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DOCTORAL THESIS

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Sveučilište u Zagrebu
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ANTIMIKROBNA UČINKOVITOST I PROPUSNOST RAZLIČITIH MATERIJALA ZA BRTVLJENJE NA SPOJU IMPLANTATA I NADOGRADNJE

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SUMMARY

Peri-implantitis is an inflammation around a dental implant. Bacteria settle in the tissues around the implant and inside the implant due to the passage of microorganisms through the microgap at the connection of the implant and the prosthetic abutment. Persistent inflammation over time causes bone resorption over time, which can lead to implant loss. There are different ways to treat and prevent peri-implant diseases. One of the ideas of preventive measures is the use of sealing materials and antimicrobial materials to decontaminate the implant abutment interface and consequently prevent the colonization of microorganisms and their impact on the occurrence of peri-implant diseases. A review of the current literature shows a lack of independent studies on this issue. The purpose of this study was to evaluate the antimicrobial efficacy and permeability of different types of the sealing materials at the connection of prosthetic abutment and dental implant in static conditions and the influence of implant and prosthetic abutment platform type on sealing quality of different materials. Furthermore, the influence of prosthetic abutment fabrication method on microbial leakage at the implant abutment interface in static conditions was evaluated. The use of different sealing materials showed no difference in the antimicrobial effect and permeability for microorganisms compared to the negative control groups. Implant abutment interface platform type did not significantly influence the sealing efficacy regardless of the sealing materials use. There was also no statistically significant difference between original and third – party prosthetic abutments regarding sealing efficacy. The clinical implication of this research is that it offers an answer to the question whether the routine use of such materials is justified.

Key words: dental implant; dental implant–abutment design; implant–abutment connection; microbial colony count; peri-implantitis

PROŠIRENI SAŽETAK

Svrha rada

Periimplantitis upalna je bolest koja zahvaća periimplantatna tkiva što dovodi do postupnoga gubitka potpornoga mekog tkiva i kosti. Bakterije koloniziraju tkiva oko implantata te unutrašnjost implantata uslijed prolaza mikroorganizama kroz mikropukotinu na spoju implantata i protetske nadogradnje. Svrha je ovoga istraživanja evaluacija antimikrobne učinkovitosti i propusnosti različitih vrsta materijala za brtvljenje mikropropusnosti na spoju protetske nadogradnje i dentalnoga implantata u statičkim uvjetima te utjecaja tipa platforme, tj. geometrije spoja implantata i protetske nadogradnje na kvalitetu brtvljenja različitih materijala. Klinička implikacija ovoga istraživanja evaluacija je opravdanosti rutinske uporabe sredstava za brtvljenje spoja implantata s protetskom nadogradnjom

Materijali i postupci

U ovom istraživanju korišteno je 100 titanskih dentalnih implantata i 100 originalnih protetskih nadogradnji, od toga su formirane dvije velike skupine po 50 implantata u svakoj s obzirom na tip veze implantat – protetska nadogradnja.

Skupinu A činili su implantati GC Aadva Standard (GCTech.Europe GmbH, Breckerfeld, Germany) promjera 4,0 mm s koničnim tipom veze s protetskom nadogradnjom uz promjenu platforme.

Skupinu B činili su implantati Zimmer Biomet Taperd Screw-Vent promjera (Zimmer Biomet Dental, Palm Beach Gardens, Florida, USA) 4,1 mm s koničnim tipom veze s protetskom nadogradnjom bez promjene platforme.

U svakoj od velikih skupina (A i B) formirane su tri ispitne skupine s obzirom na različiti materijal za brtvljenje, i to kako slijedi: A/B 1. GapSeal gel (Hager & Werken, Duisburg, Germany); A/B 2. Oxysafe gel (Hager & Werken, Duisburg, Germany); A/B 3. Flow.sil (Bredent GmbH & Co.KG, Senden, Germany) po 10 implantata u svakoj. Formirana je jedna pozitivna kontrolna skupina od 10 implanata s klorheksidinskim gelom (Curasept ADS 350 gel, Curaden International AG, Kriens, Switzerland) i negativna kontrolna skupina bez sredstava za brtvljenje, također od 10 implantata.

Priprema dentalnih implantata

Eksperiment se proveo zasebno za implantate s promjenom i bez promjene platforme na spoju implantata i protetske nadogradnje. Isti postupci slijedili su za obje vrste implantata.
Dentalni implantati i odgovarajuće originalne protetske nadogradnje uklonjeni su iz komercijalnoga pakiranja u sterilnim uvjetima. Nakon toga implantati su fiksirani u sterilni stegač od nehrđajućeg čelika da se omogući čvrsto okretno djelovanje kod zatezanja protetske nadogradnje (po preporuci proizvođača) te da se implantati zadrže u vertikalnom položaju. Prije postave protetske nadogradnje u implantate je dodano sterilnom mikropipetom 0,3 µl sterilne BHI (Brain heart infusion) otopine koja je služila kao hranidbeni medij ukoliko dođe do prodora bakterija i gljiva. Zatim se uz sam rub implantata dodalo testirani, ovisno u kojoj su skupini (GapSeal, Oxysafe, Flow.sil, CHX gel), materijal za brtvljenje u jednom sloju nakon čega se fiksirala protetska nadogradnja po preporuci proizvođača (20 N/cm za GC Aadva implantate odnosno 30 N/cm za Zimmer Biomet implantate). Kod negativne kontrolne skupine nije aplicirano ni jedno sredstvo, nego su fiksirane protetske nadogradnje po preporuci proizvođača.

Kontaminacija dentalnih implantata

Za kontaminaciju dentalnih implantata korišteni su sojevi Staphylococcus aureus i Candida albicans izolirani iz kliničkoga uzorka u Kliničkom zavodu za mikrobiologiju, prevenciju i kontrolu infekcija Kliničkog bolničkog centra Zagreb. Pripremila se zajednička bakterijska suspenzija od bakterija i gljiva. Denzitometrom se odredila gustoća od 0.5 McFarlanda. Bakterije i gljive zajedno su uzgojene na jedinstvenom mediju za uzgoj (BHI-bujon) tijekom 72 sata.
Svi sklopovi dentalnih implantata i protetske nadogradnje uronjeni su u Eppendorf tubice s otopinom (0,3 ml) kontaminiranom sa S. aureus i C. albicans s gustoćom od 0,5 McFarland do iznad spoja implantata i nadogradnje s tim da je otvor za pristup spojnom vijku ostao iznad razine otopine kako bi se eliminirao utjecaj prodora kontaminirane suspenzije uz sami vijak ortogradnim putem tijekom 14 dana u aerobnim uvjetima.
Na uzorcima iz negativne kontrolne skupine nije primijenjen tretman, nego je sklop implantat – nadogradnja uronjen u kontaminiranu otopinu tijekom 14 dana u aerobnim uvjetima. Pozitivna kontrolna skupina tretirana je antiseptičkim gelom (CHX gel), a zatim uronjena u kontaminiranu otopinu tijekom 14 dana u aerobnim uvjetima.

Nakon 14 dana inkubacije uzorci su bili uklonjeni iz epruveta pomoću sterilnih kliješta, zatim su bili uronjeni u 70 % alkohol u trajanju do 3 minute, kako bi se spriječila vanjska kontaminacija, i osušeni sterilnom gazom te nakon toga pažljivo rastavljeni nakon montaže u vertikalnom položaju u sterilni stegač.

Nakon rastavljanja uzoraka, unutrašnje površine implantata uzorkovane su s 3 sterilna papirnata štapića koji su potom bili uronjeni u Eppendorf epruvete koje su sadržavale 0,5 ml sterilne BHI (brain heart infusion) otopine. Sadržaj svake epruvete se zajedno s papirnatim štapićima promiješao u Vortex miješalici kako bi se uklonile bakterijske i stanice gljive.

Uzorci su, s kompletnim sadržajem epruvete, naneseni na hranjive mikrobiološke podloge s 5 % krvnog agara i inkubirane 48 sati na 37 °C. Nakon toga su rezultirajuće kolonije bile identificirane i kvantificirane.

Mjerama ishoda promatrana je potpuna odsutnost testirane bakterije i/ili gljive nakon tretmana. Prisutnost bakterije i/ili gljive nakon tretmana smatrana je pozitivnim rezultatom. Samo potpuna odsutnost testirane bakterije i gljive smatrana je kao negativni rezultat.

Rezultati

Kako bi se kvantitativno opisali uzorci korišteni u ovoj studiji, rezultati su određeni na temelju učestalosti prodora mikroba u deskriptivnu svrhu. Pozitivan rezultat označavao je prisutnost C. albicans ili S. aureus kroz spoj implantata i nadogradnje, dok je potpuni izostanak ovih mikroorganizama smatran negativnim rezultatom.

Prema učestalosti mikropropuštanja S. aureus i C. albicans za ravni i konusni tip spoja implantata s protetskom nadogradnjom, svi materijali za brtvljenje uspoređeni su s pozitivnim i negativnim kontrolama na S. aureus i C. albicans infekcije pomoću Fisherovog egzaktnog testa. Vrsta konekcije implantata i nadogradnje nije imala utjecaja na kontaminaciju, adheziju i umnažanje bakterije S. aureus i nije bilo statistički značajnog poboljšanja uz korištenje različitih materijala za brtvljenje u usporedbi s kontrolnim podskupinama jer su p-vrijednosti bile iznad razine značajnosti ($p > 0,05$). GapSeal bio je jedino sredstvo za brtvljenje koje je bilo značajno učinkovitije u usporedbi s podskupinom negativne kontrole ($p = 0,008$). Isti zaključak može se izvesti i za kontaminaciju kvascem C. albicans jer nije bilo statistički značajnoga poboljšanja s bilo kojim materijalom za brtvljenje osim GapSeala u usporedbi s podskupinom negativne kontrole ($p = 0,000$). Različite vrste spoja implantata i nadogradnje nisu imale utjecaja na mikropropuštanje prema p-vrijednostima Fisherovog egzaktnog testa.

