeframes suitable for the clinical practice. Mechanical tests conducted following the ISO-5833 demonstrated that the cement has a compressive strength value (ca.8MPa) comparable to human vertebral bodies. A radiopacity comparable to commercial reference was observed and micro-computed tomography analysis evidenced homogenous distribution of the radiopaque phase throughout its volume. In vitro release experiments revealed that the biomaterial can sustainably deliver Sr2+ ions up to 28 days and also functional ICOS-Fc when polymeric nanoparticles were introduced. Scratch tests with B16-F10cells proved that the ability of ICOS-Fc to inhibit OC migration was maintained also when grafted on Sr-MBG. A weight loss of about35% was detected after 1 month of immersion in Tris-HCl. Finally, the biocompatibility and the efficacy have been assessed both in vitro and in vivo in healthy and osteoporotic mice with 2% new bone volume fraction(BV/TV)formation after 28 days.

BIOACTIVE GLASS HYBRIDS : TUNABLE PLATFORM MATERIALS FOR MULTIPLE BIOLOGICAL ACTIONS

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Among the various attempts to enhance bioactive glasses (BG), organic/inorganic hybrids (O/I) are one of the smartest, being able to combine chemical homogeneity at the sub-micrometer scale, toughness and elasticity, bioactivity and resorbability, and organic and inorganic networks that possibly interact or interconnect. O/I hybrids based on BG are obtained from the sol-gel process, that has to be conducted in a solution containing the polymer of interest to successfully achieve the polymerization of the inorganic silicate network around the organic chains. This two-pots-that-are-finally-combined-into-one synthesis yields several advantages, such as the opportunity to introduce dopants that are soluble either in the BG sol or the polymer sol, and therefore address multiple challenges such as stimulation of cell activity or inhibition of bacteria, making O/I BG hybrids a versatile and tunable platform for a variety of biological actions.In this communication, we will report some case studies illustrating how O/I silicate hybrids can benefit from inorganic dopants (metal ions), organic dopants (nutrients used in nutritional strategies for bone health), or even conventional antibiotics, to obtain materials with osteostimulating properties or active against bone infection. Strategies to fabricate porous scaffolds will be discussed, following processes that are compatible with the use of organic materials and thermosensitive compounds.

MONITORING IN VIVO BONE REGENERATION USING BIOMIMETIC SCAFFOLDS FUNCTIONALIZED WITH MAGNETIC NANOPARTICLES

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Bone is a connective tissue responsible for supporting and protecting organs and facilitating mobility. Fortunately, unlike other tissues, bone can regenerate and self-repair without scars when the damage is of limited size. Otherwise, implantable osteoconductive biomaterials can be used to promote and support bone healing. To this end, hybrid biomaterials have been synthetized through a nature-inspired biomineralization process that allows to reproduce chemical-physical properties and behaviour are very close to those of natural tissue. They represent an ideal solution to promote cell adhesion and proliferation as well as bone tissue regeneration. During the biomineralization process, a nanostructured Mg-doped hydroxyapatite (MgHA) phase was nucleated on the organic template (Collagen, Coll) which exerts the control on the formation and growth of the mineral phase through multiple mechanisms and determines the formation of a hybrid construct [1]. On the other hand, as bone is a dynamic tissue that constantly remodels, specific investigations are needed to assess bone self-healing and correct graft placement during implantation in vivo. Instead of histological techniques being time-consuming and labour-intensive, non-invasive imaging techniques (i.e CT, SPECT and MRI) are effective to monitor the repair and fate of host–material interactions and to follow the evolution of the implanted materials over time in vivo [2]. In this research hybrid scaffolds (Coll/MgHA) have been functionalized with magnetic nanoparticles (MNPs) to follow in vivo cell integration differentiation by using the non-invasive MRI techniques. MNPs used as contrast agent were previously coated with 3-(triethoxysilyl) propylsuccinic anhydride (TEPSA) to obtain a thin polysiloxane shell presenting carboxylic acid functions (TEPSA-MNPs) in order to avoid owing aggregation and adverse physiological reactions [3]. Different labelling protocols (simultaneously and post-synthesis) were evaluated to achieve a homogeneous functionalization with MNPs without losing the properties of the hybrid scaffold. "Simultanously" approach demonstrated to be the better protocol to functionalize the scaffold with MNPs resulting in a device endowed with high interconnected porosity, homogenous MNPs labelling that allow a good MRI visualization, no cytotoxicity effect and increased cell proliferation.

TAILORABLE LOW TEMPERATURE GELATIN-SILOXANE BIOMATERIALS AS PROMISING SCAFFOLDS FOR DRUG DELIVERY AND TISSUE REGENERATION

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Sol-gel silica-based hybrids combine an inorganic compound, silica, with an organic one to obtain the right properties for tissue regeneration. These hybrid materials can be produced at low temperature, which allow to encapsulate and release bioactive molecules, acting as a drug delivery system and enhancing tissue regeneration. In this study, gelatin has been combined with tetraethyl orthosilicate (TEOS), which were cross-linked with (3-Glycidyloxypropyl)trimethoxysilane (GPTMS). It is hypothesized that the physic-chemical and its drug delivery properties can be tailored by adjusting the composition of the hybrid. To tailor these properties, sols were prepared using two different H2O/TEOS molar proportions and different volume ratios of TEOS:GPTMS. Furthermore, three different amounts of gelatin were added to the sols to produce the hybrids. Their ability to form the gel at low temperature and to maintain the shape once dried was assessed. Once the gels were formed, their stability and mechanical properties were studied. Additionally, due to the low temperature and mild pH conditions, doxycycline was incorporated to evaluate the subsequent release capacity of the formed gels. Results show that the amount of water mainly determines the ability to form a gel. Moreover, a high amount of gelatin and a low amount of crosslinker increase the degradation rate and the water uptake of the hybrids. The mechanical properties are also positively affected by increasing amounts of gelatin, and also by increasing cross-linker concentration. The release rate of the doxycycline molecule showed a similar tendency as degradation, being faster as the gelatin amounts increased and slower as the cross-linker concentration raised. These results demonstrate that the different parameters of the silica-gelatin hybrids during preparation can be adjusted to tune the properties and drug release rates, making them promising biomaterials to use as drug delivery scaffolds for tissue regeneration.

BIO-COMPOSITE CEMENT BASED ON CALCIUM PHOSPHATE, CALCIUM CARBONATE, BIOACTIVE GLASS AND POLYMER: PHYSICOCHEMICAL, MECHANICAL AND RHEOLOGICAL PROPERTIES

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Calcium phosphate cements (CPCs) are extensively used for bone replacement and regeneration in orthopedic surgical applications, due to their natural bone-like compositions, excellent biocompatibility, and osteoconductivity1. However, their applications are relatively limited due to their low mechanical strength and biodegradability, and poor rheological properties. Adding some inorganic or organic compounds to the CaPs formulations represents an interesting strategy to improve their physicochemical, rheological, and biological behavior2. In this context, the current work focuses on the formulation of new composite cement based on CPCs, and bioactive glass (BG), associated with sodium alginate hydrogel (Alg). The composition, microstructure, setting, rheological, and mechanical properties of the obtained composite cement were investigated. The formulated composite cements consist of nanocrystalline carbonated apatite analogous to the mineral part of the bone. The evaluation of setting properties showed that BG participates crucially in the setting reaction as a calcium and phosphate ions source and serves as a setting accelerator. The rheological evaluation revealed that injectability was slightly improved with increasing BG content compared to CPC, reaching a value close to 100% when combined with Alg hydrogel. The anti-washout property appeared to be weak for the CPC with or without BG. However, this property was significantly improved by introducing Alg hydrogel. The addition of Alg induced an increase in the compressive strength about twice (7.2 MPa) higher than that of the reference CPC (4.0 MPa). According to the above findings, the addition of BG acts as a setting accelerator leading to a fast apatite formation, while the introduction of Alg as a rheological promoting agent improves the injectability and cohesion. The combination of BG and Alg as additives is an interesting strategy for the development of composites with appropriate compressive strength, cohesion, and injectability, giving the possibility of using them in minimally invasive surgical techniques.

Keywords

Alginate, Bioactive glass, Biocomposites, Bone cement, Calcium phosphates.

SYMPOSIUM 8
INNOVATIVE PROCESSES AND CERAMICS

KEYNOTE LECTURE

INNOVATIVE BIOMORPHIC TRANSFORMATION PROCESSES YIELDING NANOSTRUCTURED 3-D BIOCERAMICS WITH SUPERIOR BIOACTIVITY AND MECHANICAL PERFORMANCE

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The regeneration of load-bearing bone segments is a critical need, still unmet due to the lack of bone scaffolds capable of inducing extensive osteogenesis and vascularization, and with appropriate mechanical performance. Indeed, conventional fabrication methods do not allow accurate control of compositional and structural changes, particularly during the consolidation of calcium phosphate bioceramics, where irreversible crystal growth and chemical stabilization occurs, reducing the scaffold bioactivity. The present work describes the unique properties of a large hydroxyapatite (HA) scaffold obtained by biomorphic transformation of natural wood structures, obtained thanks to the application of heterogeneous gas–solid reactions acting in the 3-D state. Thanks to this unpreceded approach, the scaffold shows enhanced bioactivity, induced by the synergistic effect of bone-like composition, lamellar nanostructure and hierarchical osteon-mimicking architecture exhibiting wide interconnected porosity from the nano to the macro scale. We found that these features enable continuous exchange of bioactive ions from and to the scaffold when soaked in physiological body fluids, as chemical signals supporting osteogenic cell differentiation. In this respect, bioreactor studies show overexpression of various osteogenic genes with the biomorphic scaffold in comparison with sintered hydroxyapatite scaffold with similar porosity extent. Moreover, the hierarchical, channel like porosity, closely resembling the osteon structure, was found to facilitate the crosstalk between mesenchymal and endothelial cells, very promising to promote vascularization in the whole scaffold volume. Furthermore, such a hierarchical architecture was found to induce damage-tolerant mechanical performance, unusual for a pure ceramic material, that permit screwing and easy fixability during implantation in bone defects. We observe that these properties make biomorphic ceramics as unique materials laying between ceramics and woods, when depicted in Ashby maps. The biologic and mechanical performance so far observed are very promising for application as scaffolds to regenerate load-bearing segmental bone defects.

SUSPENSION PLASMA SPRAY AND COLD SPRAY AS THE NEXT GENERATION OF PROCESSES TO COAT IMPLANTS WITH BIOACTIVE FUNCTIONALIZED APATITE

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Atmospheric Plasma Spraying (APS) is the most common technique, approved by the FDA, to cover orthopaedic titanium-based implant surfaces with hydroxyapatite (HA - Ca10(PO4)6OH2). However the current trend is to produce thin (<50 μm) and bioactive coatings in accordance with ISO 13779-2. Achieving all these characteristics is challenging and requires implementing other techniques than APS. The work, carried out over the past ten years, is to evaluate two alternative thermal spraying processes: suspension plasma spraying (SPS) and cold spraying (CS).SPS makes it possible to produce HA nanostructured coatings whose chemical composition can be modulated by incorporation of ions having a bactericidal effect (Ag) and bone growth-stimulating ability (Sr). Their mechanical and biological properties were compared to a reference APS coating: adhesion energy 4 to 12 times greater and affinity of the same order both with proteins (albumin) and mesenchymal cells. It has been demonstrated that the coatings contain up to 0.35 wt% silver, with no evidence of cytotoxicity. Tests have shown that the size of silver nanoparticles has a more significant effect than their concentration on their antibacterial potential (S. aureus, S. epidermidis and E. coli). Strontium is incorporated relatively uniformly for concentrations between 3 and 5 wt% regardless of the doping process and shows no effect on the biological or mechanical properties of the deposit.Cold spray process is also suitable for the production of hydroxyapatite coatings, but above all it has shown its ability to allow the development of a biomimetic apatite deposit. This apatite is metastable and nanostructured which makes it very sensitive to temperature. The CS process preserves all the characteristics of this material which has a very high reactivity and therefore a high bioactivity.

Acknowledgments

This work was supported by Région Occitanie (REVAMITIC project, CLE 2016) and Agence Nationale de la Recherche (ArchiCaP project, ANR-15-CE19-0021).

DEVELOPMENT OF AN INNOVATIVE "COATING-FROM" APPROACH FOR CERAMIC BIO-ACTIVATION, BY HIGH PRESSURE CO_2

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Biphasic macroporous HA/β-TCP scaffolds (BCPs) are widely used for bone repair. However, the high-temperature of the shaping process limited bioactivity of the HA and β-TCP phases (low solubility of HA[1], restricted surface area, low ion release).

Strategies were developed to coat such BCPs with biomimetic apatite to enhance bioactivity. However, this can be associated with poor adhesion[2], and metastable solutions[3] may prove difficult to handle at industrial scale. Alternative strategies are thus desirable to generate a highly bioactive surface on commercial BCPs.

In this work, we developed an innovative "coating from" approach for BCP surface remodeling via hydrothermal treatment under high pressure CO_2, used as a reversible pH modifier and with industrial scalability. Thanks to a set of complementary tools including FEG-SEM, solid state NMR and ion exchange tests, we demonstrate the remodeling of macroporous BCP surface with the occurrence of dissolution-reprecipitation phenomena involving biomimetic CaP phases.

The newly precipitated compounds are identified as bone-like nanocrystalline apatite and octacalcium phosphate (OCP), both known for their high bioactivity character favoring bone healing[4].

In the context of such bone substitute, therefore important surgery, adding anti-bacterial properties could be of interest [5]. Aiming to avoid the use of antibiotic, we showed the possibility to dope the remodeled BCPs with antibacterial Cu^{2+} ions to convey additional functionality to the scaffolds, which was confirmed by in vitro tests. This new process is appealing for enhancing the bioactivity of commercial BCP scaffolds via a simple and biocompatible approach.

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MICROWAVE APPLICATION TO BIO-SCAFFOLD PREPARATION

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Microwave ceramic sintering is a well-established methodology for sintering ceramics (see Fang). Due to its characteristics related to energy spare, quickness and also for the mechanism of sintering which is different form the "conventional" sintering technologies.

In the present work we used this technology in order to prepare scaffolds with controlled porosity using nano hydroxyapatite and silica glass (as a foaming agent) for bone regeneration application. The morphology of the scaffold was analyzed and also preliminary in vitro biocompatibility test was performed. The results were promising showing the achievement of the target porosity and also the indication of a good biocompatibility of the scaffolds obtained with human osteoblast cells.

The advantages from the microwave technology employed are multiple. The first immediate advantage was the quickness of the preparation (a few minutes in comparison with traditional methodologies) but also the possibility to let the material expand in a confined mold (with the possibility to obtain scaffolds corresponding to the mold with extreme precision). Further investigation and optimization of this very promising process is on the way.

Microwave sintering of ceramics, composites and metal powders, Editor(s): Zhigang Zak Fang, In Woodhead Publishing Series in Metals and Surface Engineering, Sintering of Advanced Materials, Woodhead Publishing, 2010, Pages 222-248, ISBN

KEYNOTE LECTURE

ADDITIVE MANUFACTURING OF INORGANIC-ORGANIC HYBRID MATERIALS FOR MEDICAL APPLICATIONS

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Two photon polymerization is an additive manufacturing approach that involves the use of ultrashort laser pulses to selectively polymerize photosensitive resins, resulting in fabrication of microscale and/or nanoscale structures. The nonlinear nature of two photon absorption enables additive manufacturing of features with features below the diffraction limit. A structure with an arbitrary geometry may be fabricated by polymerizing a photosensitive resin, which is translated in three dimensions using micropositioning system. The use of two photon polymerization to prepare many types of medically-relevant structures, scaffolds for tissue engineering and microneedles for drug delivery and sensing, out of inorganic-organic hybrid materials (e.g., organically-modified ceramic materials) will be considered. In addition, use of in vitro studies and in vivo studies to assess the functionality of the two photon polymerization-created structures will also be discussed. Our results indicate that two photon polymerization is an attractive additive manufacturing approach for scalable production of many types of microstructured and nanostructured medical devices.