Zaključak

Na temelju rezultata ovog istraživanja može se zaključiti da tip spoja implantata i nadogradnje nije imao značajan utjecaj na mikropropuštanje, zadržavanje i umnažanje testiranih mikroorganizama. Dodatno, GapSeal značajno smanjuje mikropropuštanje, osobito protiv *Candida spp*. infekcija. Unatoč uvjerljivim prednostima primjene GapSeala, potpuno hermetičko brtvljenje nije postignuto ni s jednim od sredstava za brtvljenje testiranih u ovoj studiji. Trebalo bi provesti daljnja klinička istraživanja s duljim razdobljima praćenja kako bi se procijenili učinci upotrebe različitih materijala za brtvljenje na različitim tipovima spoja implantata i nadogradnje.

Ključne riječi: dentalni implantat; dizajn nadogradnje na implantatu; spoj implantat – nadogradnja; mikrobiološko brojanje kolonija; periimplantitis

The list of abbreviations

IAI	implant-abutment interface
BHI	brain heart infusion
C. albicans	*Candida albicans*
S. aureus	*Staphylococcus aureus*
CFU	colony forming units

CONTENT

1. INTRODUCTION

1.1. Peri-implantitis

With a mean survival rate of 94.6% and a mean success rate of 89.7% during follow-up in the period of 10 years (1), dental implant therapy is considered safe and predictable. Biomechanical or mechanical problems are responsible for the failure of implant therapy. Dental implant issues are typically split into two categories: early complications, which are mostly surgical and anatomical, and late complications, which are caused by periimplantitis and/or prosthodontic failures (2).

Dental implants are surrounded by soft and hard tissue that is chronically and progressively infected with peri-implantitis, which causes the loss of the supporting bone. It is caused by the same bacteria that cause periodontitis (1).

The crucial aspect of implant therapy is the connection between implant and abutment since, from a mechanical standpoint, it is the weakest part and, from a biological standpoint, a micro-gap can allow bacteria to flow out. The micro – gap is a narrow space between the internal part of the implant and the prosthodontic abutment. It is considered inevitable in two – piece implants and it frequently serves as a reservoir for various comensal and pathogenic bacteria, particularly those that are micro-aerophilic or anaerobic in nature. The progression of these bacteria causes tissue inflammation and bone resorption (3, 4). Thus, micro – gap is a critical area in microbial colonization and the starting point of peri – implant pathology (3).

In addition to peri-implant infections at locations with deeper probing depths, periapical peri-implantitis lesions have also been found in several case studies. A periapical radiographic radiolucency, together with or without concurrent clinical signs of inflammation such as redness, edema, fistula, and/or abscess formation, was a prevalent feature of the affected implants. According to most of the research, periapical endodontic lesions at neighboring teeth and retrograde periimplantitis are inevitably connected (5-7).

1.2. Microbiology of peri – implantitis

Both healthy and affected implant sites have been found to have periodontopathogenic bacteria, although peri-implantitis has been linked to greater counts of 19 bacterial species, including *Porphyromonas gingivalis* and *Tannerella forsythia* (8, 9). Additionally, observational studies have shown that peri-implantitis is more frequently associated with opportunistic pathogens like *Pseudomonas aeruginosa and Staphylococcus aureus (S. aureus*), fungal organisms like *Candida albicans and Candida boidinii*, as well as viruses like the human cytomegalovirus and

Epstein-Barr virus (10-13). This, therefore, indicates that the microbiota of peri-implantitis lesions is relatively diverse and complex and has not been thoroughly investigated using culture-independent methods.

Most recent systematic studies have concentrated on the relationships between the clinical state at implant sites and various cytokines (i.e., proinflammatory/anti-inflammatory/osteoclastogenesis-related) and chemokines detected in the peri-implant cervicular fluid (14,15). Much of the research concentrated on evaluating interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF – α). According to a meta-analysis, compared to healthy implant sites, the release of IL-1 was shown to be considerably higher at mucositis and peri-implantitis sites. The IL-1 levels between the peri-implant mucositis and peri-implantitis sites did not differ significantly, though. Additionally, peri-implantitis locations had significantly higher TNF- levels than healthy implant sites (14). However, neither IL-4, IL-10, nor osteoclastogenesis-related (RANKL) cytokines were significantly different in levels between healthy and peri-implantitis regions in the majority of the included investigations Accordingly, in a systematic review by Faot et al. (14) it was found that the measurement of proinflammatory cytokines (mostly IL-1) in the peri-implant crevicular fluid would be useful for differentiation between peri-implant health and disease.

1.2.1 Strategies for the prevention and treatment modalities for peri-implantitis

Patients should be aware of the value of good oral hygiene after dental implants have been placed and implant-prosthodontic therapy is finished. Professional plaque and biofilm removal must be done at routine check - ups to eliminate the bacteria and fungus that are the primary causes of peri-implant diseases (16).

The space that exists between the implant and the superstructure can be reduced but not eliminated. The marginal gap, which ranges in size from 14 to 160 μm, is unable to fend against bacterial invasion from the oral cavity (16). This is the reason that products like gold foil, self-hardening silicone materials, petroleum jelly, antibiotic gels, chlorhexidine gel, Paladur®, and Ledermix® that are applied to the gap to prevent peri-implantitis have been put on the market. It should be kept in mind that this therapy is brief and needs to be repeated regardless of which of the preparations for treating this crucial area are used.

A mix of systemic antibiotic medication and some surgical or non-surgical treatments are utilized in the treatment of peri-implant infections given their recognized etiology.

1.2.1.1 Mechanical methods

Mechanical treatment of the infected implant surface effectively removes the formed biofilm while sacrificing the original micromorphology of the implant surface. The most clinically represented methods are: *implantoplasty, metal curettes and air abrasion.*

Implantoplasty is recommended to completely level the exposed section of the implant using rotary tools when the titanium surface of the implant is exposed to the oral cavity and contaminated with microorganisms (17). Lang et al. (18) first suggested this method with the intention of lessening the roughness of the titanium surface and, subsequently, plaque adherence, as it has been demonstrated that plaque accumulates more on rough surfaces than on smooth or moderately rough surfaces. According to Valderrama et al. (17), the coating on the implant's surface can be fully removed by utilizing diamond polishing tools. It is also indicated that there is no difference between the drill used for implantoplasty, diamond powder, and carbide polisher or carbide drills alone because all of them provide comparable polished surfaces.

Streptococcus sanguius bacteria, an essential early colonizer in the oral cavity, are reduced in quantity and roughness on the implant surface by using *metal curettes* (19). In comparison to titanium curettes and ultrasonic scalers with plastic inserts, metal curettes can remove an average of 0.83 micrometers of surface material from a rough surface after 20 seconds of usage (20).

With the help of compressed air and an abrasive powder, such as sodium bicarbonate or the amino acid glycine, air abrasion can remove the biofilm or extrinsic stains from the teeth. Numerous tests have shown that this device, which uses a mixture of water, air, and powder under pressure ranging from 414 to 689.5 Pa, is effective in cleaning the previously contaminated implant surface (17).

1.2.1.2 Chemical methods

Disinfection of the implant surface with chlorhexidine preparations and photodynamic therapy with a diode laser are the most common chemical methods that have become established in clinical practice.

Chlorhexidine gluconate is the most important antiseptic in periodontology (21). Because of its bactericidal properties, its main uses include treating localized periodontal pockets as an antiseptic or reducing the number of bacteria before or after surgical procedures. Numerous studies that have been published have examined its use in cleaning an implant surface that has been involved in peri-implantitis (17).

Light-activated disinfection and photo-dynamically activated chemotherapy are alternate names for photodynamic therapy (aPDT). "Light-induced inactivation of cells of microbes or molecules" is the commonly accepted definition (22). This method is used in dental care to eliminate microorganisms by applying photosensitive dyes that are activated by light of a specific wavelength (23). It consists of three fundamental components: oxygen, a non-toxic photosensitizer, and harmless visible light. Oxygen is changed into highly reactive ions and radicals that obliterate bacteria (17). Light absorption by bacteria, laser wavelength, exposure period, the size of the treated region, and the organic matrix of the biofilm are some variables that can impact the success of photodynamic therapy (23). Its one drawback is that some dyes may not distinguish between bacteria and host cells, which could harm the tissues around them (24). One potential benefit of photodynamic therapy over traditional antibiotic therapy is that it is a local therapy, limiting the adverse effects of utilizing systemic antibiotics by only treating the affected areas that need antibiotic treatment with dyes and illumination. Additionally, there is no evidence of target microorganisms developing resistance following photodynamic therapy (22).

1.2.1.3. Regenerative procedures

Regeneration is the process of restoring the structure and function of a lost or damaged tissue. Guided tissue regeneration and bone grafts (autologous, homologous, heterologous, and alloplastic) are examples of regenerative treatments for peri-implant disorders (25). The fundamental idea behind using bone grafts is the presumption that the material contains cells that create the bone (osteogenesis), cells that stimulate bone formation (osteoconduction), or substances that induce bone growth (osteoinduction), which would stimulate alveolar bone

regrowth and the development of a new fastener (26). Placing a physical barrier to ensure the repopulation of periodontal ligament cells is known as guided tissue regeneration (GTR). Physical barriers include membranes, which can be either natural or artificial, resorbable, or non–resorbable. The desirable membrane characteristics include biocompatibility, cell-occlusion properties, integration with host tissues, clinical manageability, space-making ability, and adequate mechanical and physical properties (27). In more severe cases of peri-implantitis, regenerative techniques are performed in conjunction with non-surgical therapeutic options (28). Probing, X-ray analysis, measuring the new bone, and histological analysis are used to determine whether regeneration was successful.

1.3. Types and role of implant – abutment interfaces in peri-implantitis

During the fixation of a transmucosal prosthodontic abutment to a dental implant a microgap is formed between the abutment and the implant. Inflamed soft tissue may develop in front of the implant-abutment junction because of microbial colonization of the microgap at the IAI and the establishment of a bacterial reservoir (2, 29, 30). Inflammation at the implant site and bone loss may be influenced by the presence of a microgap at the IAI (31-35). Construction of two-piece implant systems presents a significant problem in preventing microbiological leakage at the IAI. Eliminating microbial leakage reduces inflammatory responses, which in turn increases peri-implant crestal bone durability (33). Implant abutment connections can then be categorized as either internal or external. According to geometrical characteristics and the connection between the internal aspect of the implant and the abutment, internal abutment connections can be further divided into three categories: clearance-fit connections, conical connections, and combination (Figure 1) (36). To prevent friction between the components, clearance-fit connections have parallel barriers between the abutment piece and the matching internal aspect of the implant (Figure 2). The implant-abutment interface, which is made up of a flat abutment surface resting on a flat implant surface (flat-to-flat interface or butt joint), is used in clearance-fit connections. Clearance-fit connections use geometric patterns such triangular, polygonal, notches, and lobes to stop the abutment and implant from rotating while also making it easier to position the abutment during the prosthetic phase of implant therapy. "Index" is a common term for the insertion of geometric, antirotational properties (37). The implant platform diameter and the diameter of the abutment at the interface can be the same or different in size. Platform switching occurs when the abutment diameter is smaller than the implant platform diameter (38). To provide a tapered contact between the abutment and the implant, internal

conical connections are made up of a conical component of the abutment, which is placed at the matching portion of the internal aspect of the implant (Figure 3). Geometric elements can be added to the abutment's apical section in addition to its conical portion to provide prosthetic orientation and antirotational qualities (37). A retention screw is used to secure abutments to the implant (abutment screw). The length of the taper section of the abutment, the total amount of abutment surface area in contact with the internal aspect of the implant, the geometry of the antirotational features, and variations in the taper angle can all contribute to variations in the internal conical connections.

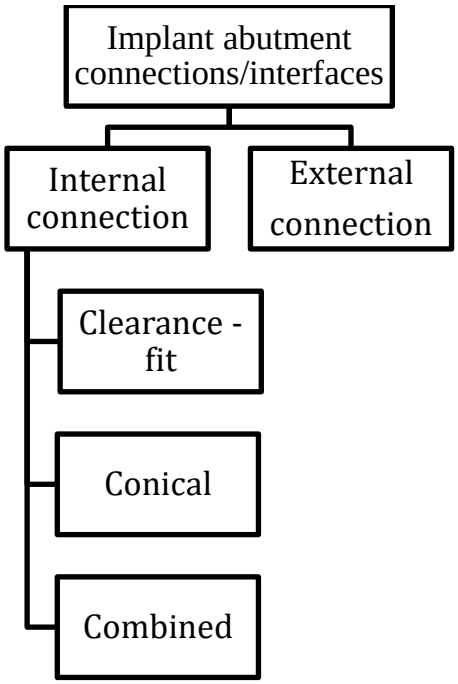

Figure 1. Categorization of implant abutment interfaces based on their geometry.

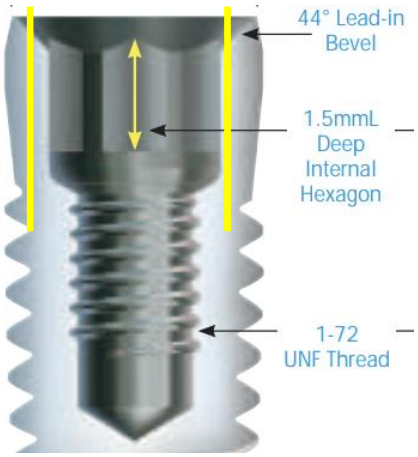

Figure 2. Clearance – fit type of implant abutment interface. (Reprinted with the permission of the manufacturer)

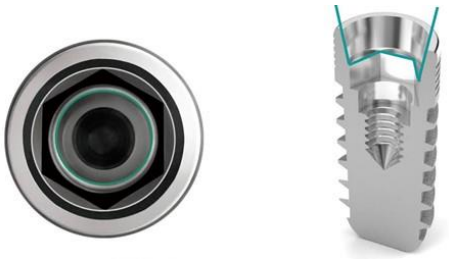

Figure 3. Conical type of implant abutment interface. (Reprinted with the permission of the manufacturer)

1.3.1 Microleakage at implant - abutment interface and methodological aspects of in vitro studyes for its evaluation

The risk of bacterial infiltration into the internal components of dental implants through the implant-abutment contact has been thoroughly assessed in *in vitro* conditions. Reviews examining the methods of those *in vitro* experiments as well as systematic reviews have been carried out (26, 27). Two major techniques were used in *in vitro* investigations to assess the likelihood of bacterial infiltration at the implant–abutment interface. One technique involves injecting a microbial agent into the implant's interior, putting the implant and abutment together, then monitoring if the bacteria are released into the environment afterward (outgrowth model). The alternate method involves constructing the implant and abutment system in sterile conditions, dipping the system into a bacterial solution, and determining if bacteria have

penetrated the internal aspect of the implants (ingrowth model). These two fundamental techniques have been applied both with and without the usage of loading conditions. There are drawbacks to both the ingrowth and outgrowth approaches. For instance, the primary flaw in the outgrowth model is a mistake made during the implant's internal inoculation phase. To prevent contamination of the implants' exterior after assembling the implant-abutment complex, the precise amount of bacterial solution for the particular type of the implant system must be calculated. False-positive results may result from inoculation errors. For this reason, some investigations assess possible contamination of the implants' exteriors before the actual trial (28). The key methodological difficulty in the ingrowth model is to put together and take apart the implant-abutment complex in sterile settings and collect a microbiological sample from the internal aspect of the implant without contaminating the exterior aspect of the implant. More complex models have been created, enabling sampling of the implant's inside without removing the abutment. This is accomplished with the use of prefabricated channels that give cannulas access to the implant's interior (39). In most *in vitro* investigations assessing the possibility of bacterial penetration into the implant-abutment contact no load was applied on the dental implants (40-43). The main disadvantage of no loading strategy is that bacterial transmission into and out of the implant-abutment contact isn't evaluated. There is a strong proof that loading increases that risk in a study that examined the risk of bacterial penetration into the implant interface under loading and nonloading settings (44). The key benefit of conducting the experiment without loading conditions is the use of variety of microorganisms. Since the studies can be carried out in anaerobic environments, they can also include anaerobic organisms. Bacterial penetration into the implant-abutment interface is a common observation for all types of implant-abutment connections, according to experiments carried out under nonloading conditions. Compared to the implants with internal clearance-fit connections, the implants with internal conical connections tend to show a lower risk of bacterial penetration into the implant–abutment interface (45-49). When testing the sealing ability of the implant-abutment interfaces, the application of loading pressures provides more difficult experimental conditions. Because these tests are more difficult and expensive to carry out, there are fewer investigations that have been done (39,44,50-54). Steinebrunner et al. (50) conducted the first investigation utilizing loading conditions to assess the sealing ability of various implant-abutment interface configurations. In that study, after being loaded with 1 200 000 cycles and 120 N, all specimens for all kinds of connections (external clearance fit, internal clearance fit with silicone washer, internal clearance fit, internal combined) eventually showed microbial penetration through the implant-abutment interface (50). There was no evaluation of internal conical connections in that

investigation. It was shown in a later investigation, under stress settings, that the two alternative internal conical connections have different risks for bacterial penetration through the implant-abutment contact (51). In addition, in studies comparing loaded and unloaded implants, it was found that loading might make it more likely for germs to infiltrate the implant-abutment contact (44,54). All internal conical connections have some bacterial leakage through the implant-abutment interface, but this risk may not be the same for all of them. *In vitro* tests on implant part contamination involve a variety of different bacterial species and methodologies. Since bacterial endotoxin molecules are smaller than complete bacteria and may be able to penetrate the implant-abutment contact more easily, research using endotoxins from bacteria has shown this method to be extremely sensitive to contamination (52,55). Overall, *in vitro* research using various experimental designs suggests that it is challenging to completely avoid bacterial penetration across the implant-abutment contact. In comparison to implants with external and internal clearance-fit connections, the implants with internal conical connections function better in non-loaded and loaded testing settings. Smaller-molecule tests, such as those using bacterial endotoxins, show endotoxin penetration in all specimens analyzed for implants with internal, conical connections (55).

1.3.2 Materials for sealing implant - abutment interface

In vitro and *in vivo* conducted studies have shown the existence of live bacteria inside dental implants, as well as the infiltration of liquids and microorganisms into all internal spaces through the microgap created between the implant and the prosthetic abutment, which can be a source of contamination for the tissues surrounding the implant (44,51,53). The body's defense system can remove the biofilm formed by the bacteria on the external surface, but the internal colonization of the implants at the interface between the parts proceeds, leading to further infection and damage to the periodontal tissue as well as bone resorption (56). The administration of silicone sealant and chlorhexidine varnish at the cervical implant section is recommended to limit these infiltrations; nevertheless, both are ineffective for periods longer than 5 weeks, proving that they are unable to seal the IAI (57). Due to this, numerous materials have undergone extensive research to seal the screw access channel and safeguard the abutment screw either during the temporary period or during the final restoration. To safeguard the abutment screw head and ensure the proper sealing of IAI, compounds, such as gutta percha (GP), GapSeal gel and PTFE-based materials have been utilized (40,58-61). Although these materials produce good outcomes, they are only a short-term solution, because they only

successfully prevent bacterial invasion for brief periods of time. On the other hand, the data about sealing agents designed for use at the IAI itself remains scarce in the contemporary literature (62). There is evidence that GapSeal significantly reduces bacterial growth at IAI (60). This material consists of highly viscous silicone base with thymol added for antiseptic property (63). Oxysafe gel is another material developed and sold by the same manufacturer with active oxygen molecules and it is primarily used in treatment of deep periodontal pockets and peri-implantitis (64). Furthermore, Flow.sil is a product based on poly-dimethyl-siloxane matrix with appropriate mechanical properties providing stable and rigid microbial barrier which also appeared on the market as a potential widely used sealant (65). However, the efficacy of the aforementioned materials is poorly documented, with limited evidence in the literature. As a result, there is no clear agreement as to the best material for preventing biofilm growth and sealing against abutment-implant area infiltration. The hollow areas created by implant abutment interface in screw restorations may function as conduits and reservoirs, hosting and encouraging the colonization of microbial species present in the oral biofilm. According to research in the literature, the internal colonization of the implant following the osseointegration phase is related to different implant systems regardless of the type of the connecting platform (30). There is currently no agreement or set protocols for this purpose for the use of sealing materials to prevent the formation of biofilm in IAI due to the range of potential locations of microbial penetration in the implant abutment connection and different materials employed.

2. GENERAL AND SPECIFIC AIMS

The aim of this research was to evaluate the antimicrobial efficiency and permeability of different sealing materials at the connection of implants and prosthetic abutments in static conditions.

The additional aim was to see the influence of the type of platform, that was, the geometry of the connection between the implant and the prosthetic abutments to the sealing quality of various sealing materials.