INVESTIGATION OF THE MICROWAVE SINTERING OF CARBONATED HYDROXYAPATITE FOR BONE RECONSTRUCTION

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With improved solubility and biodegradation rate, carbonated hydroxyapatites (CHA) are promising bioceramics for filling bone defect compared to unsubstituted hydroxyapatite (HA, Ca10(PO4)6(OH)2). Carbonate ions in the HA lattice can substitute either for OH- through thermal treatment under CO2-rich atmosphere to form A-type CHA (CAHA) or for PO43- through synthesis by wet precipitation to form B-type CHA (CBHA). Controlling the amount and location of CO32- as well as the microstructure, which both affect the biodegradation, depends on the fabrication process and remains challenging. The sintering of CBHA is usually achieved through resistive sintering under a CO2-rich atmosphere to prevent thermal decomposition. This atmosphere causes an enrichement of A-type CO32- known to hinder densification. Sufficient consolidation thus requires high temperature or longer dwell time which may be accompanied by thermal decomposition or grain growth. To overcome these limitations, we investigate the microwave (MW) sintering which allow faster processing time.In this work, the sintering of CBHA containing 0.8 mol.% of CO32- was studied in a MW multimode cavity under air. Pellets starting from CBHA alone and from CBHA mixed with carbon powder to generate in-situ a CO2-rich atmosphere were considered. Sintering were performed at 900 °C and 1000 °C with different ramps and dwell times. Final densities were ranged between 70 and 85 % of theoretical density. From XRD and FTIR results, it was not possible to sinter a pure CBHA. With carbon, substitution of A-type CO32- appeared. The presence of carbon was thus effective for generating a CO2-rich atmosphere but insufficient, in our conditions, to avoid the decomposition. A new project that has just started will allow us to develop MW sintering under a controlled CO2-rich atmosphere. Eventually, the parts produced will be the subject of cellular and acellular biological tests.

Keywords: Microwave sintering, Carbonated hydroxapatite, Thermal stability

TUNABILITY OF COMPOSITE SCAFFOLDS FOR BONE SUBSTITUTION VIA THE FREEZE-CASTING PROCESS

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Numerous scaffolds have been developed to replace bone defects in non-bearing sites. Ideally, such scaffolds should: i) stimulate the new bone formation (cell differentiation, proliferation, adhesion) iii) exhibit adequate macropore size and volume (cell colonization, angiogenesis), iv) maintain mechanical properties during handling. Some inorganic materials, such as calcium phosphate or bioactive glasses, partially meet of some of these criteria. Nevertheless, the mechanical cohesion of such powders is often obtained after high temperature treatment that prevents the shaping of metastable materials or their association with active molecules. These drawbacks can be avoided using polymeric-based composites. Such porous composites could be obtained by various processes such as electrospinning or robocasting.

The present work is focused on the freeze-casting technique that is based on the control of solvent crystal nucleation/growth and its subsequent sublimation. Through several examples, the aim of this contribution is to highlight correlations between formulation parameters (nature/size of inorganic fillers, nature/functionalization/length of polymers, solvent nature/amount), the process parameters and the final properties of scaffolds. Several (nano)fillers were synthesized (co-precipitation, sol-gel) and fully characterized (XRD, NMR, DLS, granulometry, TEM), associated with synthetic or biosourced polymers and freeze-cast. Resulting scaffolds were studied via SEM, porometry, X-Ray microtomography, SAXS and compression tests. It was demonstrated, for instance, that the PDLLA-grafting [1] onto the surface of bioglass nanoparticles improves their dispersion and their spatial distribution or recovery rate [2]. We also showed that the apatite/PLGA ratio has a strong influence on the Young's modulus and in vitro degradation properties [3]. Porosity orientation and size have also been proven to be process-driven. Finally, appropriate (low temperature) processes allowed to integrate ionic/ molecular active agents in fillers (LDH /bioglass/apatite).These results demonstrated that well-chosen smart (nano)filler/polymers associated to the freeze-casting technique lead to well-controlled scaffolds with tunable properties allowing them to adapt to bone pathologies.

Acknowledgments

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BIOCOMPATIBLE CERAMIC POWDERS DESIGNED FOR SLS APPLICATIONS: A FEASIBILITY STUDY

Christos Softas (1), Sooraj Hussain Nandyala (1), Md Towhidul Islam (2), Ifty Ahmed (2), David Grossin (3), Artemis Stamboulis (1)

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Utilising additive manufacturing (AM) technologies for biomedical applications is a highly novel alternative method to the traditional manufacturing processes; the intricate and tailored geometries required for these applications, is highly attainable through direct laser sintering/melting AM techniques. Hydroxyapatite has been widely used in the biomedical field due to its high biocompatibility, osteo-conductivity and bonding ability [1,2]. The same applies for biomedical glasses, which are a unique class of materials well known for their excellent biomedical properties [3]. This study focused on the development of a hydroxyapatite and low melting temperature La2O3-doped borosilicate glass powder designed for Selective Laser Sintering (SLS).

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ONE-POT SYNTHESIS AND CELLULAR INTERNALIZATION OF SPHERICAL HYDROXYAPATITE NANOPARTICLES

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Hydroxyapatite nanoparticles (nano-HAPs) have been frequently used as component in a wide variety of bone graft substitutes for both dental and orthopedic surgery. Numerous fabrication methods have been proposed, however, controlled one-pot synthesis of well-dispersed spherical nano-HAPs remains a major challenge. These spherical nanoparticles are hypothesized to exhibit favorable properties for the design of colloidal formulations, e.g. colloidal composite gels. Therefore, we have investigated the influence of synthesis parameters on the physicochemical properties and cellular internalization of spherical HA nanoparticles.In our research, the nano-HAPs of controllable size, aspect ratio and crystallinity were synthesized using a one-pot aqueous precipitation reaction between calcium acetate and trisodium phosphate at different precursor addition speed, pH and temperature at a fixed Ca/P molar ratio of 1.67 [1]. FTIR was used to confirm the molecular structure of the precipitated nanoaprticles and their crystallinity and estimated crystallite size were analyzed by X-ray diffraction (XRD), Figure 1A&B. Scanning electron microscopy (SEM, Figure 1C), energy dispersive spectroscopy (EDS) and dynamic light scattering (DLS) studies confirmed the formation of spherical nanoparticles with average size of 50 ~ 250 nm. The Ca/P ratio of the nanoparticles ranged between 1.55 and 1.67 upon assessment using inductively coupled plasma mass spectrometry (ICP-MS). The nano-HAPs were post-modified with citrate ions to control their surface charge and colloidal stability via optimization molar ratios and addition time of citrate salt [2]. Cytotoxicity (CCK-8 assay) and uptake of synthesized nano-HAPs on MC-3T3 (murine, preosteoblast) revealed their nontoxic nature at low concentrations and ability to be internalized by preosteoblast MC-3T3 cells (Figure 1D). In conclusion, our study contributes to the development of a simple and controlled methodology to synthesize well dispersed spherical nano-HAPs. These nano-HAPs can be effectively internalized by cells, which justifies further application as delivery vehicle for therapeutics in nano- and regenerative medicine.Figure 1 (See attachment) Figure 1: (A) FTIR and (B) XRD spectra of nano-HAPs synthesized at different temperatures, (C) SEM imaging of nano-HAPs and (D) internalization of nano-HAPs in murine MC3T3 preosteoblast cells. White colors resulted from co-localization of purple-stained nano-HAPs and green-stained lysosomes.

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SEPTEMBER 23RD

PLENARY LECTURE

COSMETICAL AND COSMECEUTICAL BIOCERAMIC MATERIALS IN THE THIRD MILLENNIUM BETWEEN FAKE AND FACTS

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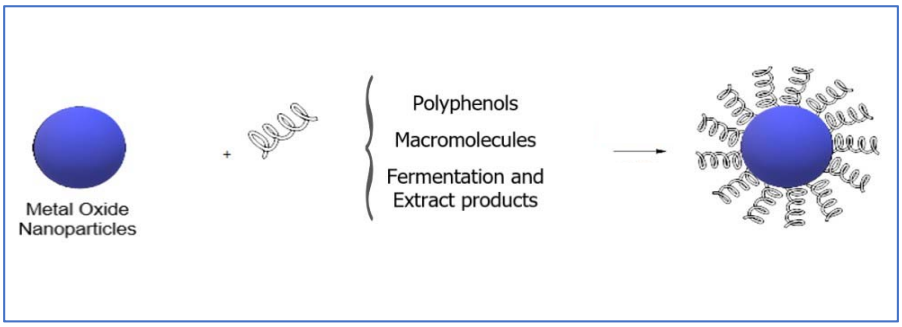
Cosmetic pleasantness of product containing inorganic UV filters depends on an unwanted whitening effect which is inversely correlated to their size. To overcome this effect, diameter has been reduced over the years to nanosize, increasing the surface area and biological reactivity thus carrying questions, amplified by fake news and social media, about the safety towards human and environment. Following a "safe and sustainability by design" approach we designed a new class of optimized sunscreen UV filters by chemical functionalization of ZnO and TiO_2 with polyphenols, both natural and synthetic (i.e. Oxisol, Ellagic Acid and Ferulic Acid), macromolecules and fermentation/extract products in the aim to reduce side effects of nano UV filters. If compared with the simple physical mixture, the new coated filters show different properties and benefits: a higher SPF (ISO 24443:2012), a better cytotoxic profile (MTT and NRU assay), a radical scavenging action (PCL assay) and an improved safety profile with a strong reduction of photocatalytic activity (Acid blue 9 test). In conclusion, our SbD approach represent a new generation of UV filters bonded with booster molecules (by means of synergistic effects) as the best compromise in the conscientious UV protection and respect of the environment.

This work was supported by EU Community's HORIZON-2020 framework programme H2020 (GRANT. Nr. 862195 project: SBD4Nano https://www.sbd4nano.eu and GRANT. Nr. 953206, project BIONANOPOLYS https://www.bionanopolys.eu).



SYMPOSIUM 10
BIOCERAMICS FOR SOFT TISSUE APPLICATIONS AND SKIN CARE

KEYNOTE LECTURE

INNOVATIONS FROM THE HORIZON 2020 EUROPEAN SCREENED PROJECT: IRON-DOPED HYDROXYAPATITE NANOPARTICLES (FEHA NPS) FOR MAGNETIC GUIDANCE OF RAT THYROCYTES ONTO THYROID LOBE-LIKE 3D COLLAGEN SCAFFOLDS

Roberto Toni (1,2), Silvia Panseri (3), Elisabetta Campodoni (3), Giusy Di Conza (1), Fulvio Barbaro (1), Giulia Remaggi (1,4), Lisa Elviri (4), Giulia Spaletta (5), Monica Sandri (3)

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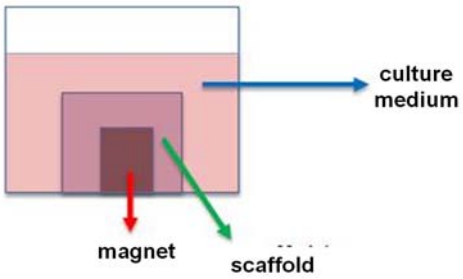
The Horizon 2020 European SCREENED Project (#825745,https://www.unipr.it/notizie/interferenti-endocrini-luniversita-di-parma-vincitrice-di-un-finanziamento-europeo-horizon) is focussed on developing 3D *in vitro* assays based on 3D organoids engineered with mammalian thyroid cells, for screening the effect of low doses Endocrine Disruptors (EDs). Exploiting cellular internalization of CNR-ISTEC fabricated, biocompatible and bioresorbable iron-doped hydroxyapatite nanoparticles (FeHA NPs) (1) we used the certified adult rat thyrocyte cell line FRTL5 (CLS n.500407) whose thyroid-specific proteomic profile is fully comparable to that of adult male rat primary thyrocytes (2). FIRTL5 monolayers were challenged for 6 hs with FeHA NPs; commercially available SPION FLUIDMag NPs were used as control. Following uptake of both NPs types, cell viability was assessed at 6, 24 and 48 hs whereas evaluation of internalized NPs was immediately achieved by ICP analysis. Using both NPs, FIRTL5 cells remained viable in extremely high percentage. Similar, intracellular accumulation of NPs resulted close to maximal saturation with both types of NPs. For magnetic guidance, both unmagnetized and magnetized FIRTL5 cells were grown inside an *ad hoc* engineered culture chamber having at its center a 3D cylindric and porous collagen scaffold mimicking a decellularized rat thyroid lobe, harboring at its core a 3x3 mm static magnet generating a magnetic field of 320 mT (figure).



Light fluorescence, immunocytochemistry, and ultrastructural (TEM, SEM) microscopies revealed that magnetized cells densely accumulated onto the 3D scaffold, colonizing the empty follicular-like cavities. Collectively, we succeeded in setting an innovative 3D bioartificial thyroid lobe-like organoid based on thyroid epithelial cells magnetized with FeHA NPs, and their guidance onto a 3D collagen scaffold surrogating a decellularized 3D thyroid lobe matrix, thus providing a new assay model for *in vitro* testing low doses of EDs.

Acknowledgments

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HYBRID PARTICLES FOR THE LOCAL TREATMENT OF COMPLEX WOUNDS WITH HIGH RISK OF INFECTION

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Calcium phosphate apatites are present in all vertebrates, as bone and teeth biominerals. Bio-inspired analogs can be prepare in the lab and their intrinsic biocompatibility and submicron to nanoscale dimensions along with a high surface reactivity may open the way to a wealth of biomedical applications ranging from bone regeneration to nanomedicine (hematology, oncology, dermatology…). Previous data demonstrated the possibility to prepare colloidal apatite particles stabilized by an organic corona of aminoethylphopsphate (AEP) or phosphonated ethyleneglycol (PEGp); and in vitro evaluations confirmed their very low cytotoxicity, good hemocompatibility and non-proinflammatory potential [1-3].With the exception of the treatment of acne [4], these particles have been little studied in dermatology. In this field, wound healing remains a challenge, especially for patients with a high risk of infection and/or wound healing disorders (e.g. diabetics, immune-suppressed, burns victims, elderly patients, etc.). In this context, the present work aims at combining for the first time two types of bio-inspired compounds, namely apatite particles and peptides, to develop bioactive hybrid colloidal particles capable of exhibiting antimicrobial, pro-healing and/or anti-inflammatory properties via the selection of peptides and the possible incorporation of bioactive ions into the apatite structure. These particles, presenting a peptide external corona, can then be incorporated in wound care formulations (e.g. impregnated onto bandages or used as a gel or spray) to allow optimal local delivery to the affected skin. We will present here this bio-inspired proof of concept of hybrid peptide/apatite particles, as well as selected results showing the relevance of these systems in the general context of complex wound healing.

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REINVENTING CALCIUM PHOSPHATES FOR SKIN TISSUE ENGINEERING: CONTINUOUS MANUFACTURING OF TAILORED PARTICLES

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Calcium plays an important role in barrier function repair and skin homeostasis [1]. In particular, calcium phosphates (CaPs) are well established materials for biomedical engineering due to their biocompatibility [2]. To generate biomaterials with a more complete set of biological properties, previously discarded silk sericin (SS) has been recovered and used as a template to grow CaPs [3],[4]. Crucial characteristics for skin applications, such as antibacterial activity, can be further enhanced by doping CaPs with cerium (Ce) ions [5]. The effectiveness of cell attachment and growth on the materials highly depends on their morphology, particle size distribution, and chemical composition [6]. These characteristics can be tailored through the application of oscillatory flow technology, which provides precise mixing control of the reaction medium.Thus, in the present work, nano-hydroxyapatite (HAp)(CaP1), nano/micro-HAp/brushite (CaP2), micro-brushite (CaP3), CaP/SS and CaP/SS/Ce particles were fabricated using a novel modular oscillatory flow plate reactor (MOFPR) in a continuous mode (WO/2017/175207).Through MTT assay, it was evidenced CaP1, CaP2, CaP3 and Ca-SS composites promote cell viability of human dermal fibroblasts (HDFs), contrary to what was observed in highly crystalline CaP3 particles (Fig. 1 A, B). Moreover, regarding DNA quantification, the addition of SS significantly increases the amount of DNA produced (Fig. 1 B2). According to confocal microscopy, after 7 days the cells were more scattered and exhibit several contact points. The samples of a commercial HAp, CaP1 and CaP2 exhibit a very symmetrical and oriented cytoskeleton. In the composite particles with sericin, the CaP-SS also evidence cells with a well-organized cytoskeleton (Fig. 2). Thus, it was shown that HAp-based nanoparticles with rod/plate shape promote adhesion and proliferation.This work represents a first step towards the reinvention of CaPs for skin TE, demonstrating the importance of physicochemical and compositional characteristics of CaPs in the biological response of skin cells.