Research hypotheses:

1. There is no difference in the antimicrobial effect and permeability for microorganisms that cause peri-implant diseases regarding different types of sealing materials compared to the negative control group

2. There is no difference in the sealing ability of different sealing materials regarding the type of platform or the geometry of the IAI compared to the negative control group.

3. Evaluation of Antimicrobial Efficacy and Permeability of Various Sealing Materials at the Implant–Abutment Interface—A Pilot In Vitro Study

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3.1 Abstract

Statement of problem: The microenvironment of the oral cavity is altered when an implant, a biocompatible foreign body, is inserted into the mouth. Bacteria settle in the tissues in and around the implant due to the passage of microorganisms through the microgap at the connection of the implant and prosthetic abutment. To prevent colonization of the implant by microorganisms, one idea is to use sealing and antimicrobial materials to decontaminate the implant–abutment interface and close the microgap.

Purpose: The purpose of this study was to evaluate the antimicrobial efficacy and permeability of different types of sealing materials at the implant–abutment interface, under static conditions.

Materials and methods: Three different sealing materials (GapSeal gel, Oxysafe gel and Flow.sil) were used for sealing the implant–abutment interfaces in 60 titanium dental implants, which were first contaminated with a solution containing Staphylococcus aureus and Candida albicans for 14 days under an aerobic condition.

Results: Results showed that a complete seal against bacterial infection was not formed at the implant–abutment interface, while for fungal infections, only GapSeal material helped to prevent microleakage.

Conclusions: Findings of this *in vitro* study reported that application of sealing material before abutment connection may reduce peri-implant bacterial and fungal population compared with the interface without sealing material.

3.2 Introduction

Modern dental implantology is based on the principle of osseointegration and all current implant systems use biocompatible materials based on titanium, zirconium oxide or tantalum. Over the years, many different shapes and surfaces of implants have been produced, which enable better loading of the implant and increase the area of its surface in contact with the alveolar bone (1). By inserting the implant, we introduce a biocompatible foreign body into the mouth, which changes the microenvironment of the area of the oral cavity into which it is inserted. Changes in this environment lead to the settlement of microorganisms in the peri-implant mucosa, which has less resistance and weaker vascularization than natural teeth. When coupled with reduced oral hygiene, this results in ideal conditions for the development of peri-implant diseases, especially after biofilm formation (2). The connection between the implant and the prosthetic abutment is the weakest point from a mechanical point of view, and from a biological point of view it is a microgap. Bacterial infection and biomechanical factors, which are associated with implant overload, are the two main factors, which lead to the development of peri-implant diseases (3,4). Peri-implant diseases themselves are divided into peri-implant mucositis, when inflammation occurs only at the level of the mucosa, and peri-implantitis, where the inflammatory process also affects the bone (3). For this reason, preparations that fill the microgap and, therefore, participate in the prevention of peri-implant diseases have appeared on the market over the years. A material called GapSeal (Hager and Werken, Duisburg, Germany) was developed at the University of Dusseldorf. The material is based on a highly viscous silicone base and thymol, which allows it to have long-lasting softness and efficient sealing of the implant interstitial space. Given the impossibility of removal by rinsing, and only being removable by mechanical means, it should provide long-term protection against reinfection from inside the implant. Oxysafe (Hager andWerken, Duisburg, Germany) is a material from the same manufacturer that contains active oxygen molecules, which should provide antimicrobial activity, but the sealing effect is questionable. Flow.sil (Bredent GmbH and Co.KG, Senden, Germany) is a product based on poly-dimethyl-siloxane derivatives that provide stability and rigidity, however, it has questionable antimicrobial activity. Chlorhexidine preparations (CHX) are a broad-spectrum bisguanide antiseptic with proven activity in the prevention and treatment of peri-implant mucositis (5). To the best of our knowledge, there are no recently published studies that investigate the abilities of fungi to penetrate through these new microgap sealants. As a starting point, this research was done in static conditions. This study could provide a clinical benefit by providing evidence for or against the routine use of

microgap sealants. If a particular agent shows a beneficial effect, the next step is to conduct the same study under dynamic loading conditions. The aim of this study is to evaluate, under static conditions, the antimicrobial efficacy and permeability of different chemical materials designed to seal the microgap found at the implant–abutment interface.

3.3 Materials and methods

In this study, 60 titanium dental implants and 60 original prosthetic abutments were used and divided into two main groups regarding bacteria and fungi. GC Aadva Standard Implants (GCTech.Europe GmbH, Breckerfeld, Germany) of 4.0 mm diameter, with a conical connection to the prosthetic abutment, and a platform-switch were used. There were 3 test groups formed (with 6 implants in each) for different sealing materials as follows:

1. GapSeal gel (Hager and Werken, Duisburg, Germany);

2. Oxysafe gel (Hager and Werken, Duisburg, Germany);

3. Flow.sil (Bredent GmbH and Co.KG, Senden, Germany).

One positive control group of 6 implants with chlorhexidine gel (Curasept ADS 350 gel, Curaden International AG, Kriens, Switzerland) and one negative control group without sealants (6 implants) were also formed.

3.3.1. Preparation of dental implants

Dental implants and corresponding original prosthetic abutments were removed from commercial packaging under sterile conditions. After being removed from the sterile package, the dental implants were placed in a vertical position in a sterile clamp using sterile forceps. They were then fixed in a sterile stainless steel clamp (Figure 1) to allow swivel action when tightening the prosthetic abutment at 20 N/cm (as recommended by the manufacturer) and to keep the implants in a vertical position (Figure 2). Prior to the installation of the prosthetic abutment, 0.3 µL of sterile brain heart infusion (BHI) solution (brain infusion solids (12.5 g/L), beef heart infusion solids (5.0 g/L), proteose peptone (10.0 g/L), glucose (2.0 g/L), sodium chloride (5.0 g/L) and disodium phosphate (2.5 g/L), pH 7.4 ± 0.2 at 25 °C) was added to the implants, using a sterile micropipette, to serve as a nutrient medium if bacteria and fungi

penetrate. The test material was then applied to the edge of the implant, depending on the group (Oxysafe, GapSeal, Flow.sil, CHX gel, (Figures 3–5), after which a prosthetic abutment was installed according to the manufacturer's recommendations (20 N/cm for GC Aadva implants (GCTech.Europe GmbH, Breckerfeld, Germany)) (Figure 6). In the case of the negative control group, no test material was applied, but a prosthetic abutment was installed according to the manufacturer's recommendation.