MEDICATED HYBRID REGENERATIVE MEMBRANES FOR SYNERGISTIC DRESSING AND HEALING OF DEEP AND INFECTED SKIN WOUNDS

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Regenerative medicine is a rapidly expanding area of medical science, aimed at improving the regeneration process through a multidisciplinary approach which integrates knowledge of cellular and molecular biology, pathophysiology and materials science. In the framework of wound healing, this strategy could allow overcoming the existing hurdle associated with deep wound care, particularly in the presence of a concomitant infection and make available an appropriate treatment that still remains an open challenge. In this scenario, this research was focused on the development of medicated 3D biomimetic and bioresorbable hybrid patches for the treatment of infected skin wound able to conjugate a prolonged and targeted antimicrobial activity to defeat the infection with a regenerative ability deriving from epidermal tissue mimicry and physical support to tissue regrowth. Specifically, collagen and gelatin ideal for their compositional and structural affinity with connective tissues and excellent hydrophilicity were combined with chitosan, selected for its structural characteristics and inherent antimicrobial properties. These biopolymers were used to assemble mono- and multi-layer patches whose composition, structure and porosity were modulated to obtain 3D porous matrices with tailored chemical and structural features, high hydrophilicity and organized porosity [1]. Additionally, the replication in lab scale of a nature inspired biomineralization process permitted the realization of bio-hybrid matrices, where biomimetic mineral calcium phosphates (CaP) were grown directly on the polymer in a nanostructured way, exponentially increasing its bioavailability. The doping of CaPs with bioactive and antimicrobial metal ions, such as Mg^{2+}, Cu^{2+}, $Fe^{2+/3+}$, Zn^{2+}, enables their delivery in situ at the local basic pH caused by the infectious status [2, 3]. To boost their antimicrobial potential, the patches were also medicated with Vancomycin that was appropriately adsorbed on the matrix. The degree of porosity and pore size of the 3D matrices were engineered with freeze-casting processes and conceived with the ability of drug adsorption and ions release, favorable to fluid retention and cell infiltration. The chemical stability, the degradation timing of patches and the drug release profile, was controlled and modulated by cross-linking treatments involving biocompatible agents and physical methods. In conclusion, 3D multifunctional devices with graded composition and structure were successfully created, associating physical stability, hydrophilicity, skin breathability, tailored multi-scale porosity, tuned bio-degradation and drug release profiles. The resulting 3D patches, addressed to advanced approaches for skin wound healing, are designed to be applied directly to the wound and transmit multiple signals to the complex cell system involved in the healing process, fostering regeneration of the damaged epidermal tissue and resolution/prevention of microbial infection. Moreover, allowing the local administration of antibiotic drugs and metals avoids many concerns about the possibility of obtaining effective dosages without inducing adverse allergic sensitization reactions and selection of resistant bacteria.

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MODIFICATION OF NANO TITANIUM DIOXIDE (TIO2) BY BIOSURFACTANTS FOR SAFETY AND SUSTAINABLE COSMETICS

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Engineered nanomaterials (ENMs) used in several applications including medicine and cosmetics need further investigation for their functional performance and safety towards humans and environment. Although, surface modification introduced as a solution, it may result with the loss their functionality [1]. Therefore, safe-by-design (SbD) approach aims to maximise the functional performances while minimising their impact on human and environment [2]. European Commission supports the SbD concepts of nanomaterials in nanotechnology field [3]. One of the projects supported by EC is SbD4Nano. In this project, one of the nanomaterials evaluated is TiO2 NPs, which is typically used in cosmetics as sun light blocker. Nevertheless, the photo-induced excitation of TiO2 NPs in UVB region (290-320 nm) may cause short-term catalytic reactions under the sun light [4]. Photocatalytic redox reactions occurred on the TiO2 NPs surface may cause the degradation of organic formulation components in contact and produce reactive oxygen species resulting in adverse effects on human health [4, 5]. The coating of ENMs with a suitable molecular barrier to insulate them may help to decrease their toxicity while keeping their function intact [6]. In this study, biosurfactant such as sodium surfactant as an anionic surfactant and its undissociated forms were used as surface modifiers due to their very low toxicity, and biodegradable features. After the optimization of modification procedures, the characterization of modified species by using spectroscopic, thermal and images techniques, as well as the evaluation of photocatalytic effects and toxicity, it was found a dependence of in vitro responses by the molecular structures used to coat their surface. This study suggests an experimental strategy to modify TiO2 NPs surface and demonstrate how this decreases their potential toxicity by preserving their solar barrier capacity, so implementing a SbD approach in a very relevant and appealing application.

Acknowledgments

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SYNTHESIS, CHARACTERIZATION, AND ION RELEASE PROFILE OF BINARY SIO2–AG2O MESOPOROUS BIOACTIVE GLASS SUBMICRON PARTICLES

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Biomaterials are primarily intended to support, repair, or regenerate injured tissue with a low immune response by the host [1,2]. In the skin, a wound is associated with the rupture of its continuity due to injuries, diseases, or physical damage, and which is often infected by microorganisms [3,4]. For potential application as bioceramic in assisting epithelial tissue, the Bioactive Glass (BG) has shown properties such as biocompatibility, and the possibility to carry antibacterial agents such as silver (Ag), whose effectiveness has already been extensively discussed [5–7]. In this work, spherical and submicron particles of BG with different concentrations of silver ions were obtained by a simplified sol-gel methodology. Morphological (TEM) and particle size (DLS) analyses demonstrated the obtaining of the binary system (SiO2-Ag2O) spherical particles with sizes between 119-220 nm. TEM revealed the formation of silver nanoparticles (AgNP) as a second phase, also confirmed by X-ray diffraction (XRD). The Zeta Potential indicated a surface charge close to -30 mV, demonstrating the conditions of a colloidal dispersion considered stable. The ion release assay confirmed the dissolution capability of the BG structure, demonstrating its potential as a carrier for antimicrobial agents. Moreover, X-ray photoelectron spectroscopy (XPS) tests showed the presence of Ag+, indicating the probability of incorporation of silver ions in the silicate structure in addition to its presence in the metallic form. Therefore, the sol-gel methodology employed presents a simplified way to obtain submicron, spherical, and mesoporous particles of binary BG (Si-Ag), with potential application as bioceramics in composite antibacterial dressings.

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BIODEGRADABLE CHITOSAN-BASED SCAFFOLDS CONTAINING BORATE BIOACTIVE GLASSES

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In recent years, a growing attention has been paid to the development of wound dressings from biodegradable polymers. The wound dressings that can deliver various biologically active agents represent a new generation of materials for wound therapy. Chitosan is one of the biopolymers that stands out for its favourable properties such as non-toxicity, biocompatibility, and hemostasis activity. The present work studies a combination of chitosan-zinc complex with borate bioactive glass to obtain a foam-based platform for dual delivery of biologically active ions. Chitosan was successfully chelated with zinc via the in-situ precipitation method and served as a soft matrix. The multifunctional biocomposites containing borate bioactive glasses were produced by freeze-drying. The resulting foams exhibit a morphology suitable for wound healing applications. The biodegradation of the composites in lysozyme-containing media was investigated for up to 7 days. The cytotoxic response was assessed using stromal cells. Additionally, in vitro scratch assay was performed with keratinocyte-like cells. A positive effect of the foams on cell viability and migration was observed. The novel biocomposites show promising properties for their use in wound treatment.

SYMPOSIUM 11
BIOGENIC CERAMICS & CIRCULAR ECONOMY

KEYNOTE LECTURE

SUSTAINABLE BIOCERAMICS AND BIOMATERIALS PRODUCED USING NATURAL WASTES AND BY-PRODUCTS FROM AGRICULTURE AND FOOD PROCESSING

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As part of the ever increasing importance of developing a sustainable, circular economy, as well as often for sound economic reasons, there is a growing interest in, and demand for, products produced from the valorisation of wastes and by-products of industry and agriculture. This is especially true for biomaterials, where often animal or plant wastes / by-products can offer existing natural sources for many useful products. Probably the best known bioceramic example is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp), the major component of human and animal bones, used for bioimplants and bone scaffolds. This can be synthetic HAp made via chemical reactions between calcium and phosphorus-containing compounds, but there is increasing development and interest in using biogenic HAp obtained from sustainable natural sources, such as animal and fish bones. These are often by-products of the food industry, which can be valorised by extracting HAp-based compounds. These naturally-sourced materials exhibit biocompatibility comparable to, or superior than, that of standard commercial synthetic materials. Indeed, several commercial products derived from animal bones are already available on the market. We have carried out much research on the conversion of wastes from the fisheries industry (fish bones and scales) into a range of HAp-based biomaterials. We have also shown that HAp produced from such waste materials can be photocatalytic, with associated antibacterial properties, and we have also produced other antibacterial phosphate compounds from these by-products. HAp from natural sources with added iron oxide can absorb UV radiation with applications as sunscreens, and when combined with chitosan, a natural polysaccharide biopolymer with antibacterial properties which can be extracted from seashells and crustacean and insect carapaces, a material suitable for wound dressings was produced. A possible alternative approach to producing porous scaffold-like structures is to use a natural template to create biomorphic HAp materials. Cork bark is a highly porous material with a 3-dimensionally ordered microstructure (3-DOM), and is highly sustainable as the tree is not harmed during harvesting. We have used cork wastes as a sustainable template to produce biomimetic calcium carbonates and HAp with hierarchical porosity. In this presentation I will give an overview of our work in this area.

THE CIRCULAR ECONOMY OF CALCIUM PHOSPHATES: SOURCES, OPPORTUNITIES AND APPLICATION IN COSMETIC.

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During the last 30 years, green and sustainable chemicals capable to replace dangerous or brown ones have been sought by researchers worldwide. In this context, calcium phosphates (CaPs) have gained interest due to their wide range of applications and to their intrinsic bio and eco-compatibility. CaPs are a family of materials that are ubiquitous in the biosphere, and they are found in water bodies, soils, vegetables and indeed in humans and animals, where they constitute the mineral part of bones. For this reason, they are recognized as biologically safe and are considered standard materials for biomedical applications. The possibility to control their size, morphology, and chemical composition is one of the reasons of their successful application in different fields.As an example, CaPs have been recently proposed as a bio- and eco-compatible alternative to the chemicals used in sunscreens to protect the skin from harmful UV radiation, the so-called UV-filters, which are associated to health risks for both people and the environment. We found that CaPs produced according to a circular economy approach can be used as effective SPF boosters in combination to commonly used UV-filters to obtain more eco-friendly and safer sunscreens. Moreover, CaPs contain around the 20 wt.% of phosphorous, which is a diminishing resource on the planet as its reserves are being quickly depleted by agricultural and electronic industry demands. For this reason, phosphorous was included among the elements with limited availability and future risk to supply by the European Chemical Society. In this view and in consideration of their large-scale utilization for large-scale applications such as cosmetic, the recovery of CaPs from circular economy sources such as water and food by products is of primary importance.Acknowledgment: The author acknowledges the CNR research project “SEARCULAR” for providing financial support to the present work.

STRONTIUM/SILVER-CO-SUBSTITUTED HYDROXYAPATITE DERIVED FROM BIOGENIC SOURCE WITH NON-CYTOTOXIC AND ANTIBACTERIAL PROPERTIES: A MULTIFUNCTIONAL BIOMATERIAL

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Hydroxyapatite (HAp) is the most widely used calcium phosphate for hard tissue applications due to its compositional similarity to biological apatite, biocompatibility and bioactivity. As bone apatite is a multi-substituted carbonated HAp containing trace elements, one of the used approaches to improve the biological and physicochemical properties of HAp is ionic substitution. To achieve a good balance between cell biomaterial interactions and anti-bacterial properties, a Sr-substituted HAp with potential regenerative properties was co-substituted with Ag+ ions to offset the cytotoxicity of higher substitution levels of Ag+ ions and stimulate osteogenic growth. A series of single-substituted HAp with Sr2+ and Ag+ ions, and Ag/Sr-co-substituted HAp with different substitution degrees (0, 1, 2.5 and 5 mol%) were obtained by wet precipitation method from cuttlefish bone in order to obtain biomimetic multi-substituted HAp. Rietveld refinement studies revealed an increase of cell volume (Å3), a-axis (Å), b-axis (Å) and c-axis (Å) for all obtained HAp powders, due to larger ionic radius of Sr2+ (1.12 Å) and Ag+ (1.15 Å) in comparison to substituted Ca2+ (0.99 Å) ion. The element mapping reflected the uniform distribution of the Ca, P, Ag and Sr elements in the precipitated powders. The zeta-potential values of the Ag/Sr-co-substituted HAp samples revealed that all the samples were negatively charged and the obtained zeta-potential values expressed a downward trend with increasing buffer pH. The antibacterial effect was evaluated by inhibition zone and spread plate analysis, while the surface of samples was observed after inhibition zone analysis by scanning electron microscopy. The antibacterial effect was confirmed for all HAp powders substituted with Ag+ ions against Gram-positive S. aureus and Gram-negative E. coli, representative bacteria in clinical bone infections. Further, the non-cytotoxicity was confirmed by using human embryonic kidney 293 and human mesenchymal stem cells.

HYDROXYAPATITE-CHITOSAN 3D SCAFFOLDS MADE FROM NATURAL AND SUSTAINABLE SOURCES

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The use of natural and sustainable sources in the chemical processes is becoming an issue of increasing importance; in addition to this, often natural materials have features (i.e. structure or morphology) which could lead to enhanced performances. Within this frame, in this work we report about the preparation of hydroxyapatite-chitosan 3D scaffolds made from natural and sustainable sources.Hydroxyapatite (HA) is a calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$) widely used in biomedicine as bone substitute; in the present work HA was prepared from $CaCO_3$ obtained using cork as a template agent. Cork is the bark of the Quercus suber L., and it possess a porous honeycomb structure, which was maintained by $CaCO_3$. Chitosan (Cs), on the other hand, is a polysaccharide of natural origin, derived from chitin, which is extracted from the shells of crustaceous, i.e. by-product of the food industry. The 3D scaffolds were prepared with in situ HA formation in a Cs solution, which was successively crosslinked and freeze dried. Their morphology was assessed with Scanning Electron Microscopy (SEM) methodology, and a statistical comparison was performed between Cs and Cs-HA scaffolds (pore size, shape and distribution). Moreover, functional properties such as mechanical behavior, antibacterial activity and cytotoxicity were also assessed. Results showed that these 3D scaffolds have great potential for biomedical use, as biocompatible and antibacterial structures.

COLD SINTERING OF MUSSEL SHELLS-DERIVED HYDROXYAPATITE/BIOPOLYMER COMPOSITES FOR BONE TISSUE REGENERATION

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Nowadays, waste recovery and low energy-demanding production routes are of great interest in line with the circular economy philosophy. In this work, discarded mussel shells (MS) were exploited as a raw material to synthetize calcium phosphates at low temperature. MS have a great potential as a precursor for bioceramics due to the presence of substitutional ions such as Sr2+, Mg2+ and K+ which can promote cells' activity. As a calcium carbonate source, crushed shells were transformed into nano-crystalline hydroxyapatite (HAp) via dissolution-precipitation synthesis; shells were mixed with phosphoric acid, hydrochloric acid and sodium hydroxide solutions under controlled stirring speed, pH and temperature. The system was heated up to 40°C and mixed up to 6 h. The obtained suspension was then centrifuged and the white precipitate was washed with distilled water until all NaCl residues were removed. After that, the white slurry was immersed in liquid nitrogen for 10 min and lyophilized for 72h. As-synthesized HAp nano-powders were then consolidated by the cold sintering (CS). CS is an innovative technique that allows to densify ceramic materials at low temperature (< 350°C) under an external applied pressure and, usually, with the aid of a transient liquid phase. In the present work, CS was exploited to produce of HAp-based composites at near room temperature by applying up to 1 GPa pressure. Biopolymers such as chitosan and gelatin were added as filler (5-10 vol%) in the ceramic matrix to mimic bone composition having in mind the application of the composite scaffold for bone tissue regeneration. The as-synthesised powder and sintered composites were analysed by XRD, FT-IR, SEM, pycnometry, thermogravimetric and differential thermal analyses. Mechanical tests and preliminary in vitro assessment were also carried out in view of the biomedical applications.

HYDROTHERMAL SYNTHESIS TOWARDS A MORPHOLOGICAL AND STOICHIOMETRIC CONTROL OF HYDROXYAPATITE NANOPOWDER

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Hydroxyapatite is the main inorganic phase of the natural bone and it can be used as essential raw material in tissue engineering and regenerative medicine, in the production of bone substitutes owning biomimetic, osteoconductive and osteoinductive properties.The control of the nanocrystalline structure of hydroxyapatite, during its synthesis, is fundamental for the success in several applications, like the realization of scaffold by sintering, drug delivery system, bone and tooth fillers, adsorptive materials, prosthetics, in vitro implants, etc.This work aims to provide an overview for the realization of nanometric hydroxyapatite powder with the desired morphology of the crystals, through hydrothermal processes (synthesis in aqueous solution, at high pressure and temperature, in a closed system). Compared to other techniques, in this conditions hydroxyapatite precipitates from an overheated solution, with better control over the regulating of the speed and uniformity of nucleation, growth and maturation, which affect the particle size, the morphology and aggregation of crystals, the thermal stability and stoichiometry. It is discussed how some hydrothermal synthesis parameters (e.g. pH, temperature, starting precursors) are fundamental to control these characteristics. For example, based on the pH condition, crystalline growth along the hydroxyapatite c-axis leads to preferential growth up and different morphologies, from plate to nanorod crystals, obtained under neutral, acid or basic conditions. Furthermore, the control of growth direction of hydroxyapatite is necessary for simulate the crystallization of bone minerals within the collagen fibrils in bone tissue, according to a preferential orientation. SEM-EDS, XRD, ICP-OES and FTIR spectroscopy techniques have been used to characterize the particle size distribution, the growth direction, the chemical composition and the crystalline phases. In addition, the saturation index of reaction systems under different conditions is considered in order to explore the mechanism of formation of hydroxyapatite.

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POSTER SESSION

P01

DECIPHERING THE INTERACTIONS OF MAGNETIC IRON-DOPED HYDROXYAPATITE NANOPARTICLES WITH BONE CELLS

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Strong coupling between nanotechnology and cell/molecular biology leaded to a breakthrough in medicine due to the opportunities in designing a tailored approach in response to different disease. Magnetic nanoparticles (NPs) have attracted the attention of scientific community for drug or gene delivery, DNA/biomolecules separation, hypothermal treatment of tumours, contrast agents for imaging, tissue engineering and theranostic applications. Here biomimetic, biodegradable and cytocompatible NPs fabricated by doping hydroxyapatite (HA) with Fe ions (FeHA) were tested. FeHA NPs were prepared by a neutralization process using FeCl2 and FeCl3 as a source of Fe2+ and Fe3+ ions1; HA NPs and commercial fluidMag NPs (Chemicell) were used as control groups. Bone cells were cultured with 100 μg/ml NPs up to 72 hours. The molecular pathways of cellular response (apoptosis/necrosis, ROS production and autophagy) to NPs were investigated. The mechanism of internalization by Caveolae and Clathrin-mediated endocytosis were studied. In a pilot in vivo experiment the biodistribution of NPs was evaluated. The data clarifies the intracellular fate of the FeHA NPs and open prospective for their use as injectable and guidable nanoparticles to a desired body site by an external magnetic field and with the possibility to functionalized them with biomolecules/drugs for medical applications2.1.

P02

SURFACE CHARGE AND WETTABILITY DEPENDENCE OF THE STRUCTURE OF HYDROXYAPATITE COATINGS

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Hydroxyapatite (HAp) due to its compatibility with natural human bone and teeth has been broadly used as a bone biomaterial in orthopedics and dentistry. However, on its own HAp has poor mechanical properties therefore its use as a coating is more favorable for implantation at a load bearing sites. To further improve the properties of HAp coatings additional processing such as surface charge can be created with electrical polarization in electrical field [1]. Electrically charged HAp ceramics can improve cell growth both in vitro [2] and in vivo [3]. This research will evaluate how different structures of HAp coatings influences capacity of surface charge and wettability.In this research, 3 types of HAp coatings with different structures will be reported. Coatings were thermally sprayed using spherical HAp powder to form three structural states – amorphous and two types of crystalline (random crystal orientation and oriented crystal orientation) structures. X ray diffraction measurements showed the structural states within the coatings XRD (see Fig. 1A). Each coating was electrically polarized at 300 °C to create a positive or negative surface charge. Surface charge was measured using thermally stimulated depolarization currents (TSDC) and calculated from TSDC curves (see Fig. 1B). As a complementary method for measuring surface charge, contact angle measurements investigated improvement of surface wettability. Higher wettability of HAp surfaces has been linked to increasing osteoblastic adhesion [2].

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P03

FUNCTIONALIZED HYDROXYAPATITE DECORATED WITH TUNGSTEN OXIDE NANOPARTICLES

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In the last decades calcium phosphates-based materials have gained a prime spot in the bone tissue engineering.[1] In particular, Hydroxyapatite (HA) is widely studied for bone regeneration due to its high similarity to the inorganic phase of bones. Moreover, it is biocompatible, osteoconductive and osteoinductive.[2] Among the largest problems in orthopaedic surgery there are postoperative infections that often lead to severe pain and, in some cases, to more operations. These infections are largely caused by Gram-positive bacteria such as Staphylococcus aureus.[1] To avoid the use of antibiotics, and prevent the development of antibiotic resistance, many studies focus on the functionalization of HA with nanoparticles (NPs). Some of NPs prominent features are their capability to stimulate osteogenic and angiogenic activities, and their potential antimicrobial properties. Several metals and their oxides have been found suitable for this application such as silver, zinc, titanium and tungsten.[2][3] Despite the research into nanosized tungsten and its derivatives is scarce, some studies show a good antimicrobial activity against S.aureus.[3] This work focuses on the adsorption of WO3-NPs on three different substrates: i) HA; ii) HA functionalized with Polyacrylic acid; and iii) HA functionalized with Polyethylenimine. The W amounts in the final materials significantly vary between the two functionalized substrates while HA ranks among the two (Figure). The apatitic crystal phases and crystallinity are scarcely influenced by the treatment and the consequent presence of nanoparticles. Meanwhile, the presence of WO3 alters the zeta potential of the crystals. Furthermore the antibacterial properties of the WO3 NPs have been found as a function of their amount, even when they are associated to HA crystals. Data showed that the viability of a mammalian cell line (Vero cells) is slightly reduced only in the sample with the highest W content, although none of the samples showed cytotoxicity at the experimental conditions.[1] A.Ewald et al. / Acta Biomaterialia 2011, 7, 4064–407[2] Boanini E, et at. /European Journal of Pharmaceutics and Biopharmaceutics 2018, 127, 120[3] R.K. Matharu et al./ Nanomaterials 2020, 10, 1017

P04

THE EFFECT OF ION-DOPING ON THE RHEOLOGY OF HYDROXYAPATITE SUSPENSIONS FOR BONE REGENERATION

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Synthetic hydroxyapatite (HA) is widely considered as a reference material for bone regeneration, due to its high mimicry with natural bone inorganic matrix1. However, the fabrication of 3D apatite scaffolds with adequate porosity and mechanical strength for applications in load-bearing site is still a big challenge. The biological functions of the natural apatite include the ability to exchange bioactive ions with the physiological environment, contributing to modulate the bone cell metabolism. The processing of synthetic hydroxyapatite is affected by its chemical composition, since ion doping affects the crystal size and ordering, the powder particle size, as well its morphology and surface charge. These properties in turn impact on their rheological properties, relevant when processing ceramic powders into water-based slurries for the production of 3D scaffolds. In the present work, apatitic phases doped with bioactive ions such as Mg^{2+}, Sr^{2+}, Zn^{2+} were synthesized by wet methods and aqueous powder suspensions were prepared to be applied with direct foaming and 3D printing techniques. We compared the rheology of synthetic and ion-doped HA suspensions on the basis of different powder calcination temperature, powder and dispersant concentration, to identify the best conditions to achieve stable slurries. The stability of HA suspensions was assessed by pH and ζ-potential measurements, as well as viscosity and viscoelasticity tests. We found that, among the various investigated parameters, the calcination temperature strongly impacts on suspension stability, while dispersant showed a clear transition as the powder concentration increased.

P05

NOVEL MINERAL-ORGANIC BONE ADHESIVE COMPOSITIONS AND ADHESIVE STRENGTH EVALUATION THROUGH HYDROXYAPATITE CEMENT BASED BIOMIMETIC IN-VITRO TEST SYSTEM

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Introduction

Despite decades of research, no satisfying bone adhesive material has been developed yet.[1] Recent papers have shown that certain magnesium phosphate-based cement compositions and addition of phosphoserine (OPLS) might yield mineral-organic bone adhesives.[2,3] Extensive research on the matter requires reliable test setups to evaluate potential materials. Commonly, samples from animal bones are used for adhesive tests. Bone samples vary greatly depending on their origin and must be mechanically processed into test specimens at great expense, severely limiting the number of tests. A further limitation is the inability to perform long-term tests, as specimens decompose during storage. So far, there are no standards regarding processing and testing. Here, a completely synthetic biomimetic approach based on a calcium phosphate cement is used to perform a high-throughput pre-study with the possibility to evaluate long-term stability, before finalising results by testing on natural bone. Both approaches complement each other and allow effective bone adhesive research.

Methods

Hydroxyapatite samples were produced by mixing α-TCP with 2.5 % Na2HPO4-solution in a powder-to-liquid ratio of 3 g/mL and subsequent setting in milliQ-water for 7 days. Bone samples were cut from bovine femur corticalis. Cylinders (Ø5 mm) were glued to 20x10x5 mm plates and sheared off with a universal testing machine (Z010, Zwick Roell, USA) using a custom-made test setup (Fig. 1). Various new bone adhesives consisting of (OPLS) and magnesium compounds were tested.

Results & Discussion

Adhesive strengths of different mineral-organic adhesives were evaluated using a test system based on synthetic hydroxyapatite. Although no specific adhesive strength values for natural bone can be predicted, trends can be used find suitable candidates for further evaluation on bone (Fig. 2). This way, new adhesive compositions based on (amorphous) trimagnesium phosphate, magnesium oxide, phytic acid and OPLS were shown to provide adhesive strengths up to 10 MPa on bone.

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P06

SYNTHESIS, STRUCTURE AND DYNAMICS OF SELF-ASSEMBLED HYDROXYAPATITE NANORODS

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Hydroxyapatite (HA) is a calcium phosphate widely used in bone tissue engineering thanks to its biocompatibility and strong mimicry with the main inorganic component of human hard tissues (e.g. bone and teeth), in turn eliciting excellent osteogenic character that enables the development of biomaterials suitable for regenerative medicine, without using any growth factors. Relevant aspects determining the apatite bioactivity are related to multiple ion substitutions in the lattice structure and to specific crystal organization both affecting cell chemotaxis and bio-resorption behaviour. In the present work apatite nanocrystals were synthesized by different routes to investigate the effect of different ions doping such as $Mg^{2+,}$ CO_3^{2-}, Sr^{2+} and Zn^{2+} as well as the effect of specific crystal orientation, on the fate and metabolism of stem cells towards specific phenotypic differentiation. To this purpose, wet synthesis routes carried out at body temperature or under hydrothermal conditions were applied for the synthesis of apatite nanoparticles, particularly using organic molecules as templates driving the nucleation pathway and specific crystal orientation of the apatitic phase. With this latter process we could obtain self-assembled hydroxyapatite nanorods, of which we investigated the chemico-physical and biologic properties, in terms of cell adhesion, proliferation and specific phenotypic differentiation. The enhanced ability to trigger signalling mechanisms instructing cells to activate and sustain the natural physiologic metabolism is a key element to the design of novel biomaterials capable to regenerate biological tissues such as bone.

P07

EFFECT OF GALLIUM ON CALCIUM PHOSPHATE CERAMICS PHASE COMPOSITION

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INTRODUCTION

Calcium phosphates (CaPs) doped with different metal ions have potential as antibacterial material promoting bone regeneration. Gallium (Ga) has been studied since the 1970s as an effective treatment of bone disease. Therefore, Ga doped hydroxyapatite (HAp) could be used as an antibacterial agent. The aim of the study was to investigate the effect of Ga on HAp properties and phase composition after high-temperature treatment

METHODS

Ga-doped HAp was synthesized with a wet chemical precipitation method with a concentration of gallium 2wt%, 4wt%, 6.3wt% and 8wt%. CaO, H3PO4 and Ga(NO3)3*xH2O were used as raw materials. Synthesis was performed at 45 °C and the final pH was 6.95±0.05. The synthesized powders were characterized by X-ray Diffraction analysis (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and specific surface area (SSA) was measured with N2 adsorption system BET method. To reveal phase composition, the powders were heat-treated at temperature > 600 °C, and analysed by XRD and Rietveld method.

RESULTS

The XRD patterns of the obtained Ga-doped HAp have characteristic peaks of apatite. Decreased crystallinity and no additional phases were detected. The Ga-doped HAp compared to HAp has a higher SSA, indicating a decrease in particle size. The XRD patterns of the heat-treated powders showed the formation of biphasic CaPs with the composition of HAp and α-TCP (Fig.1). Moreover, the α-TCP phase ratio increased with increasing concentration of Ga. Fig.1 XRD patterns of the GaHAp heat-treated at 1100 °C

CONCLUSIONS

Gallium ions have an inhibitory effect on the formation of crystalline hydroxyapatite crystals of non heat-treated samples. The presence of gallium ions decreases the temperature of $\alpha \rightarrow \beta$ phase transition and the formation of α-TCP was detected.

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P08

SYNTHESIS OF DOXORUBICIN-FUNCTIONALIZED BIOMIMETIC-MAGNETOLIPOSOMES AS DRUG DELIVERY SYSTEMS IN VITRO AND IN VIVO

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Nanotechnology has become very attractive for its applications in different fields, comprising biology, medicine, and oncology, since cancers are still the second cause of morbidity and mortality in western countries, and thus require new therapeutic strategies. In this context, magnetoliposomes (LP-BMNPs), i.e, liposomes containing biomimetic magnetic nanoparticles (BMNPs) are particularly interesting since they can be functionalized with chemotherapeutic drugs as doxorubicin (DOXO) and can be manipulated by an external gradient magnetic field (GMF), besides being multifunctional highly biocompatible platforms. Moreover, when they are exposed to an alternating magnetic field (AMF), they can develop hyperthermia, mediating thermoablation of tumor cells, which are more sensitive than healthy cells to heat. Herein, BMNPs were synthetized in presence of the MamC protein from Magnetococcus marinus MC-1, functionalized with DOXO and then encapsulated in liposomes obtaining LP-(+/-DOXO)-BMNPs. Nanocomplexes were extensively characterized [transmission electron microscopy analysis (TEM), hydrodynamic radius, ζ-potential, Fourier-transform infrared (FTIR), colloidal stability and specific absorption rate (SAR)] and their effective functionalization with the different moieties was shown not to alter the structural-functional properties of the BMNPs. In vitro biological tests were then performed on 4T1 cells: biocompatibility was analysed by MTT assay and LP-BMNPs (+/-GMF) were found to be not toxic to cells while GMF enhanced the cell uptake of LP-BMNPs and the cytotoxicity of DOXO-BMNPs. LP-BMNPs resulted biocompatible when injected iv in BALB/c mice. The application of GMF on 4T1 cells-induced tumor after each of the sequential iv administration of LP-DOXO-BMNPs enhanced tumor growth inhibition when compared to other treatments. These promising results show the suitability of the LP-BMNPs as magnetic nanocarriers for local targeted chemotherapy and as possible future agents for hyperthermia and photothermia paving the way for the development of powerful approaches for cancer therapy suggesting a tumour multiple attack by different combined strategies.