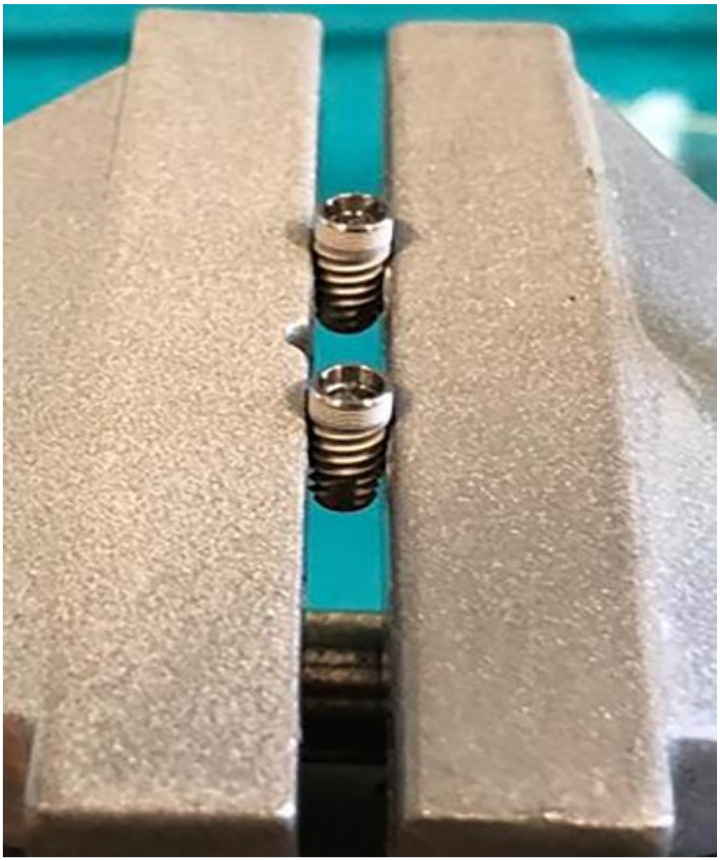

Figure 1. *Dental implants placed in a sterile clamp.*

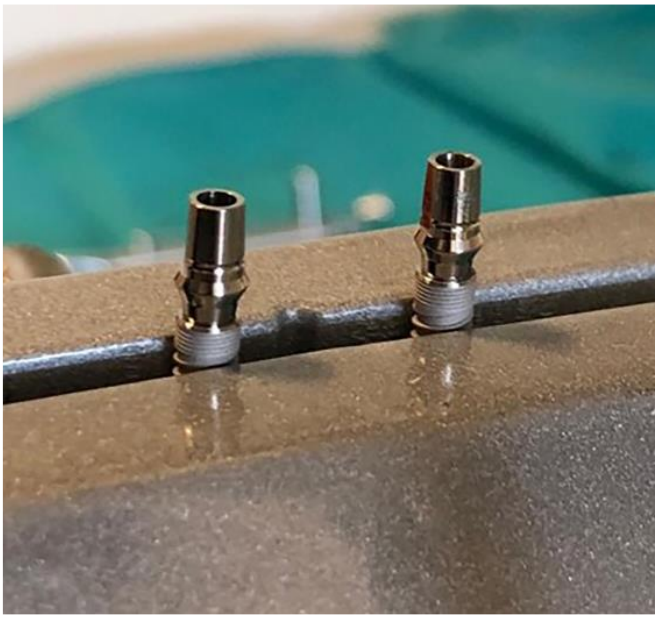

Figure 2. *Implant–abutment compound in a sterile clamp.*

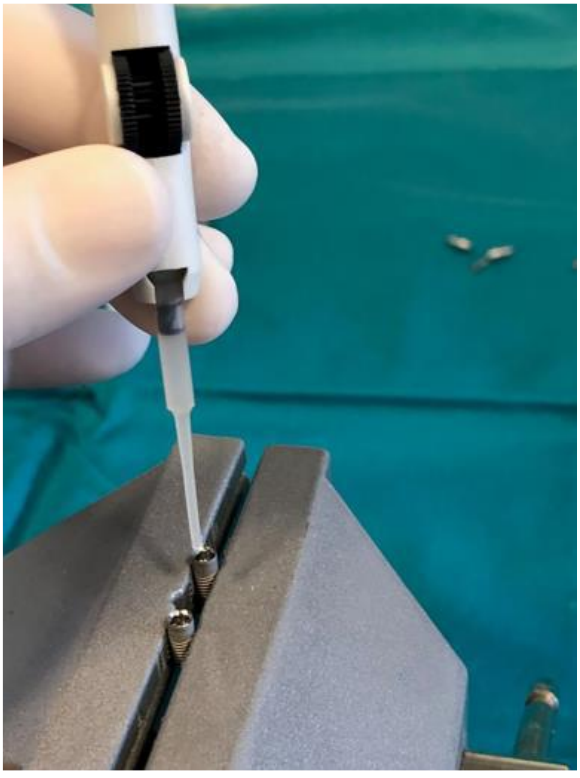

Figure 3. *GapSeal gel application.*

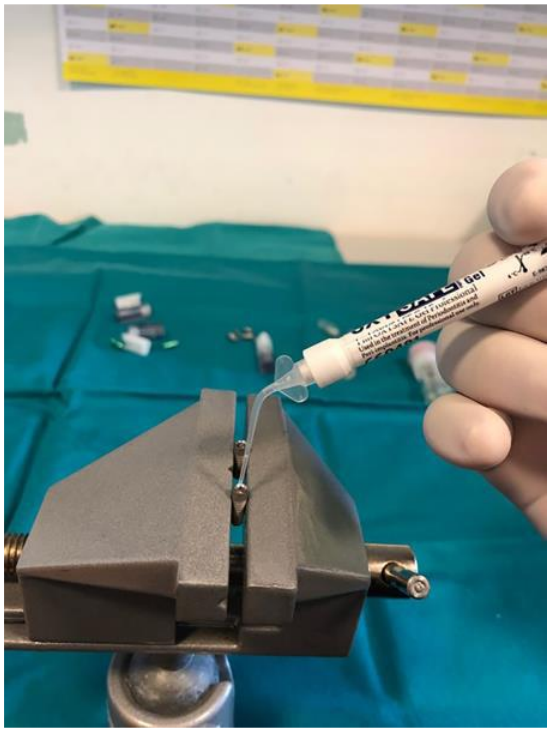

Figure 4. *Oxysafe gel application.*

Figure 5. *Flow.sil material application.*

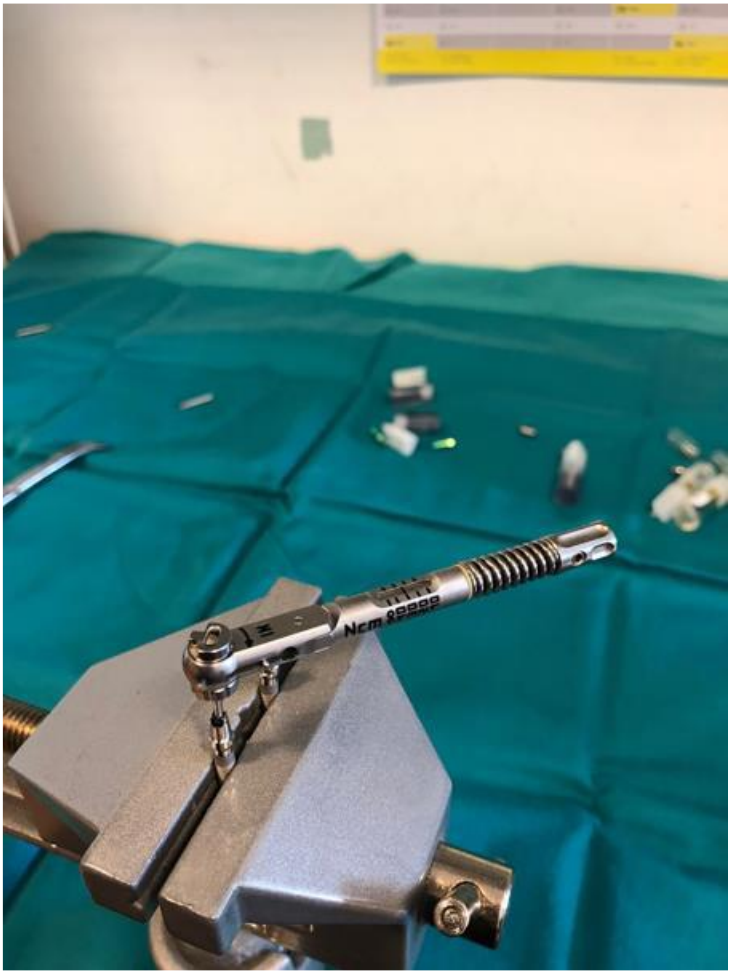

Figure 6. *Tightening of a prosthetic abutment.*

3.3.2. Contamination of dental implants

All microbiological procedures were performed at the laboratory of the Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb. Staphylococcus aureus and Candida albicans strains isolated from a clinical sample at the Clinical Hospital Center Zagreb were used for contaminating the dental implants. The bacteria and fungi were grown separately in Columbia Agar for 72 h and then, using thioglycolate broth, a bacterial and fungal suspension was prepared for each of the microorganisms and mixed together in a joint suspension. A density of 600 nm, equivalent of 1 ×108 CFU/mL (colony forming units per milliliter), was set by optical densitometer (Densimat, Biomerieux, Marcy l'Etoile, France). All dental implant and prosthetic abutment assemblies were immersed for 14 days, under aerobic conditions, in

300 µL of mixed bacterial and fungal suspension (containing S. aureus and C. albicans at a density of 0.5 McFarland), which covered the implant neck abutment (Figure 7).

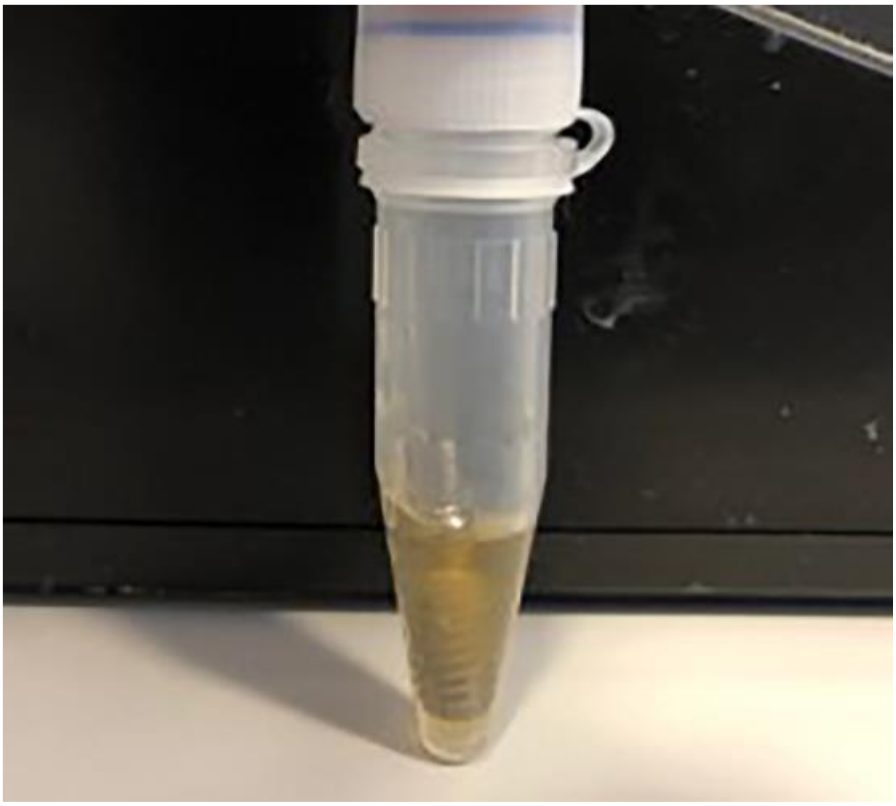

Figure 7. *Implant assemblies immersed in a solution contaminated with S. aureus and C. albicans, in Eppendorf tubes.*

The opening for screw access remained above the level of the suspension to eliminate the impact of the penetration of the contaminated suspension along the screw itself. No sealant or antiseptic treatment was applied to the negative control group samples, but the implant - abutment assemblies were immersed in the contaminated solution for 14 days, under aerobic conditions with an incubation temperature of 35^0C. The positive control group was treated with an antiseptic gel (CHX gel) and then immersed in the contaminated solution for 14 days, under aerobic conditions.

After 14 days of incubation, the samples were removed from the tubes using sterile forceps, then immersed in 70% alcohol for up to 3 min to prevent external contamination. Samples were then dried with sterile gauze before being carefully disassembled in an upright position, in a sterile clamp. After disassembling the samples, the inner surfaces of the implants were sampled using 3 sterile paper sticks/points (Absorbent points, DENTSPLY Maillefer, Tulsa, OK, USA)

(Figure 8), which were then immersed in Eppendorf tubes containing 0.5 mL of sterile phosphate buffered saline (PBS). The contents of the tube, along with the paper sticks, were vortexed for 60 s (Corning® LSE™ vortex mixer, Corning, NY, USA) to remove bacterial and fungal cells (Figure 9).

Figure 8. *Sampling with paper points.*

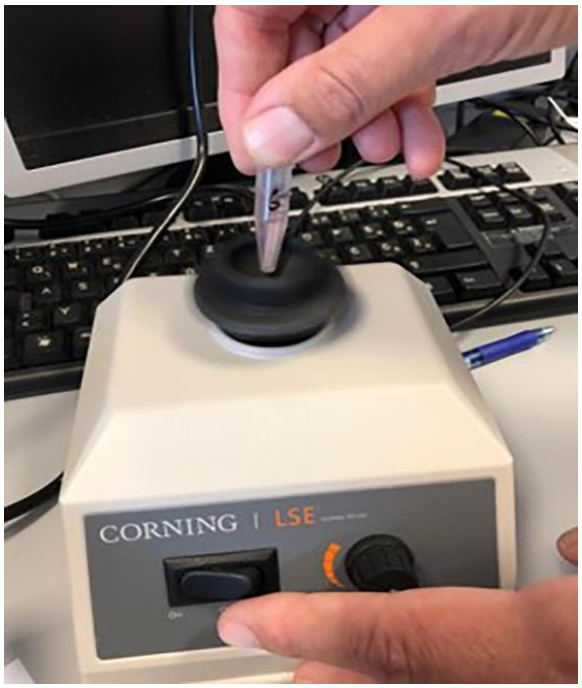

Figure 9. Vortexing.