P09

TELLURIUM-CONTAINING CHALCOGENIDE GLASSES FOR APPLICATIONS AS INFRA-RED PHOTODETECTORS IN BIOMEDICINE

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Chalcogenide materials have aroused great interest due to their ability to be repeatedly transformed between glass and crystalline states. Thus, they can find potential application in electronics, optoelectronic memory and neuromorphic computing. This provoked our interest in the study of the electrical properties and microstructural evolution of Te-containing chalcogenide bulk glasses. The glasses are prepared by applying traditional melting-quenching technique and main goal of the research was to determine whether these materials have potential for application in biomedicine.In this work we report on the investigation of the physicochemical and electrical properties as well as on the microstructure of the prepared materials. The microstructures of the as prepared and obtained after the electrical properties samples are imaged by using scanning electron microscopy. The electrical properties are investigated by impedance spectroscopy at different frequencies (from 10Hz to 500kHz) and temperatures (from room temperature (25°C) to 150°C and witness semiconducting behavior. From the slope of the obtained Arrhenius plots the thermal band gap energy is calculated.Keywords: electrical properties, chalcogenide glasses, infra-red detectors.

P10

PREPARATION OF THE ANTIBACTERIAL COMPOSITES BASED ON METAL/METAL OXIDE NANOPARTICLES AND CALCIUM PHOSPHATES

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Research of antibiotic alternatives is particulary important given the increasing number of nosocomial infections caused by antibiotic-resistant bacteria that may develop after implant surgery. Advances in the preparation of biocompatible implants with antibacterial properties are of special interest as they have a local effect and could potentially reduce the use of systemic antibiotics. In this regard, composites based on calcium phosphates (CaPs) and metal or metal oxide nanomaterials have immense potential, as CaPs are chemically and crystalline similar to bone mineral and metal/metal oxide nanoparticles generally do not promote bacterial resistance (1). In this study, silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles were selected for their antimicrobial properties (2). The novelty of this study is the preparation of new antibacterial composites by precipitating CaPs with the addition of AgNPs or ZnONPs at different concentrations, as an alternative to ion-substituted CaPs. Two types of AgNPs differing in capping agents were synthesised, namely Polyvinylpyrrolidone (PVPAgNP) and Sodium bis(2-ethylhexyl) sulphosuccinate (AOTAgNP) stabilised AgNPs (3). The ZnONPs were obtained commercially. The CaPs' precipitation progress in the presence of NPs was followed by monitoring the changes in pH. The precipitates were collected at predetermined period of time, filtered and characterised by scanning electron microscopy (SEM), powder X-ray diffraction (PXRD), and Fourier transform infrared spectroscopy (FTIR). Calcium-deficient hydroxyapatite (CaDHA) was identified in all precipitation systems, including one without NPs. From the SEM micrographs, it was evident that the capping agents had a significant effect on the morphology of the precipitates formed in the different systems. Therefore, it became clear that the formation of CaP composites with metallic nanoparticles should be comprehensively investigated, as different parameters may affect the process and properties of the formed materials differently. Additional clarification and determination of these parameters is of great importance for the further development of orthopaedic implants.

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P11

FORMULATION OF A LIPID-OLIGONUCLEOTIDE/CALCIUM PHOSPHATE CEMENT FOR A LOCAL RELEASE OF ACTIVES AGAINST BACTERIAL RESISTANCE

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For decades, calcium phosphate cements (CPC) have been used as bone substitutes due to several practical characteristics (biocompatibility, bioactivity toward bone cells, ease of shaping, possible injectability, etc)[1]. However, infection risks during bone surgery are high. Existing research demonstrates the enhancement of CPC bioactivity by combining it with different ions (Cu^{2+}, Ag^{+}…)[2] or antibiotics (gemtamicine, vancomicine…)[3,4]. Although the antibacterial effect of these functionalized CPC is proven[5], few studies explored the bacterial resistance which may be associated to such a local treatment, which is a significant global public health concern[6]. Developed to reduce the threat of antibiotic resistance, lipid oligonucleotides (LONs) effectively decrease the minimum inhibitory concentration of antibiotics, such as ceftriaxone (CFX), a commonly used third-generation cephalosporin[7]. Our research aims at formulating a new bioactive mineral cement (vaterite/brushite), including both CFX, to provide an antibacterial effect, and LONs for a local simultaneous release, preventing bacterial resistance to antibiotics. First, the self-organizing behavior of LONs in Ca^{2+}-rich solutions was studied using DLS. Large objects (~10000 nm) were observed, likely showing an interaction between LONs' phosphate backbone and Ca^{2+} ions.Next, physicochemical characterization of the cement was achieved upon the introduction of either CFX or LONs, before formulating CPC with both active agents, in order to study first CPC behavior with each molecule. The addition of CFX prolonged the cement setting time, whereas LONs had a shortening effect. During setting, a faster rate of transformation of vaterite/brushite into apatite was observed using FTIR-ATR measurements, supporting prior findings using other antibiotics[8]. Conversely, LONs seemed to delay this conversion, likely due to its interaction with the crystal surface, as demonstrated with DNA and calcium phosphate[9]. These interactions were highlighted by the absence of LONs release and induced higher friability of the LONs-loaded cement, while CFX was released (NanoDrop UV-Visible spectrophotometry) from CFX-loaded cement.Further experiments are in process for optimizing the cement formulation, enhancing both the release of the active agent and the presence of LONs. Assessment of bacterial resistance against resistant E.coli strains (Tck12 and Ec3536) using such LONs-CFX-loaded cement is currently under investigation.

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P12

SYNTHESIS AND CHARACTERIZATION OF OCTACALCIUM PHOSPHATE PREPARED BY CO-PRECIPITATION METHOD

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The current report aims to prepare octacalcium phosphate (OCP, Ca8H2(PO4)6.5H2O). The biological occurrence of OCP has been found in dentine and it is also known as the precursor of apatite crystals in bone and teeth. Moreover, OCP possesses superior osteoconductivity than hydroxyapatite and tricalcium phosphate [1]. The synthesis of OCP is found to be very challenging and time-consuming. A minor variation in synthesis conditions adversely affects the precipitation of OCP [2]. The current study is aimed to optimize the necessary reaction conditions for the synthesis of OCP. The reaction between calcium and phosphate salts resulted in the formation of OCP within four hours. The formation of low crystalline OCP (Fig. 1a) was noticed and the characteristic peaks matched the standard ICDD pattern. FT-IR revealed the presence of necessary functional groups associated with OCP and scanning electron microscopy (Fig. 1b) revealed broken plate-like surface morphology. It was found that the pH and temperature played a key role during OCP precipitation. Thus, the synthesis time for OCP was reduced from days to hours.

P13

EVALUATION OF B TRICALCIUM PHOSPHATE-BASED SCAFFOLD COATED WITH POLYHYDROXYNONANOATE FOR BONE TISSUE ENGINEERING APPLICATION

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Porous scaffolds are 3D-support materials for efficient cell and tissue growth. In the case of treatment of bone defects, the use of β tricalcium phosphate (βTCP) provides biocompatibility, osteoconductivity, and biodegradability. However, the brittleness and low mechanical strength of porous ceramic materials limit their range of applications. To overcome these disadvantages, a variety of polymeric coatings on bioceramic scaffolds have been proposed [1,2]. An interesting idea is covering βTCP-based scaffolds with biocompatible and biodegradable polyhydroxyalkanoates (PHAs). In our study we used elastic, medium chain length PHA - polyhydroxynonanoate (PHN).The βTCP scaffolds were obtained using the polyurethane foam replica method. Next, the ceramic specimens were infiltrated with PHN solution in chloroform and dried. The phase composition, microstructure, porosity, compressive strength, and bioactive potential of the prepared materials were evaluated.The obtained scaffolds had a network of connected spherical pores with sizes between about 100 - 800 μm. SEM observations confirmed a smooth and uniform layer of PHN on βTCP. The polymeric coating did not significantly influence the porosity of scaffolds (~70 vol%). The compressive strength of βTCP-PHN was higher (5.2 ± 0.7 MPa) in comparison to uncoated βTCP (3.7 ± 0.9 MPa). Crack-bridging of the polymer improved the mechanical stability of the scaffolds. Furthermore, the formation of an apatite-like layer on the composites immersed in simulated body fluid confirmed their bioactive potential. Thus, the obtained βTCP-PHN scaffolds might be a promising candidate for bone tissue engineering application. Further in vitro and in vivo studies are still necessary.

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P14

SILVER AND SILICON MODIFIED B TRICALCIUM PHOSPHATE AND POLY(3-HYDROXYBUTYRATE)-BASED SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Calcium phosphate (CaP)-based scaffolds are promising materials in bone tissue regeneration due to their biocompatibility, bioactivity, and similarity to inorganic part of natural bone. The incorporation of metallic ions into CaPs has been intensively studied due to the additional therapeutic or bioactive functions that these elements may provide. It is well-known that silver containing biomaterials offer antibacterial properties. Otherwise, silicon-modified CaPs may boost in vitro and in vivo bioactivity. Therefore, the aim of this study was to obtain and characterize silver and silicon modified β tricalcium phosphate (AgSi-βTCP) scaffolds, non-coated and coated with biocompatible poly(3-hydroxybutyrate). The degradation products of P(3HB) may act as the nourishing agents for bone tissue.AgSi-βTCP powder was synthesized via the wet precipitation method and AgNO3 and silicon tetraacetate were used as silver and silicon sources, respectively. The amount of silver was 1%wt. and silicon 0.3 wt.%. The polyurethane foams were impregnated in ceramic slurry, dried and sintered at 1150 °C. The obtained ceramic specimens were infiltrated with 5% P(3HB) chloroform solution, dried and subjected to further studies. The developed scaffolds were investigated by X-ray diffraction (XRD), X-ray fluorescence method (XRF), scanning electron microscopy (SEM), hydrostatic weighing, and compression tests (universal testing machine Instron). Initial AgSi-βTCP powder and bioceramic sinters consist of one crystalline phase - βTCP. The presence of silver and silicon in the materials was confirmed by XRF and SEM-EDS studies. The open porosity of the materials (~ 65 vol.%) was close to the total porosity (~70 vol.%). SEM observations also demonstrated that the scaffolds were covered with polymer P(3HB). AgSi-βTCP/P(3HB) composites possessed higher compressive strength (5.1 ± 1.7 MPa) compared to uncoated scaffolds (3.5 ± 0.8 MPa). Composite materials possessed also higher surgical maneuverability. Further in vitro studies are necessary.

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P15

INFLUENCE OF SILICON ON THE PROPERTIES OF Β TRICALCIUM PHOSPHATE-BASED BONE TISSUE ENGINEERING SCAFFOLDS

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Different types of calcium phosphates (CaPs): hydroxyapatite (HAp), tricalcium phosphate (TCP) or biphasic calcium phosphates (BCP) have been commonly applied in bone tissue engineering due to their resemblance to the mineral phase of bone. Currently, many efforts have been made to enhance the properties of CaPs by doping them with various ions[1,2]. Silicon plays a beneficial role as bioactive element which enhances in vitro and in vivo performance of bioceramics[3]. Therefore, the aim of this study was to obtain and characterize silicon modified β tricalcium phosphate (Si-βTCP) scaffolds for bone tissue regeneration. Si-βTCP powder was synthesized via the wet precipitation method and silicon tetraacetate was used as the silicon source. The amount of silicon was equal 0.3 wt.%. Scaffolds were prepared via the polyurethane foam replication method. Tree types of matrices with different pore sizes were impregnated with ceramic slurry, dried and sintered at 1150 °C. The developed scaffolds were investigated by X-ray diffraction (XRD), X-ray fluorescence method (XRF), scanning electron microscopy (SEM), hydrostatic weighing and compression tests (universal testing machine Instron). Highly porous silicon modified βTCP scaffolds with different pore sizes were successfully obtained. XRD analysis confirmed that the initial powder and the bioceramic scaffolds consist of one crystalline phase i.e. βTCP. The lattice parameter c of Si-βTCP slightly decreased from 37.3988 Å (βTCP) to 37.3969 Å (Si-βTCP), while parameter a slightly increased from 10.4346 Å (βTCP) to 10.4372 Å (Si-βTCP). The presence of silicon in the materials was confirmed by XRF and SEM-EDS studies. The obtained scaffolds possessed total porosity of around 70 vol% with the spherical pores ranging from 100 to 1000 μm depending on the type of polyurethane foam used. Scaffolds possessed compressive strength from 2.8 ± 0.6 to 3.2 ± 0.6 MPa. To assess the biological performance of the scaffolds, further in vitro studies are necessary.

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P16

PROCESSING AND IN VITRO BIOLOGICAL EVALUATION OF HYDROXYAPATITE SCAFFOLDS MIMICKING HUMAN TRABECULAR BONE ARCHITECTURE

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Hydroxyapatite (HA) ceramic scaffolds are commonly used as bone graft substitute. The osteoconductive properties of HA allow cell colonization and new bone formation within the interconnected porous network of the implant. Pore geometry as well as surface topography are known to influence cell behavior [1,2]. Design of scaffolds is a challenge to improve biological properties and extend the applications of HA ceramics in the field of bone tissue engineering. Various architectures, especially including those produced by additive manufacturing have been studied [3,4]. In this work we investigated the processing and in vitro properties of HA ceramic scaffolds mimicking human trabecular bone architecture. Samples of human tibial trabecular bone of 1 cm3 were collected (University Hospital Center Limoges) and scanned by X-Ray microtomography to generate 3D model database. From this computer aided design, HA ceramic scaffolds were shaped layer-by-layer (layer thinness = 100 ◎m) by additive manufacturing using laser stereolithography (SLA). Green ceramic parts were then sintered to obtain dense ceramic scaffolds. Shape and size of the resulting parts were compared to the model (wall thickness, size and geometry of the porous network) using image analysis in order to adjust the settings of SLA and produce reliable ceramic architectures mimicking the natural trabecular bone. Human mesenchymal stem cell (hMSC) and human umbilical vein endothelial cell (HUVEC) cultures were performed to investigate the biological properties of these scaffolds (cell attachment and proliferation, differentiation of hMSC, activation of HUVEC).Results show the efficiency of SLA to produce ceramic scaffold architectures mimicking that of the natural trabecular bone (figure 1) with promising biological behaviour.

P17

MODULAR OSCILLATORY FLOW REACTOR AS A NEW TECHNOLOGY FOR THE CONTINUOUS PRODUCTION OF CUSTOMIZED CALCIUM PHOSPHATES FOR TISSUE ENGINEERING

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Several technologies and synthesis routes have been implemented to produce calcium phosphates (CaPs) with distinct characteristics for tissue engineering (TE) [1–3]. However, produce CaPs in a controlled way still represents a challenge. Oscillatory flow reactors are a technology ready to deliver in terms of mixing intensification in multiphase systems. In particular, continuous processes improve control over the reaction conditions and can be implemented at an industrial scale [4]. The aim of the present work work was to assess for the first time the influence of the oscillation amplitude (x0: 4, 8 and 18 mm), frequency (f: 1.9, 4 and 6 Hz) and residence time (τ: 3.3, 6.6 min) on the final CaP physicochemical properties in a novel modular oscillatory flow plate reactor (MOFPR) (WO/2017/175207) (Fig.1). CaP synthesis was carried out by a simple precipitation route under near-physiological conditions of temperature (37ºC) and pH (8-6), described in previous work [5]. XRD, laser diffraction, SEM, FTIR and Ca/P ratio were performed to the different experimental conditions to obtain fully characterized CaP particles. For lower f (1.9 Hz), different x0 (4, 8 and 18 mm) lead to the production of distinct nano-hydroxyapatite (HAp) particles with a sharper, elongated or even rod-like morphology. At higher f (4 and 6 Hz), the x0 does not seem to have a significant influence on the particle's morphology. Plate-like and nano-HAp particles with traces of brushite were obtained at 4 Hz, while only HAp nanorods were identified at 6 Hz. τ affects the reaction yield but not the particle characteristics, which can result from the higher nucleation generated by more contact area between the reactant solutions at higher flow rates. Thus, the properties of the synthetized CaPs using specific mixing conditions provide a new window for the rational design of tailored CaP for TE (Fig 2).

P18

CALCIUM CARBONATE/HYDROXYAPATITE MICROPARTICLES AND OSTEOBLAST RESPONSES

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Introduction

Calcium carbonate (cc)-hydroxyapatite (HA) porous microparticles with a nano-structured lamellar surface have gained a lot of popularity as a promising material for clinical applications. The objective of this study is to evaluate the effects of cc-HA microparticles on osteoblast-like cells to be used as a bone-regeneration biomaterial.

Materials and Methods

The conditioned medium was prepared by the incubation of cc-HA particles in cell culture medium at various ratios at 37 °C. X-ray diffraction and Fourier transform-infrared spectroscopy were used for particles characterization before and after the immersion in cell culture medium. The concentration of Ca ions released from cc-HA particles was measured. Osteoblast-like cells were cultured in the conditioned medium of cc-HA. The cell responses were evaluated by cell morphology observation, proliferation test, and osteoblast differentiation test.

Results and Discussion

The characterization of cc-HA shows that the particles after the incubation in cell culture medium had more crystalline structure and that there are no differences in crystal phases. The osteoblast-like cells adhered and spread to flat shape on cell culture plates in conditioned medium with different concentrations of cc-HA. Cell proliferation was negatively affected by adding the cc-HA particles. The cell proliferation and differentiation were inhibited in the conditioned medium with higher amount of cc-HA but rescued with lower amount of cc-HA.

Conclusion

cc-HA microparticles adversely affect the cell proliferation, however, they could promote osteoblast differentiation especially with lower concentrations.

P19

CALCIUM PHOSPHATE NANOPARTICLES AS CARRIERS OF THERAPEUTIC PEPTIDES

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Calcium phosphate is a natural biomineral and the major inorganic constituent of bones and teeth. Therefore, it possesses excellent biocompatibility as well as biodegradability. Due to their unique physiochemical properties, calcium phosphate nanoparticles (CaP NPs) are extensively exploited in the biomedical field as carrier of several biomolecules, including peptides, proteins, and nucleic acids. In this regard, peptides are exceptionally selective and efficacious for the treatment of a broad range of diseases. Peptide-loaded CaP NPs are often prepared *via* biomineralization. The greatest advantage of mineralization routes relies on the encapsulation of the selected biomolecule. The biomineralized peptide generally provides nucleation sites, so that inorganic mineral phase can form around the peptide itself creating a protective shell. Being trapped inside the nanoparticles, the bioactive agent is both stabilized and protected from degradation in biological fluids.
Among various peptides for biomedical applications, antimicrobial and cardio specific peptides are particularly interesting since they represent a valuable alternative to conventional treatments. Moreover, they can contribute to overcome important clinical limitations including antibiotic resistance and non-specific biodistribution of traditional drug products. Within this context, we have investigated a new peptide-based therapeutic approach for the treatment of cystic fibrosis bacterial colonization and cardiovascular diseases, using colistin which is one of the most active antimicrobials against Gram-negative bacteria[1], and a therapeutic mimetic peptide that improves myocardial contraction and results in restoration of cardiac function[2], respectively. Peptide-loaded CaP NPs were prepared exploiting the biomineralization approach, using a mineralizing solution containing Ca^{2+}, Mg^{2+} and PO_4^{3-} ions. Several experimental conditions were tested, by varying the reaction time, temperature, and pH as well as the drug concentration. Colloidal stability, morphology, size as well as drug loading and release were evaluated to identify the best candidate to be tested *in vitro* and *in vivo*.

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P20

HYBRID NANOSTRUCTURED TI-DOPED HYDROXYAPATITE-BASED UV FILTER TO DEVELOP ECO-SUSTAINABLE SUNSCREENS

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The sunlight is essential for our well-being, it is responsible for regulating our internal clock, metabolism and for the production of vitamin D, essentials for healthy bones. However, excessive exposure to the sun can be dangerous, thus, protecting ourselves from solar radiation is of fundamental importance1 for our health, to avoid skin photo-aging and the onset of malignant tumors2. An ever-increasing attention to the protection of human health and marine environment3 has led, in recent years, to an increase in the regulatory aspects concerning this sector, which are becoming always more severe. With these evidences, this research would like to offer an innovation to this sector of cosmetic by designing innovative and eco-sustainable hybrid UV-physical filters obtained by a nature-inspired biomineralization process.In particular, titanium-doped-hydroxyapatite (TiHA) was nucleated on an organic matrix, i.e. alginate, to develop an efficient biomimetic UV physical filter, biocompatible, biodegradable and without photocatalytic effect. It was fully investigated by chemical-physical and morphological analysis, particularly the results obtained by UV-VIS spectroscopy have shown its excellent reflectance and absorption properties. Moreover, since titanium is present as Ti4+ ions, rather than titanium dioxide (TiO2), inside the HA crystal structure, any photocatalytic effect was revealed which it is important to avoid the photo-degradation of ingredients in sunscreen formulations and thus the formation of reactive species (e.g. ROS), which leads to greater skin tolerance with a reduced risk of sensitization. In addition, the presence of the organic matrix allowed an easy suspension in sunscreen formulation that from the in vitro (with fibroblast cells) and in vivo tests have shown an optimal cytocompatibility, good protection efficiency, activity as booster of organic filters and excellent safety. To conclude, by means of a nature inspired approach it was possible to develop an innovative safe and effective UV-filter useful to formulate eco-sustainable sunscreens and to avoid the use of traditional TiO2-based mineral filters. Since being mainly formed by hydroxyapatite, the main component of human bones, this material is biocompatible and fully-biodegradable in sea water, with the release of harmless ions, therefore, if after use its end in coastal waters, it is not responsible to damage marine ecosystems.

P21

BIORESORBABLE AND ANTIMICROBIAL REGENERATIVE PATCHES FOR WOUND DRESSING AND HEALING

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Given the critical economic and social issue that wound management represents, in the last decades the field of wound healing has gained great attention from the researcher worldwide. Especially chronic wounds seriously impact the health care system, due to their inability to proceed through the normal healing process, and their prevalence rate, that, in developed countries, is about 1-2% of the population1. With the growing interest in the development of biomaterials and the regenerative approaches, many forms of dressings (membranes, foams, and hydrogels) show great potential in wound management by promoting wound healing as well as skin regeneration. Many requirements are essential to obtain an effective device, such as biocompatibility, ability to maintain good O2 and water permeation thanks to its porous structure, and antimicrobial activity. The last, in particular, represent a very attractive challenge for the researcher, namely the possibility to exploit novel antibiotic agents, such as antimicrobial peptides (AMPs) or ion-doped hydroxyapatite (e.g. Cu2+, Mg2+, Zn2+, Fe2+/3+), released directly on the infection's site, thereby helping to reduce the antibiotic resistance and the topical application of antibiotics2-3.In this work we have designed and developed a multi-layer antimicrobial patch with optimized characteristics in terms of biocompatibility, bioresorbability and mimicry of the skin microenvironment thus to promote tissue regeneration. To this aim were used biopolymers such as chitosan and gelatine, widely exploited in wound healing4, glycerol as a plasticizer to contrast the brittle nature of the films, and tannic acid as cross-linker to control the chemical stability and degradation timing of the devices. Each layer was designed choosing different composition and combining different fabrication methods (namely solvent casting and freeze-drying) to obtain a tuneable device. The top layer is a skin-type layer with function of protecting the skin and avoiding bacterial infection, followed by a middle layer loaded with an AMP, aimed at the release of the antimicrobic agent, and a bottom, porous membrane with the function to absorb the wound fluid, help to modulate the AMP release, and provide both antimicrobial and regenerative ability thanks to the incorporation of ions-doped hydroxyapatite.

P22

CONTROLLED RELEASE OF TETRACYCLINE FROM INJECTABLE APATITIC BONE CEMENTS

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Self-hardening and injectable calcium phosphate cements (CPCs) represent valuable biomaterials for bone regeneration applications [1]. Their preparation involves the mixing of solid powder and liquid components, which then self-harden in vivo and transform into nanocrystalline apatitic phases. However, bone infections still represent a major challenge to face during the implantation surgery, especially due to worldwide bacterial resistance to antibiotics. If a bone infection occurs, the clinical protocols mostly include oral administration of antibiotics, which however is characterized by limited bioavailability of the drug, provoking the occurrence of systemic adverse side effects [2]. In this scenario, the development of biomaterials with regenerative ability and effective antibacterial and antibiofilm properties is particularly demanding. The present work describes a novel approach to prepare Sr-doped apatitic CPC enriched with hydroxyapatite nanoparticles (HA-NPs) as carriers of tetracycline (TC), intended as broad-spectrum antibiotic, but also chemically close to anthracyclines, a wide group of first-line antitumor drugs. The drug-loaded CPCs were obtained by mixing proper amounts of TC-loaded nanoparticles (TC-NPs) to Sr-doped α-tricalcium phosphate powders and aqueous solutions enriched with sodium alginate. The injectability, setting times and drug release profile of these final pastes were optimized according to the clinical need. The antibacterial and antibiofilm effectiveness of native and TC-loaded cements was evaluated by microbiological tests assessing the reduced viability of Staphylococcus aureus and Escherichia coli, among the most diffuse bacterial strains in nosocomial infections, proving significant bacteriostatic, bactericidal and anti-biofilm properties in the native cement and complete eradication of bacterial cultures in the TC-loaded device. The presence of TC-loaded apatites allowed to prolong the drug release profile in physiological conditions, if compared with the formulation without NPs. These results are relevant in the view of design and development of new injectable CPC formulations more effective to improve the patient safety and clinical outcomes, thus aiding to contrast the steady increase of bacterial resistance to antibiotics.

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P23

FACILE SYNTHESIS OF SINGLE PHASE WHITLOCKITE; COULD BE A BEST SUBSTITUTE FOR OTHER CALCIUM PHOSPHATES

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Bone whitlockite (WH) exists in the collagen matrix along with hydroxyapatite (HA) and plays a vital role during earlier stages of bone development. It is present in short-range order and is difficult to identify in the bone, as compared to HA mineral, which covers 80% of the bone inorganic phase. It has the same structural analogy as β-TCP, but detailed structural and crystallographic analyses of bone have shown that β-tricalcium phosphate (β-TCP) is merely a synthetic analog of bone whitlockite. WH contains magnesium at Ca (IV), Ca(V) positions, and HPO42− on a threefold axis in a rhombohedral crystal lattice. Its biocompatibility, functionality, negative surface charge, mechanical strength, and stability in physiological solvents make it an ideal bone substitute for other CaPs. It has magnesium as a major component that has a strong affinity with integrin protein. Integrin protein plays a vital role in bone tissue integration. It is bioresorbable and biodegradable and the rate of degradation complements with regeneration. Although precipitation of the WH phase has been reported recently in many publications, the effect of various reaction parameters like pH, heating conditions, heating time, aging, and feed rate on WH phase formation and stability have not been investigated in detail. This investigation is extremely important for the bulk preparation of this important bio-mineral. In this work, we report the synthesis of single-phase WH using different facile methods. The effect of different reaction parameters and the presence of therapeutic metals, in the reaction system, on WH phase purity is also studied in detail. Lastly, a biological evaluation of WH for bone tissue regeneration and drug delivery application is done. The physicochemical and biological characterization results suggest this material could be an ideal substitute for other CaPs.

P24

NOVEL EXTRACTION ROUTES OF COLLAGEN AND HYDROXYAPATITE FROM FISHBONES, AND THEIR APPLICATIONS IN COSMETIC

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During the last 30 years, new green and sustainable chemicals capable to replace dangerous or brown ones have been researched worldwide. This trend was intense in cosmetic, where the need for natural and biologically safe materials is continuously raising. In the plethora of alternative materials, calcium phosphates (CaPs) have gained high interest due to their wide range of applications and to their intrinsic bio and eco-compatibility. For cosmetic applications, researchers focused their efforts on the recovery of CaPs from food by products, particularly from fish bones which is an excellent source of highly pure hydroxyapatite [Ca10(PO4)6(OH)2 – HAp]. However, most of the approaches employed consist in the thermal extraction of CaPs, during which all the organic matter of the fish bone is removed and the inorganic material is calcined.In this work, novel approaches to the extraction of CaPs from fishbones based on the use of enzymes and ionic liquids have been used with a double aim: (i) to preserve the bioactivity of biological HAp, and (ii) to recover both the inorganic and the organic components (e.g., collagen) of fishbones. Several parameters of the extraction processes, such as extraction time, temperature, sonication and reactant concentrations, have been screened in the attempt to optimize the extraction yield and the purity of the final products (i.e., collagen and HAp). The recovered materials – both inorganic and organic – have been characterized from the physical-chemical point of view. Finally, the extracted HAp has been used in cosmetic formulations and its performance as SPF booster has been tested in vitro. Preliminary results showed that HAp from fishbones can be used as effective SPF boosters in sunscreen formulation, where their performance may significantly differ from a sunscreen to another depending on their composition, and especially on the kind UV-filters and their concentrations in the formula.

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MACROPOROUS HYDROXYAPATITE SCAFFOLDS MODIFIED FOR SUSTAINED DRUG DELIVERY

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The regeneration of critical size bone defects caused by trauma or metabolic diseases is still a strong clinical challenge. The physiological process of bone regeneration has to be supported by a bioactive 3D scaffold, able to direct and sustain the physiological cell metabolism [1]. Particularly relevant is the need to develop bone scaffolds associating drug delivery ability, in order to favour and sustain tissue regeneration also in the presence of infections or impaired physiological conditions such as tumours [2]. This achievement would be very relevant also to prevent the systemic administration of drugs, often associated with adverse side effects, and to improve the drug effectiveness by tuned in situ delivery [3]. In this respect, a major challenge is to obtain a bioactive scaffold capable to deliver drugs with controlled release profiles.In this work, macroporous apatitic scaffolds were produced by direct foaming process with controlled drug delivery ability. Direct foaming is a template-free technique allowing the preparation of macroporous bioceramic scaffolds by incorporation of air bubbles into the ceramic suspension, followed by drying and sintering [4]. As model drug, we selected tetracycline, a broad-spectrum antibiotic frequently used in the treatment of local infection and chemically close to anthracyclines, a group of drugs commonly used as chemotherapeutic treatment of osteosarcoma. Then, sodium alginate was selected as natural biopolymer for smart coating of the porous apatite scaffolds, to obtain ability to modulate and control the drug release kinetics. Drug loading was firstly performed by drop addition on the scaffold, followed by multiple layers of cross-linked sodium alginate (alginate/CaCl2). The drug release kinetics and the mechanism governing drug release in such a layered construct were investigated. The results obtained are relevant for the design and development of new devices with local drug delivery and bone regeneration capabilities.

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P26

IN VIVO EVALUATION OF CALCIUM-PHOSPHATE CERAMICS WITH HIGHLY-INTERCONNECTED PORES USING PORCINE TIBIA DEFECT MODEL

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Calcium-phosphate ceramics have been widely used in orthopedic and oral surgery to generate new bone in defect site. Various products are commercially available and difference in porous structure are the key differentiator among the products beside composition. Porous structure of the material should allow sufficient circulation of body fluid and have enough interconnection size for the cells to go through. Our group have developed porous calcium-phosphate ceramics with highly-interconnected structure by sintering calcium-phosphate fibers. In this study, we aimed to evaluate the bone formation after implantation to porcine tibia defect model and compared the difference between hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) ceramics with similar porous structure.Porous HAp and β-TCP ceramics (total porosity: ~70%) were fabricated by firing calcium-phosphate fiber mixed with carbon beads (~150 μm) to form macropores. Interconnection size was measured by mercury intrusion technique. Each sample (ϕ4 mm × 8 mm) was implanted to tibial bone defect (ϕ4.2 mm × 8 mm) of pig (2 years old, male). After 6 weeks of implantation, undecalcified section was prepared and stained with hematoxylin and eosin (HE) stain. New bone area and remaining material area was measured by using Image J (NIH).Interconnection size of HAp and β-TCP ceramics were 23.0 μm and 15.6 μm respectively. New bone area after 6 weeks was 57.2 ± 0.8% for β-TCP ceramics and 47.6 ± 3.3% for HAp ceramics. Remaining material area was 77.1 ± 3.3% for β-TCP ceramics where HAp ceramics remained stable. From HE stained section, bone formation in HAp ceramics were inhomogeneous compared to β-TCP ceramics. Although the interconnection size was smaller for β-TCP ceramics, higher resorption may have played a positive role in homogeneous bone formation. From these results, we suggest that highly-interconnected ceramics made by β-TCP will be an ideal material to generate new bone.