Samples of the complete tube contents were applied to nutrient microbial media with 5% blood agar, then incubated for 48 h at 37^0C (Figure 10). After that, the resulting colonies were identified and quantified (Figure 11). Macroscopically distinctive colonies were confirmed with MALDI Biotyper (Bruker Daltonics, Hamburg, Germany) and the obtained results were entered into the prepared tables. The results were then determined depending on if there was a presence (positive result) or complete absence (negative result) of bacteria or fungus.

Figure 10. *Application of samples on to blood agar.*

Figure 11. *Blood agar plate ready for CFU/mL analysis.*

3.3.3 Statistical analysis

Statistical analysis was performed using the MedCalc software version 19.2.6 (Ostend, Belgium) with the traditional level of statistical significance set at $p < 0.05$. The efficiency of seals, in comparison to controls, was analyzed using a test for one proportion where proportions of infections with regard to seals were treated as observed, and that with regard to controls as prespecified proportions.

3.4 Results

Using the percentage of infection (Table 1), the seals were compared to controls with regard to Staphylococcus spp. (Table 2) and Candida spp. (Table 3) infection. With regard to Staphylococcus spp. infection, all seals were significantly more efficient compared to the negative control ($p < 0.0001$). However, only Flow.sil performed as well as the CHX gel treatment used in the positive control group ($p = 0.01$). With regard to Candida spp. infection, the GapSeal was significantly more efficient than both control groups, CHX gel ($p = 0.02$) and negative control ($p < 0.0001$). The third sealant material (Flow.sil) was more efficient only compared to the negative control group ($p = 0.002$). In contrast, the Oxysafe and the Flow.sil were significantly less efficient than the positive control ($p = 0.0002$ and $p = 0.01$, respectively).

TABLE 1. Percentage of implant-abutment assemblies that became infected in each group (n = 12 in each group)

Microbe	Sealant material			Controls	
	S_1	S_2	S_3	positive (CHX)	negative (no seal)
Staphylococcus spp.	66.7	83.3	33.3	66.7	100.0
Candida spp.	0.0	83.3	66.7	33.3	91.7

TABLE 2. Comparison between sealant materials and control groups with regard to Staphylococcus spp. infection

Seal	Controls					
	positive (CHX)			negative (no seal)		
	z	95% CI	p	z	95% CI	p
S_1	0.0	34.9; 90.1	1.00	36.4	34.9; 90.1	< 0.0001 *
S_2	1.2	51.6; 97.9	0.22	18.2	51.6; 97.9	< 0.0001 *
S_3	2.5	9.9; 65.1	0.01 *	73.0	9.9; 65.1	< 0.0001 *

* statistically significant; 95% CI – 95% confidence interval, S_1 – GapSeal, S_2 – Oxysafe, S_3 – Flow.sil.

TABLE 3. Comparison between sealant materials and control groups with regard to *Candida spp.* infection

Seal	Controls					
	positive (CHX)			Negative (no seal)		
	z	95% CI	p	z	95% CI	p
S_1	2.4	0.0; 26.6	0.02 *	11.5	0.0; 26.6	< 0.0001 *
S_2	3.7	51.6; 97.9	0.0002 *	1.1	51.6; 97.9	0.29
S_3	2.5	34.9; 90.1	0.01 *	3.1	34.9; 90.1	0.002 *

* statistically significant; 95% CI – 95% confidence interval, S_1 – GapSeal, S_2 – Oxysafe, S_3 – Flow.sil.

3.5 Discussion

This study showed that a complete seal against bacterial infection was not formed at the implant-abutment interface using the tested materials. Regarding fungal infection, only one sealing material helped to prevent microleakage. Presence of other sealing agents helped to reduce microleakage in infections with *Candida spp.* The fungi and bacteria that grew at the implant-abutment interface colonised and percolated through the microgap, then the inner space

of implant acted as a reservoir (6). Analysing the results presented in this study shows that the concept of a complete hermetic seal at the interface is not possible regarding bacterial infection. These findings are in accordance to several studies (7-9). However, the presence of a media at the interface (for example, a gel), reduces the leakage either through having antimicrobial properties or due to a sealing ability. In the negative control group where no sealants were used, the leakage is evidence-based probably due to the lack of complete adaptation between implant-abutment interface an closing the microgap. Despite this fact, according to Duarte et al. (10), screw tightening is important as time passes as this can influence increased microleakage. Leakage may depend on different methods of tightening the implant-abutment connection and the degree of leakage was found to be dependent on the closing torque. Severity of the leakage has an inverse correlation with the degree of closing torque (11). In this study, a 20 N/cm torque was used for implant-abutment connection stability, as recommended by the manufacturer for the oral cavity. In cases where the force applied to the implant-abutment interface was bigger than expected, the screw may have loosened, leading to contamination of the inner space of implants.

In 2007, Zitzman and Berglundh (2) concluded that peri-implant mucositis occurs in up to 50% of cases and peri-implantitis in 12–43% of cases. The composition of the biofilm formed on and around the dental implant shifts from that dominated by gram-positive cocci, to a greater amount of gram-negative anaerobic and facultatively anaerobic bacteria such as *Aggregaticabacter actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides forsythus, Prevotella intermedia* and *Fusobacterium nucleatum* (2). Moreover, observational studies have shown that peri-implantitis is more commonly associated with opportunistic pathogens (such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*) (12,13), fungal organisms (such as *Candida albicans, Candida boidinii, Penicillum sp, Rhadotorula sarycesis* and *Pachaces*) (14,15), and viruses (such as human-cytomegalovirus and Epstein-Barr virus) (16) suggesting a rather complex and heterogeneous infection (17,18).

Colonies of the genus *Candida spp.* were found in periodontal pockets, periodontitis, and in failed implants in studies by Reynaud et al (19) and Dahlen et al (20). *Candida albicans*, a commensal, is a major pathogen in oral and systemic candidiasis and a major fungus in the oral cavity in 20–40% of healthy individuals (21). It is considered to be a major human pathogen in clinical studies, and the incidence of skin and mucosal fungal infections has increased in recent years. In accordance with these findings, and because there is a lack of studies focused on the effectiveness of different types of sealants against leakage of this type of fungus, we decided to contaminate the inner surface of dental implants with *Candida spp.* Several *in vitro* and *in vivo*

studies (22-25) have evaluated the ability of different types of bacteria to penetrate an implant along a microgap with a prosthetic abutment, depending on the geometry of the connection itself. Quirynen et al. (22) described that connections with an external six-fold design are more prone to microorganism invasion. Jansen et al. (23) evaluated the microbial leakage of *Escherichia coli* through 13 different combinations of prosthetic augmentation and implant compounds and showed that internal compounds are more resistant to colonisation. Steinebrunner et al. (24) evaluated the bacterial colonisation of five implant systems with respect to the number of masticatory cycles. Here, they showed that implants with an internal hexagonal connection are more resistant to bacterial leakage under dynamic loading. Koutouzis et al. (25) reported that implants with an internal Morse taper connection have minimal interface colonisation after incubation in bacterial solutions of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Presented *in vitro* study, dental implants with a conical connection to the prosthetic abutment, and a platform-switch were used. It was confirmed that there is microleakage in all bacterial groups and in most fungal groups. We can conclude from these studies that all types of dental implant connections and prosthetic abutments leak bacteria and fungi along the microgap at their connection.

The biofilm made by different types of microorganisms on the external surface of implants is eliminated by immune mechanisms, while internal colonisation can persist and produce an unpleasant, malodorous taste, as well as tissue damage and infections of periodontal tissue (26,27). Trying to prevent such infiltrations in these regions, Duarte et al. (10) recommended the separate use of silicon sealant and chlorhexidine varnish at the cervical part of dental implant, but this was not effective for more than 35 days, demonstrating that they were not able to prevent microleakage. Nayak et al. (11) used GapSeal and concluded that leakage was reduced because of the viscosity of the gel which flows easily throughout the interface, resulting in a better seal. This is in agreement with our results from this study, and with those obtained by Podhorsky et al. (28), where GapSeal was also used. In our results we showed that it had a similar effect to chlorhexidine gluconate (CHX) in *Staphylococcus aureus* infection and better result in *Candida albicans* infection. Duarte et al. (10) showed that a combination of chlorhexidine gluconate with tymol varnish, one of the main components of GapSeal, could reduce the number of microorganisms in the oral cavity for a 45–63 day period, leaving 40% of implant-abutment interfaces intact.

3.6 Conclusion

Based on the findings of this *in vitro* study, we can conclude that application of sealing materials before abutment connection may reduce or prevent peri-implant bacterial and fungal populations. Further research is needed to test these materials under dynamic loading conditions.

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4. Sealing Efficacy of the Original and Third-Party Custom-Made Abutments—Microbiological In Vitro Pilot Study

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4.1 Abstract

Statement of problem: Implant–abutment connection (IAC) is a key factor for the long-term success and stability of implant-supported prosthodontic restoration and its surrounding tissues. Misfit between prosthodontic abutment and implant at the IAC leads to technical and biological complications. Two kinds of prosthodontic abutments are currently available on the market: original and third-party abutments.

Purpose: The aim of this pilot study was to test and compare the internal fit (gap) at the implant–abutment interface depending on the abutment fabrication method based on microbial leakage in static conditions and the need for the use of gap sealing material.

Materials and methods: Two groups of 40 implants were formed on the basis of the type of abutment. In each of the groups of two implant systems, two subgroups of 10 implants were formed. The tested subgroups consisted of 10 implants with sealing material and a negative control subgroups consisting of 10 implants without any sealing material. The test material, GapSeal (Hager and Werken, Duisburg, Germany) was applied in the test subgroups. The implant–abutment assemblies were contaminated with a solution containing *Staphylococcus aureus* and *Candida albicans* for 14 days under aerobic conditions.

Results: Results showed that there was no statistically significant difference regarding the microbial leakage between the original and third-party custom-made abutments, regardless of the use of sealing material.

Conclusions: It can be concluded that the abutment fabrication method has no significant influence on sealing efficacy regarding the bacterial and fungal leakage in static conditions.

4.2 Introduction

Implant-prosthodontic therapy is an established treatment modality in dental practice that provides high success rates (1). Implant–abutment connection (IAC) is recognized as a crucial factor for the long-term success and stability of implant-supported prosthodontic restoration and its surrounding tissues, with emphasis on benefits of original abutments (2). Misfit between such components presents a significant concern because it may lead to mechanical and biological complications (3). The most common and highly researched biological complication is peri-implantitis, which is influenced by plaque accumulation at the level of the IAC (4). The presence of a microgap is unavoidable in two-piece implants, and it is precisely this narrow space that makes a small reservoir of microorganisms interfering with the health of the peri-implant tissue (4). This space is considered to be a critical area in microbial colonization, and also a starting point for peri-implant marginal bone loss (5). Different implant systems use different designs for the IAC, with the main purpose of microleakage prevention and consequential inflammation of peri-implant tissues. They can be classified as internal or external, with internal being the most commonly used. The internal IAC can be further divided into clearance-fit (or straight), conical, and mixed (2). However, possible production imprecision and dynamic masticatory load can result in the aforementioned presence of a microgap and micromotion at the IAC, which directly or indirectly might cause technical damage (2). Even though there is no evidence of complete prevention of miocrobial infiltration through the IAC, there are constant efforts to achieve a tight connection between prosthodontic abutment and implant fixture (6). The microgap varies between 10 and 135 μm according to different implant systems (6,7). This is a wide range of values and, moreover, refers to original prosthetic abutments. Two kinds of prosthodontic abutments are currently available on the market for implant restorative procedures: original and third-party abutments (8). The industry claims that the original parts are better in terms of fit and reduced microleakage (8). Given the vast possibilities for combinations of variables in implant-prosthodontic rehabilitation, the abutment fabrication method should be carefully evaluated. Regarding these facts, there are materials on the market that are declared to seal the gap at the IAC in order to eliminate microleakage, thus reducing or eliminating biological complications (9). GapSeal (Hager and Werken, Duisburg, Germany) is such a material, and is based on a highly viscous silicone matrix with thymol. It remains durably viscous and can be removed only by ethanol or by mechanical means. Considering the given information, it should provide long-term protection, avoiding auto- and re-infections by possible microbial accumulation at the IAC (10).

Currently, only a limited number of investigations comparing the leakage of original and third-party abutments with the internal type of IAC are available. Therefore, the purpose of this study was to test and compare the internal fit (gap) at the IAC depending on the abutment fabrication method (original and third-party) based on bacterial and fungal leakage in static conditions. A comparison was performed for both straight and conical types of IAC. Additionally, the antimicrobial efficacy and need for the use of gap sealing material was tested.
The null hypothesis was that the abutment fabrication method would have no influence on the internal fit at the IAC, regardless of the connection type and use of a sealing agent.

4.3 Materials and Methods

4.3.1 Study design

This microbiological in vitro pilot study was approved by the Ethics Committee of the School of Dental Medicine University of Zagreb (protocol code: 05-PA-30-XII-12/2019 on 5 December 2019) and performed at the laboratory of the Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb. The microbiological preparation and sampling methodology itself was developed based on recent pilot study by Smojver et al. (9). The developed protocol has been tested repeatedly, in particular for static in vitro test conditions.
A total of 80 titanium dental implants were used in the study, of which 40 were GC Aadva Standard implants (GCTech.Europe GmbH, Breckerfeld, Germany), with a conical type of connection, and 40 were Zimmer Tapered Screw-Vent implants (Zimmer Biomet Dental, Palm Beach Gardens, FL, USA) with a straight type of connection. The implants were divided into two groups each, regarding the type of prosthetic abutment (A and B).
Group A consisted of 20 GC Aadva Standard implants (GCTech.Europe GmbH, Breckerfeld, Germany) of 4.0 mm diameter and 20 Zimmer Tapered Screw-Vent implants (Zimmer Biomet Dental, Palm Beach Gardens, FL, USA) of 4.1 mm diameter, both connected to their respective original factory-made prosthodontic abutments.
Group B consisted of 20 GC Aadva Standard implants of 4.0 mm diameter and 20 Zimmer Tapered Screw-Vent implants of 4.1 mm diameter, both connected to respective third-party custom-made prosthodontic abutments. The abutments were designed in Exocad Galway 3.0 (Exocad GmbH, Darmstadt, Germany). Computer-aided design (CAD) data were sent to computer-aided manufacturing (CAM) software (Mayka Dental 5.1,

PicaSoft, Vierzon, France) and then to a Yenadent DC40 milling machine (Yenadent, Vierzon, France). The abutments were milled from a Colado CAD Ti5 (Ivoclar Vivadent AG, Schaan, Liechtenstein) titanium alloy. In each of the groups (A and B), four subgroups of 10 implants were formed. Ten implants per group were required for the study according to the statistical power analysis. The two tested subgroups consisted of 10 Zimmer and 10 GC implants with sealing material and two negative control subgroups consisted of 10 Zimmer and 10 GC implants without any sealing material. GapSeal gel (Hager and Werken, Duisburg, Germany) was used as a sealant. According to the results obtained in the recent study by Smojver et al. (7), it showed the highest values in microbial leakage prevention, so it was the material of choice in this study (Figure 1).

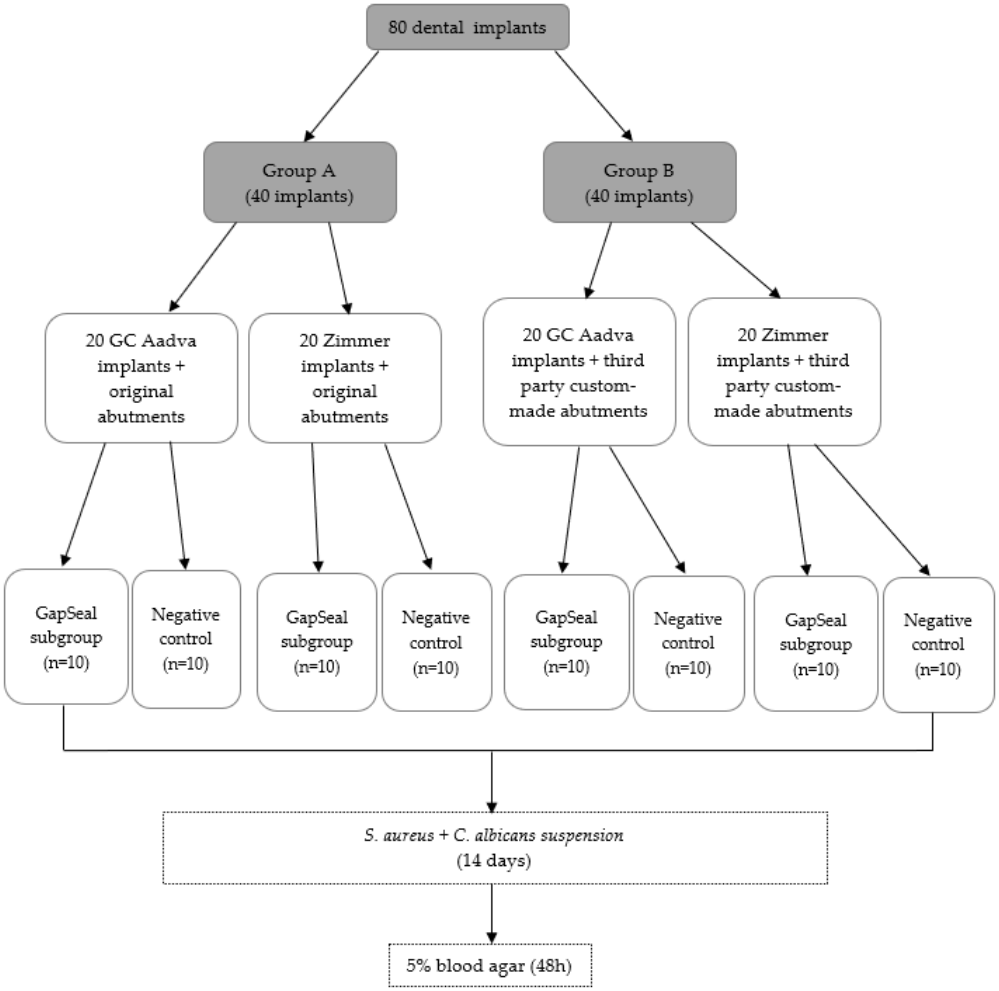

Figure 1. Flow chart illustrating the study design and division of the groups.

4.3.2. Preparation of the IAC

Each dental implant and original complementary abutment were removed from their commercial sterile packaging. Custom-made third-party abutments were sterilized in Euroklav 23 VS+ (Melag, Berlin, Germany) before use. All dental implants were placed in a strictly vertical position in a sterile stainless-steel clamp using sterile stainless-steel forceps (Henry Schein, Melville, NY, USA). Then, they were fixed in the clamp that allowed for a firm swivel action when tightening the prosthetic abutment to the values recommended by the respective manufacturer (20 N/cm for GC Aadva Standard and 30 N/cm for Zimmer Tapered Screw-Vent implants). The clamp also kept the implants in the desired vertical position (Figure 2).
Preceding the installation of the prosthetic abutment, a sterile micropipette (Merck KGaA, Darmstadt, Germany) was used to add 0.3 µL of sterile brain heart infusion (BHI) broth (calf brains (12.5 g/L), beef heart infusion solids (5.0 g/L), D-glucose (2.0 g/L), proteose peptone (10.0 g/L), disodium hydrogen phosphate (2.5 g/L) and sodium chloride (5.0 g/L) at a pH 7.4 ± 0.2 and 25 °C to the implants as a non-selective nutrient media in case of bacterial and fungal penetration. GapSeal (Hager and Werken, Duisburg, Germany) was applied to the internal surface of the implants (Figure 3) in the tested subgroups, while the negative control subgroups did not receive the treatment with sealing material. Regardless of sealant use, prosthetic abutments were installed according to the manufacturer's recommendation (Figure 4).

Figure 2. Dental implant fixed in a sterile stainless-steel clamp.

Figure 3. GapSeal gel applied on the implant.