P27

MAGNETIC BIOACTIVE GLASS-BASED 3D SYSTEMS FOR BONE CANCER THERAPY AND REGENERATION

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Several diseases, such as osteoporosis and bone cancer, are causing an increasing need for advanced bone repair materials. Bone is the second most replaced organ in the body after blood. Approximately 2.2 million bone graft procedures are performed worldwide each year at an estimated cost of $2.5 billion [1]. Malignant bone tumors are one of the main non-trauma factors resulting in critical size bone loss/defects. The treatment of such bone defects is still a considerable challenge, and it is currently recognized that the increasing request for bone substitute materials cannot be tackled solely by autogenous or allogenic bone grafts [2]. On the other hand, surgical resection often fails to completely remove the tumor, which is the main cause of postoperative recurrence and metastasis.Magnetic hyperthermia using superparamagnetic iron oxide nanoparticles (SPIONs) has emerged as a potential cancer treatment option since it is considered an effective treatment without adverse side effects [3]. On the other hand, mesoporous bioactive glasses (MBGs) dissolution products have demonstrated their effect on osteoblast cell gene expression and the potential effect on angiogenesis and neovascularization [4]. We therefore propose a new concept for bone cancer treatment and subsequent bone re-generation: an electrospun polymeric scaffold containing MBG that combines magnetic hyperthermia therapy through the incorporation of the SPIONs into the scaffold and local drug delivery The experimental work to produce the multifunctional scaffolds is divided into 3 parts: 1) production and characterization of SPIONs and MBGs individually; 2) Incorporation of these individual materials into an electrospun polyvinylpyrrolidone matrix; 3) Characterization and selection of such composites based on its bioactivity, biodegradability, biocompatibility, heating ability, and drug encapsulation/releasing profile. The composites have been produced in stages, to assess whether the materials keep their individual properties after the final blending. The composites are currently under optimization in terms of drug encapsulation and drug releasing profile.

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P28

Fe and Sr co-doped beta-tricalcium phosphate (β-TCP): Synthesis, structural analysis, magnetic and biological properties

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Beta-tricalcium phosphate (β-TCP) has been applied in the fields of medical science owing to its osteoconductive properties which stimulate the growth of new bones. Iron (Fe) follows the same physiological pathway as calcium and thus could be used for improving the cell adhesion in β-TCP in bone tissues. Strontium (Sr) can potentially play a role in active bone resorption performance. Therefore, the present study aimed to simultaneously co-doped Fe/Sr in β-TCP for various potential biomedical applications. The Fe/Sr co-doped β-TCP powder was synthesized by the aqueous co-precipitation method. Co-doping level in the range from 0.2 to 1 mol% has been studied to investigate the effect of each doping element and co-doping comparatively. The obtained powders were characterized by XRD, SEM, FTIR, VSM, and XPS. The results showed that Fe ions tended to enter the β-TCP structure by substituting calcium at the Ca (5) and Ca (4) sites, on the other hand, Sr ions entered at Ca (4), Ca (1), Ca (2), and Ca (3) in order. Furthermore, Fe/Sr co-doping could effectively promote the cell adhesion of dental pulp stem cells (DPSCs) on the surface of β-TCP.

P29

SELECTIVE LASER MELTING-SINTERING TECHNOLOGY: FROM DENTAL CO-CR ALLOYS TO DENTAL CERAMIC MATERIALS

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The general term of CAD/CAM technology (i.e., Computer-Aided Design / Computer-Aided Manufacturing) comprises several aspects, such as subtractive manufacturing processes, like milling (soft and hard milling), and additive manufacturing processes, like Selective Laser Melting, (SLM), which refers to metallic materials, or Selective Laser Sintering (SLS), which refers to glasses/glass-ceramics/ceramic, or polymeric, or related composite materials produced via powder metallurgy technique. The first step in the SLM or SLS technology is the digital design of the prosthetic restoration, whereby the patient's individual anatomical and morphological features are precisely described. The laser melting or sintering process is repeated (layer by layer) until the complete restoration item is fabricated. The SLM or SLS technique produces a wide range of dental materials, e.g., metals and alloys, thermoplastic polymers, glasses/ceramics, waxes, and thermoplastic composites. Thus, it is a promising technique for producing a variety of dental restorations, such as metal-ceramic restorations, all-ceramic restorations, maxilla-facial prostheses, functional skeletons, individual scaffolds for tissue engineering etc. Following a successful use of SLM technology in the fabrication of metal objects (such as Co-Cr alloy) for dental and orthopedic prosthetics, the interest of researchers, in the last five years, has been shifted to SLS of ceramic powders (such as SiO_2, Al_2O_3, SiO_2/Al_2O_3, ZrO_2/Y_2O_3). In this comprehensive review of the process of the SLS of glasses/glass-ceramic/ceramics materials, the emphasis focuses on biomedical/dental applications. More specifically, we present new experimental results which clearly show that this very modern additive manufacturing technique does not jeopardize the properties of the produced biomaterials at all [1-3].

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P30

CALCIUM PHOSPHATE CEMENTS AS TUNEABLE SYSTEM FOR DOXORUBICIN DELIVERY TO TREAT BONE CANCER

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Self-hardening calcium phosphates (CPCs) are considered as relevant materials for the treatment of critical bone defects requiring the use of osteogenic and osteointegrative scaffolds. In addition, bone scaffolds with controlled and sustained ability to release therapeutic agents are increasingly desired, in order to favour the bone regeneration process also when impairing diseases such as bone tumours occur [1]. Nevertheless, the achievement of therapeutically-relevant release profiles has been still a challenge.The present work shows a new approach to modulate drug release from Sr-doped CPCs, based on the addition of drug-loaded hydroxyapatite nanoparticles (HANPs). HANPs were obtained by wet chemistry process at body temperature and characterized, followed by the surface adsorption of doxorubicin (DOX), a drug commonly used in cancer chemotherapy [2,3]. Then, the as-obtained doxorubicin-loaded nanoparticles (DOX/HANPs) were mixed with Sr-doped α-tricalcium phosphate and aqueous solutions enriched with natural polymers, in proper amounts to obtain calcium phosphate bone cements with setting times and drug release profile matching clinical demands [1].The DOX release profiles from DOX/HANPs, CPC-DOX and CPC-DOX/HANPs were investigated. The addition of nanoparticles was effective in prolonging the DOX release over time. The data describing the drug release profile were further fitted with semi-empirical models, to elucidate the drug release mechanisms, suitable for the design of tuneable release systems. The biological performance of the CPC-DOX/HANPs was assessed with triple negative breast cancer (TNBC) cell line MDA-MB-231 by MTT test, exhibiting a significant decrease in the TNBC cell line proliferation rate cultured on DOX-HANPs loaded cements.The obtained results demonstrate the potential of drug loaded apatite nanoparticles as functionalizing media able to confer to nanostructured apatitic bone cements the ability of tuneable drug delivery systems, promising for more effective local treatment of bone cancers.

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DEVELOPMENT OF CORE-SHELL CALCIUM PHOSPHATE @ IRON OXIDE NANOPARTICLES FOR STIMULATED PRODUCTION OF EXOSOMES FROM STEM CELLS

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Exosomes represent a new and promising drug delivery system for medical application, as are biocompatible, have excellent tissue penetration capacity, and can deliver a plethora of therapeutic biomolecules (nucleic acids, peptides, proteins) [1]. Exosomes are natural lipidic nanoparticles secreted by cells for delivering molecules in the organism, and for this reason there is a high interest in their mass production in bioreactor. However, their harvesting from cultured cells is complex and has a very limited yield. The aim of the present work was to prepare calcium phosphate nanoparticles (CaP NPs) for the stimulated production of exosomes by stem cells. Indeed CaP NPs can easily be internalized by stem cells, and their dissolution inside the lysosomes generates an increase of calcium concentration in cell cytosol, which in turn stimulates exosomes production [2]. We also prepared core-shell nanoparticles possessing a CaP shell surrounding an iron oxide magnetite core (CaP@Mag NPs) to impart magnetic susceptibility. In this way an external magnetic field can be used to favor CaP@Mag internalization in stem cells and maximize exosome production. In addition, the use of magnetic nanoparticles and of the external field also simplifies exosome extraction and purification. Several reaction conditions were tested to produce CaP@Mag with different CaP shell thickness as well as different stoichiometry, and thus different calcium content. Afterward, the most promising CaP@Mag NPs and CaP NPs were administered to mouse primary adipogenic stem cells to evaluate biocompatibility, cell uptake, and exosomes production also in presence of an external magnetic field. Furthermore, the strengthened cells will be applied on the self-designed bioreactor system for exosome production.

Acknowledgments

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P32

BIOCERAMIC FILLER FOR SMART ANTIBACTERIAL BONE CEMENT

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Introduction

PMMA bone types of cement are mainly used to fix implanted prostheses [1,2]. The problem of the infected prosthesis could be solved due to the development of some new antibacterial bone cement [3,5].

Experimental

To determine the antimicrobial activity of the bone cement without negatively altering its mechanical performance, we modified the solid component of commercial cement based on PMMA, by combining it with antimicrobial additives like hydroxyapatite and peppermint oil, gentamicin, and silver nanoparticles incorporated in a ceramic glass matrix. The structure and morphology of the new bone types of cement reinforced with bioceramic filler were investigated by SEM and EDS. The cytotoxicity was evaluated by MTT assay using the human MG-63 cell line, while the antimicrobial properties were checked against standard strains Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans.

Results and Discussion

The addition of antimicrobial agents did not induce major structural changes in the new bone cement samples. All experimental specimens possessed a typical structure and morphology for PMMA bone cement, with a slight tendency to form agglomerates due to the use of bioceramic filler. Wettability measured by contact angle decreased by adding the antimicrobial agents. All additives used kept the contact angle values within the desired limits and showed good cell adhesion. The addition of antimicrobial agents did not significantly affect the hydration and degradation degree. The antimicrobial properties have been demonstrated for samples with hydroxyapatite and peppermint oil and silver nanoparticles. All cement types are biocompatible without any cytotoxicity effect.

Conclusions

It was observed that not only the type of antimicrobial agent is important but also the amount of bioceramic filler used. The antibacterial performance of samples with hydroxyapatite and peppermint oil and silver nanoparticles suggests that these antibacterial additives look promising to be used in clinical practice against bacterial infection.

P33

3D PRINTING OF A DOPED HAP/ Β-TCP MIXTURE FOR BONE TISSUE ENGINEERING

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3D printing is becoming an important field for shaping in the health sector through the development of various technologies and materials for different applications. It has already been used for over fifteen years for bone engineering [1] and, more recently, for soft tissue engineering, anatomic models and surgical guides. It allows to produce parts directly from medical imaging such as scanners, so it is a convenient way to personalize through shape and structure, which is an important parameter [2], and/or to assist during surgery [3]. However, the lack of diversity in the choice of materials, compared to traditional methods of bone filling, knowledge and costs are currently limiting its use in practice even if cosmetic results are better. To meet this need for material diversity, we are working on the development of photo-crosslinkable bioceramics pastes for 3D printing using a mixture of hydroxyapatite/beta tricalcium phosphate (HAP/β-TCP) Cu-doped synthesized by brushite's hydrolysis. This doping element has been chosen according to the understanding of their insertion/substitution behaviour [4] and their promising results [5] in angiogenic, antibacterial and osteogenic activities. Our mixture is composed of approximately 75/25 with x ⊚ x' ⊚ 0.05 (obtained by Rietveld refinement) : Ca10Cux(PO4)6(OH)2-2xO2x + Ca3-x'Cux'(PO4)2. The main idea of our work, is to study the influence of structure, according to exchange area, produced by 3D printing on the elemental release in the environment, by MP-AES, and the effects on the scaffold himself through a follow-up of SEM observations, mechanical properties and XRD after various periods in Simulated Body Fluid (SBF) and water. For it, we have printed various structures triply-periodic minimal surfaces like gyroid and some other geometrically computed microstructures which are interesting mechanically (similar to bone) and cellular-friendly. Figure 1: Picture of simple framed sample as printed with cross pattern (cylinder of 0.4 mm in diameter following the 4 diagonals of cube).

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POROUS CALCIUM PHOSPHATE BIOCERAMICS MANUFACTURED USING TEXTILE-BASED PROCESSES

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Introduction

In addition to the choice of material, the pore structure of the bone replacement material is of crucial importance for a high osteoconductivity and osteoinductivity. In this work, a new, simple method for the production of calcium phosphate (CaP) ceramics with anisotropic pore structure is presented, which can support blood vessel ingrowth and new bone formation.

Materials and Methods

The production of the new bone replacement material is based on cellulose-calcium phosphate hybrid hollow fibers. The CaP cellulose fibers (modified Alceru® process) were arranged in parallel, coated with CaP suspensions (Fig. 1) or infiltrated as a bundle. Bundling was done using the Kemafil® process. In a subsequent sintering process >1000 °C, the cellulose components were pyrolyzed and the anisotropically structured porous bioceramics were formed. The materials were characterised using XRD, SEM, porosity and mechanical measurements, and in vitro biological tests.

Results

Cell biological studies (LDH, ALP, MTT, angiogenesis assay) have shown that bone-forming cells easily settle on the substrates. A normal growth rate was established within 14 days of incubation, with cells also being able to grow into the long pore channels. With a porosity between 30 and 70 %, depending on the hollow fiber dimensions, the degree of compaction, the type of calcium phosphate and the sintering conditions, the compressive strength of the materials was up to 25 MPa.

Conclusion

The presented results show the high biocompatibility of the materials. In the future work, their biological properties will be investigated under conditions closer to application. Due to their structure, strength and high macro- to nano-porosity, the materials are particularly suitable for bridging larger bone defects in the area of compact bone and as interesting candidates for loading with bioactive molecules or ions.

Acknowledgements

We want to thank the German Federal Ministry for Economic Affairs and Energy (BMWi) and the German Federation of Industrial Research Associations (AiF) for funding within the IGF programme (project No. 20610 BR).Fig. 1: Embedded fibers in CaP matrix, unsintered, cross sectionFig. 2: Bioceramic colonized with human preosteoblasts, evaluation by fluorescence microscopy after 14 days.

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AMELODENTINARY COMPLEX: BIOMINERALIZATION IN ZNHA SIMULATING SOLUTION

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Enamel and dentin are the main teeth's hard tissues. Enamel is composed by 97% ceramic materials, mainly hydroxyapatite, and enamel proteins. Dentin is composed of 70% ceramic materials, collagen fibers and non-collagen proteins.1 This tissue shows lower hardness and higher fracture-toughness than enamel. These tissues have similar structures and anisotropic properties.2 Otherwise, several methods have been tested to achieve regeneration of these tissues, including biomimetic procedures. There is two routes to rebuild this tissue: the classical route that focuses on reconstruction the tissue from the constituent elements by apposition of the tissue itself as in bone grafts3 and the non-classical biomineralization pathway then emerged, emphasizing growth with pre-mineralizing elements using amorphous calcium phosphate groups as precursors. This route also considers the use of biomimetic analogs.3 This work advocates joining the two biomineralization pathways in order to promote regeneration in dental tissues. Pure hydroxyapatite (HA) and partially zinc substituted hydroxyapatite (ZnHA) pellets were considered as the dental element representative. The HA and ZnHA were synthesized by an aqueous precipitation route and pressed into pellet and then sintered at 1100ºC.4 Then a 0.4mM solution of hydroxyapatite-binding peptide (HBP) and HA powder was prepared at room temperature and applied to the samples (incubated overnight).5 In this same vehicle, a mineralizing solution of McCoy was prepared and applied again on the samples. In vitro tests have also been done with cells. The samples were characterized by SEM/EDS, XRD, FTIR. Two subgroups (negative control and HBP peptide application) were established for HA and ZnHA. The samples from each group (n=8) were incubated for the period of time: 1d, 7d and 14d. The in vitro results with cells and with McCoy's solution, proved to be consistent.

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3D BIOCERAMIC SCAFFOLDS AS CANCER-SPECIFIC INSTRUCTIVE MICROENVIRONMENT

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Worldwide cancer remains the second-most common cause of death; among bone cancers, Osteosarcoma (OS) is the most common type diagnosed in children and young adults1. The lack of specificity for the Cancer Stem Cells (CSCs) subpopulation1 and the poor in vitro-in vivo translation ability affecting traditional two-dimensional (2D) in vitro models are the main factors responsible for therapy failure2. Here two different hydroxyapatite-based scaffolds3,4 were used as instructive microenvironment to engineer three-dimensional (3D) in vitro models of osteosarcoma to improve the predictivity of preclinical studies. A ceramic scaffold4 and a hybrid biomineralized scaffold3 designed and synthesized at ISTEC-CNR were used for this study. MG63 and SAOS-2 osteosarcoma cell lines were cultured as parental cells and spheroids obtained by a well-established CSC-enrichment sphere-forming culture5,6 to obtain 3D scaffold-based in vitro model. The variability of tumoral properties in 2D serial spheroids passaging7,8 is still being investigated in term of proliferation, sphere-forming efficiency, migration and gene and protein expression. Overall, the data obtained confirmed the enrichment of a stem cell population with a strong spheroidal phenotype and the higher expression of stemness and CSC-niche markers by 3D scaffold-based model compared to 2D conditions. These results lay the basis to use the proposed scaffolds as eligible 3D microenvironments for the in vitro engineering of more complex models of tumour.

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EVALUATION OF OSTEO-CONDUCTIVE/-INDUCTIVE PROPERTIES OF A 3D-PRINTED POLYLACTIC ACID SCAFFOLD FOR BONE TISSUE ENGINEERING

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The severe bone defects due to trauma, congenital anomalies, illnesses such as cancer, or aging have always been one of the important challenges in clinical practice, and created a huge demand for more efficacy repair treatments. Bone tissue engineering (BTE) is the most used approach when predominate several pathological conditions or greater areas of damage. BTE harnesses the regenerative capability of several combinations of cells, growth factors and biomimetic materials (scaffolds and/or hydrogels) to promote bone tissue restoration. In the last years, several types of 3D-printed biomaterials have been studied to the aim to improve mechanical, structural and biological properties. Among these polylactic acid (PLA) scaffolds have been considered the most promising biomaterials due to their good processability, biocompatibility, and mechanical properties. Further, it has been demonstrated that PLA is one of the most common biomaterials approved by the Food and Drug Administration (FDA) for biomedical application. In this work, the bone tissue regeneration capability of 3D-printed PLA scaffolds was evaluated, in terms of osteo-conductivity/-inductivity. In this study, 3D PLA scaffolds (size 12x12x2 mm, strand diameter 0.4 mm and gap distance 0.4 mm) were obtained by fused deposition modelling (FDM) and their osteo-conductive/-inductive properties were analysed by culturing human fetal osteoblastic cells (hFOB 1.19) on them. To evaluate the scaffold capability to promote cell adhesion, growth and proliferation we performed MTS assay and DAPI staining; to analyse differentiation and mineralization we performed qRT-PCR by specific osteogenic markers and Alizarin red S assay. Our results suggested that 3D-printed PLA scaffold were able to stimulate cell proliferation and differentiation over time, *in vitro*. Further, we observed that cells are able to distribute along PLA scaffold fibers and to form a fibrous matrix between the gap. The results obtained, although preliminary, show that the 3D-printed PLA scaffold could be used for the design of effective biomaterials for bone regeneration. Further in *vivo* studies will be performed to confirm regenerative capabilities of these biomaterials on bone large defect in animal models.

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DETERMINING THE OPTIMUM DRYING/SINTERING ROUTES FOR NEAR-NET-SHAPE DIW PRINTED ALUMINA TOUGHENED ZIRCONIA PARTS WITH μCT CHARACTERIZATION

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Recently, there is a clear trend towards metal-free dental implants due to concerns with respect to biocompatibility and increasing aesthetic standards. Alumina toughened zirconia is a preferred ceramic for long-term dental implant applications. There are different production methods for dental implants, but additive manufacturing (AM) allows mass customization with minimum material waste.Direct Ink Writing (DIW), also known as robocasting, is an additive manufacturing (AM) technique that produces high-density parts with high precision by extruding a high solid loading viscoelastic paste through a narrow nozzle. Parts are built in a layer-by-layer fashion while the movement of the nozzle is controlled by a computer-aided design (CAD) model.Yet, only green parts are produced in this way. In order to consolidate and fully densify the DIW printed objects, an appropriate drying-debinding-sintering route is required. Especially, drying is a crucial post-printing step, since any defect introduced during drying will remain in the system in the following debinding and sintering step and affect the quality of the final product. Controlled humidity drying is preferred to avoid drying cracks and warping of the high solid loading aqueous pastes. After drying, the organic binder in the paste is removed by debinding and sintering is performed to reach full densification. Microfocus X-ray computed tomography (μCT) is a non-destructive technique that allows to characterize objects in 3D. Characterization and comparison of the quality and dimensions of the green, brown (after debinding) and sintered parts by μCT allow to perform a size calibration between the CAD models and sintered parts for a near-net-shape component manufacturing as well as the detection of larger internal flaws (residual porosity, flaw distributions, cracks, etc.). In this study, drying/sintering shrinkage and deformation were characterized with μCT and the drying parameters were optimized based on this characterization to obtain high-density monolithic alumina toughened zirconia parts.

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EFFECT OF THE HNT NANOCARRIERS DISPERSION IN SRR RELEASE IN PLA AND GN BIODEGRADABLE COMPOSITES

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Among strontium-based drugs, the Strontium ranelate (SrR) is a divalent strontium salt of ranelic acid which has an overall effect over the bone microarchitecture improvement. However, some findings reveal that the SrR affects in an opposite manner to the cell proliferation and osteoblastic differentiation, based on its concentration. Consequently, its release should be controlled. The incorporation of Halloysite nanotubes (HNT) as nanocarriers of SrR, in different polymeric matrix tailors the release of this anabolic bone-forming and anti-catabolic agent to stimulate bone growth. In this work, the role of HNT nanocarries in Sr2+ release when it is dispersed in different polymeric matrix shaoed as coatings, films and 3D scaffolds will be study. Zeta potential determination is used to stablish the drug loading (HNT-SrR) by electrostatic interaction, as well as to optimize the dispersion of HNT SrR-loaded in a gelatin (GN) and Polylactic acide (PLA) solutions. Polyethilenimnine (PEI) is used as stabilizer to buffer the suspension media, assure cargo-drug dispersion and sequential release. Biodegradable materials will be shaped by electrophoretic deposition (EPD), tape casting and fused filament fabrication (FFF), and Sr2+ release and its effect in in vitro test will be showed.

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TEA TREE LOADED COLLAGEN/HAP AEROGEL CONSTRUCT FOR CHRONIC WOUND HEALING

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Chronic wounds are slow healing skin lesions, often highly exudative, and normally associated with underlying comorbidities such as ageing, obesity, or diabetes. These wounds are usually colonized by microorganism that worsen the tissue health and delay the healing even further [1].Aerogels are three dimensional scaffolds, leading to an interconnected structure with macro and microporosity. This can provide an ideal architecture for cell colonization, as well as nutrient and oxygen diffusion [2]. These scaffolds can be tailored in terms of fluid absorption capacity, an important feature for the healing of exudative wounds. Collagen is the main constituent of the extracellular matrix of several physiologic structures and therefore, biocompatible and biodegradable [3]. It has been widely used to produce different materials for tissue regeneration [4]. It is known that calcium is a major signal regulator throughout our body. Regarding skin, calcium regulates collagen deposition, influences fibroblast migration and proliferation, regulates angiogenesis and helps to repair the skin barrier [5]. Tea Tree Oil (TTO) is a natural extract that possesses antimicrobial, anti-oxidant, and anti-inflammatory properties and has been used in many topical formulations [6]. In the present work, a novel aerogel formulation has been developed by the combination of collagen, hydroxyapatite and TTO to produce scaffolds with interconnected porosity, fluid absorption capacity, and adequate mechanical properties. Preliminary results demonstrate that the obtained hybrid material can offer a more complete and promising approach for the healing and regeneration of exudative chronic wounds, while preventing infection.

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PREPARATION OF SCAFFOLDS BASED ON CALCIUM PHOSPHATES DOPED WITH SILICA FOR BIOMECHANICAL APLICATION

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The aim of this research was to prepare porous bioceramic scaffolds based on calcium phosphates doped with silica for bone tissue engineering. Two main preparation methods were used for the creation of scaffolds – replica method and direct foaming method. The preparation process by the direct foaming method was optimized to obtain a defined structure. Calcium phosphate scaffolds containing 0–20 wt.% SiO2 were sintered and studied in terms of material characteristics (phase composition, pore size and porosity, microstructural study by scanning electron microscopy (SEM)), bioactive properties (simulated body fluid (SBF) interaction tests and tests of simulated degradation) and mechanical properties in order to evaluate the effect of silica doping. Scaffolds prepared by both methods were composed of a mixture of hydroxyapatite and/or tricalcium phosphate and cristobalite and wollastonite with comparable porosity in the range of 80–88 %. The pore size of the scaffolds prepared by the direct foaming method reached the interval of 5–250 μm opposite to template method reached the pore size up to 430 μm. The SBF interaction tests and tests of the simulated degradation confirmed the bioactive behavior of the prepared scaffolds and their ability to degrade under the simulated conditions. The scaffolds prepared by the direct foaming method showed better mechanical properties (compressive strength up to 1,8 MPa) than the scaffolds prepared by the template method. The results showed that the prepared scaffolds are suitable and promising for potential applications in bone tissue engineering

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PHOSPHORYLATED CHITOSAN AND ITS ASSOCIATION TO CALCIUM CARBONATE CEMENT FOR BONE SUBSTITUTION PURPOSE

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There is a growing interest in injectable and self-setting materials ready to be implanted through minimally invasive surgery in the field of bone repair [1]. Thus, calcium carbonate cements have been developed as a resorbable bone cement with respect to other bone cements [2]. However, their low mechanical strength and prolonged setting time limited their use in orthopaedics. The association of polysaccharides to bone cements had been exploited in order to tune their physicochemical, mechanical and biological properties. Moreover, functionalization through the chemical grafting of specific functionalities had been used to improve polymers properties [3]. The present work reports on the development of composite cements based on calcium carbonates and phosphorylated chitosan and aims to improve the setting time, rheological and mechanical properties of the cement to meet the clinical requirements and to extend the cement use in bone repair. For this, amorphous calcium carbonate and vaterite powders were synthesized by double decomposition method and then mixed with modified chitosan hydrogel as the liquid phase of the cement. The composition, microstructure, setting behavior, setting time, rheology as well as mechanical properties of the composite cements were characterized using complementary techniques. The results revealed that the cements associated with modified chitosan were mainly composed of vaterite. The formulation containing 2.5% w/w of phosphorylated chitosan exhibited a 7-fold decrease in both initial (17 min) and final (30 min) setting times, good paste rheology, as well as improved compressive strength. The in vitro evolution of the prepared cement in a simulated body fluid (SBF) revealed the formation of apatite spherulites on the surface of the designed cements indicating their potential bioactivity. Future efforts will aim to evaluate the in vitro and in vivo bioactivity of the developed cement.

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Keywords

Calcium Carbonate Cement, Composite Cement, Phosphorylated Chitosan

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SAFETY OF Β-TRICALCIUM PHOSPHATE INCORPORATING BMP-2 IN REHABILITATION OF CANCER-RELATED BONE DEFECT

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Rehabilitation of cancer-related bone defects is still a huge challenge in clinical practice due to the potential promotion of cancer development caused by bone substitutes. β-tricalcium phosphate (β-TCP) has been widely used in non-cancer-related bone defects with its promising biocompatibility and osteoconduction. Intriguingly, this material has also been reported to possess the anti-cancer property. This means β-TCP could be used for bone reconstruction in cancer cases. However, as a synthetic ceramic material, β-TCP lacks osteoinduction, weakening its compacity for bone reconstruction. To circumvent this issue and accelerate bone formation, bone morphogenic protein-2 (BMP-2), a well-known bone growth factor, can be engaged; the success of this strategy has been achieved in various studies. Nonetheless, the controversy about the role of BMP-2 in cancer progress halts the potential application of BMP-2 incorporated β-TCP in the rehabilitation of cancer-related bone defects. To investigate the safety of BMP-2 incorporated β-TCP in cancer, our group synthesized BMP-2 incorporated β-TCP and treated osteosarcoma cells (a human bone cancer cell line) with gradient concentrations of BMP-2, pure β-TCP, and BMP-2 incorporated β-TCP for 48 h. The results demonstrated that 200 ng/ml BMP-2 inhibited the viability of osteosarcoma cells and pure β-TCP also inhibited the viability of cancer cells. While this effect was reversed with an increment of the concentration of β-TCP. The BMP-2 incorporated β-TCP manifested steady inhibition on the viability of osteosarcoma cells, even at a high concentration.

Keywords

β-TCP, BMP-2, cancer-related bone defect

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SYNTHESIS OF POTASSIUM SODIUM NIOBATE (KNN) BY SOL-GEL PROCESS

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Recent advances in tissue engineering have focused on the use of biochemical and physicochemical signals to trigger specific cellular responses and encourage better biological interaction between the implant and living tissue. As a result, there has been significant interest in the development of intelligent biomaterials that can generate electrical stimuli in situ, for accelerated bone repair, healing and reconstruction. These smart biomaterials are essentially materials that contain electrically active components or materials that can be polarized. Piezoelectric ceramics may be the key to the functionalization of current implant and graft designs. They exhibit mechanically generated electrical behavior. The highest piezoelectric coefficients are currently achieved by lead-based piezoelectric ceramics (PZT). However, their high lead content makes them toxic. Due to this, other alternatives are being studied, such as potassium sodium niobate (KNN), as they have properties equivalent to PZTs. The niobates synthesis are normally reproduced by solid state reaction. However, some problems related to this ceramic come from the failure to maintain its stoichiometric composition (K0.5Na0.5)NbO3. The easy volatilization of alkaline precursors can difficult obtaining the desired phase and, thus, cause the lack of essential properties to adequately replace PZTs. In this work, the synthesis was performed by sol-gel route and the samples were calcined at 750°C and sintered at varying temperatures of 1100, 1130 and 1150°C. The characterization was performed by the X-ray diffraction technique (XRD), with refinement by Rietveld method, scanning electron microscopy (SEM) and thermogravimetric analysis (TGA). KNN phase was obtained in one of the samples; a combination of KNN and a second phase was observed in the other samples, proving that sol-gel synthesis is possible route to obtain the pure phase despite the high volatility of the alkalis.

Keywords

Sodium potassium niobate (KNN); piezoelectricity, sol-gel.

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PROMISING STRONTIUM-DOPED APATITIC BONE CEMENTS FOR THE TREATMENT OF BACTERIAL INFECTIONS

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Injectable calcium phosphate cements (CPCs) represent promising candidates for the regeneration of bone defects, thanks to their self-hardening ability, bioactive composition and nanostructure. Since they could be functionalized with different bioactive molecules, this aspect allows them to be very interesting and useful in medical applications. The aim of this part of the work was to assess the antimicrobial action of Sr-doped apatitic CPCs (SrCPCs) enriched with hydroxyapatite nanoparticles (HA-NPs) functionalized with tetracycline (TC), which is an antibiotic that inhibits bacterial protein synthesis by hampering aminoacyl t-RNA binding to bacterial ribosome. To exert its action, TC must cross bacterial cell membranes which are different depending on Gram positive or negative bacteria. In our study we used two of the main pathogens that cause infections: the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli*. Bacterial viability was evaluated with the quantitative 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay on two conditions: planktonic cultures and adherent bacteria. Both conditions were important in order to determine whether the substances released from the scaffolds could contribute to reduce bacterial viability of planktonic cultures and also the possible anti-adhesive properties of the surface itself. In addition to it, we also evaluated through an indirect contact experimental set up, whether the content of the scaffolds could affect bacterial viability. Given the interesting results we obtained against planktonic and adherent bacteria, we decided to investigate the ability of the scaffolds, through MTT assay, in preventing the formation of bacterial biofilms and reducing the preformed biofilms. The data we obtained in this work demonstrated how SrCPCs functionalized with TC can reduce the viability of both planktonic bacteria and biofilms. It was also noticed that CPCs without TC can interfere with biofilm formation, suggesting that also scaffolds features play an important role in contrasting bacterial infections. In conclusion, these cements represent promising biomaterials to use in medicine, in particular in orthopedics field.

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