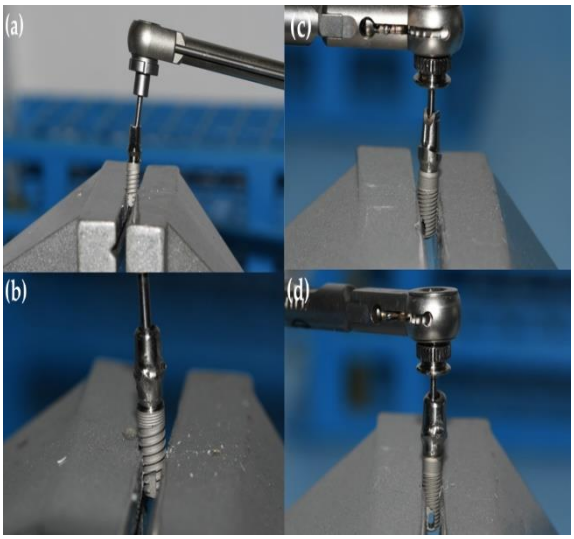

Figure 4. Tightening of the prosthetic abutment. (**a**) GC Aadva Standard implant with original abutment; (**b**) GC Aadva implant with third-party custom-made abutment; (**c**) Zimmer Tapered Screw-Vent implant with original abutment; and (**d**) Zimmer Tapered Screw-Vent implant with third-party custom-made abutment.

4.3.3 Contamination of implant–abutment interfaces

Dental implants were contaminated by *Staphylococcus aureus* and *Candida albicans* strains isolated from a clinical sample at Clinical Hospital Centre Zagreb. Firstly, bacterial and fungal strains had been grown separately in Columbia Agar for 72 h following the preparation of separated bacterial and fungal suspensions using thioglycolate broth. They were then mixed together in a joint suspension. An optical densitometer (Densimat, Biomerieux, Marcyl'Etoile,

France) was used to set a density of 600 nm, which is equivalent to 1×108 colony forming units per milliliter (CFU/mL). All dental implants with installed prosthetic abutments (implant–abutments assemblies) were immersed in 300 µL of mixed bacterial and fungal joint suspension for 14 days under aerobic conditions with an incubation temperature of 35 °C (Figure 5). The suspension contained *S. aureus* and *C. albicans* at a density of 0.5 McFarland.

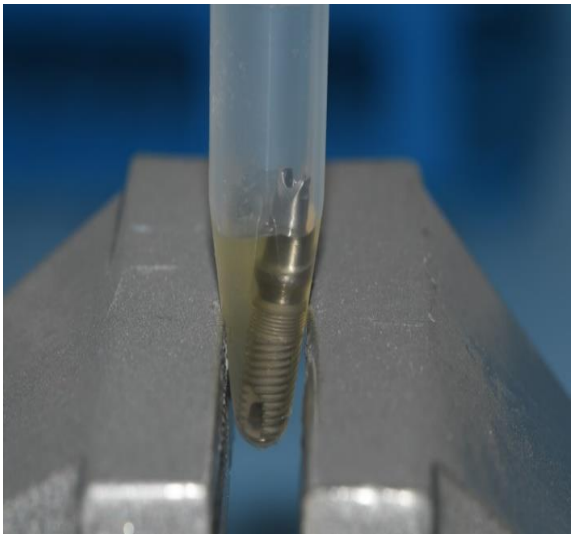

Figure 5. Implant–abutment assembly immersed in bacterial and fungal joint suspension.

The abutment screw access hole remained above the level of the suspension to eliminate the impact of the penetration of the contaminated suspension along the fixation screw itself.

The implant–abutment assemblies were removed from Eppendorf tubes after 14 days using sterile forceps, following immersion in 70% ethanol for up to 3 min to prevent external contamination. Then, the samples were dried with sterile gauze and put in a sterile clamp. They were carefully disassembled in a strictly vertical position. After the abutments were removed, samples were taken from the internal surfaces of the implants using three sterile paper points (Absorbent points, DENTSPLY Maillefer, Tulsa, OK, USA) (Figure 6), which were then immersed in the Eppendorf tubes containing 0.5 mL of sterile phosphate buffered saline (PBS) solution. The tubes with paper points were inserted into a vortex mixer (Corning® LSE™ vortex mixer, Corning, NY, USA) for 60 s to extract bacterial and fungal cells (Figure 7).

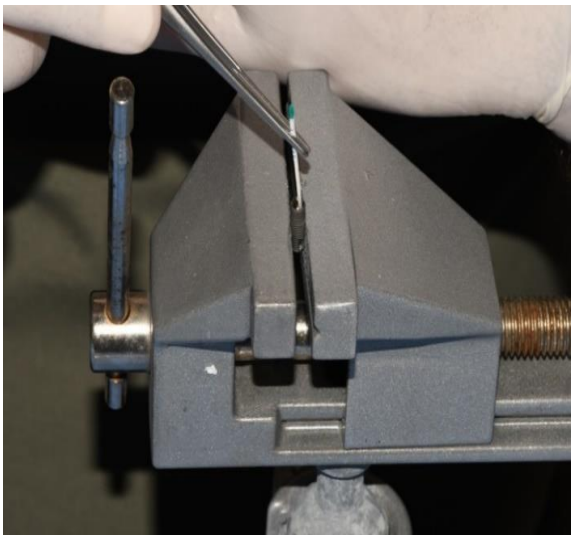

Figure 6. Sampling the implants with paper points.

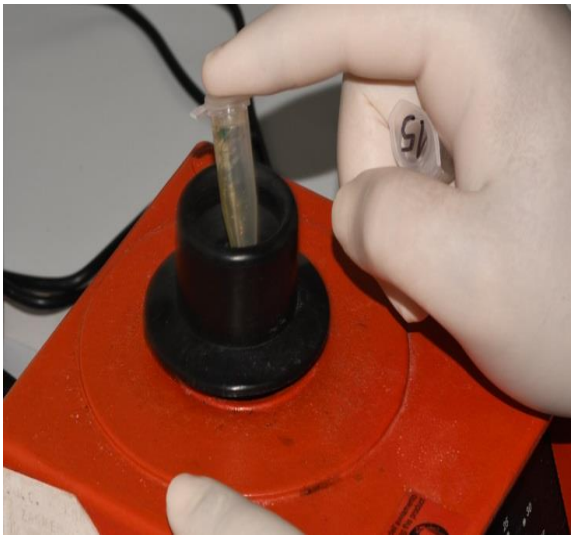

Figure 7. Vortexing.

Samples of the tube contents were applied on to 5% blood agar and incubated for 48 h at 37 °C (Figure 8). The resulting colonies were then identified, and quantification was performed. For each sample, the CFU/mL was counted. A MALDI Biotyper (Bruker Daltonics, Hamburg, Germany) was used to verify macroscopically distinctive colonies (Figure 9), and the obtained results underwent further analysis.

Figure 8. Application of the sample on to 5% blood agar.

Figure 9. Colonies of *Staphylococcus aureus* and *Candida albicans* on 5% blood agar.

4.3.4 Statistical analysis

Statistical analysis was performed using Fischer's exact test, with the traditional level of statistical significance set at $p < 0.05$. Statistical calculation was performed using MedCalc software version 20.014 (Ostend, Belgium).

4.4 Results

The results were determined based on a frequency of bacterial or fungal microleakage. The presence of *S. aureus* or *C. albicans* signifies a positive result, and complete absence of these bacteria signified a negative result.

According to the frequencies of bacterial and fungal leakage (Tables 1 and 2), the third-party custom-made prosthodontic abutments were compared to the original factory-made prosthodontic abutments with regard to infection with Staphylococcus spp. and Candida spp. (Table 3) using the *p*-values of Fisher's exact test. The abutment fabrication method had no influence on the internal fit at the IAC regarding microleakage since the *p*-values of Fisher's exact test were greater than the set level of significance ($p > 0.05$), with the lowest *p*-value being 0.4737 (Table 3). Furthermore, there was no statistically significant relationship between the original and third-party abutments with respect to the type of connection, since *p*-values changed by comparable, statistically non-significant amounts in both GC (conical connection) and Zimmer (straight connection) models (Table 3).

Table 1. The frequencies of bacterial and fungal microleakage (Zimmer Tapered Screw-Vent implants).

Microbe	Original Abutments (%)	Third-party Custom-Made Abutments (%)	Original Abutments with GapSeal (%)	Third-Party Custom-Made Abutments with GapSeal (%)
Staphylococcus aureus	80.00 (8/10)	100.00 (10/10)	50.00 (5/10)	70.00 (7/10)
Candida albicans	60.00 (6/10)	80.00 (8/10)	20.00 (2/10)	30.00 (3/10)

Table 2. The frequencies of bacterial and fungal microleakage (GC Aadva Standard implants).

Microbe	Original Abutments (%)	Third-party Custom-Made Abutments (%)	Original Abutments with GapSeal (%)	Third-party Custom-Made Abutments with GapSeal (%)
Staphylococcus aureus	90.00 (9/10)	100.00 (10/10)	60.00 (6/10)	60.00 (6/10)
Candida albicans	60.00 (6/10)	80.00 (8/10)	20.00 (2/10)	30.00 (3/10)

Table 3. Comparison of Fisher's exact test values for microleakage between original and third-party custom-made prosthodontic abutments.

Implant	Zimmer		GC	
Fisher Exact Test (*p*-Values)	*Staphylococcus aureus*	*Candida albicans*	*Staphylococcus aureus*	*Candida albicans*
Original prosthodontic abutment	H0 accepted (0.4737)	H0 accepted (0.6285)	H0 accepted (0.5000)	H0 accepted (1.0000)
Third-party custom-made prosthodontic abutment	H0 accepted (0.6499)	H0 accepted (1.0000)	H0 accepted (0.6285)	H0 accepted (1.0000)

* Statistically significant ($p < 0.05$). ** Null hypothesis: the abutment fabrication method would have no influence on the internal fit at the IAC, regardless of the connection type and use of a sealing agent.

There was no statistically significant relationship between the original and third-party abutments regarding microleakage when gap sealing material was used (Table 4). Data in Table 4 suggest there was more of an impact with sealing material usage in GC implants when compared with Zimmer implants ($p = 0.0867$ for Staphylococcus aureus in GC and $p = 0.2105$ in Zimmer implants), although it was not statistically significant.

Table 4. Comparison of Fisher's exact test values for microleakage with and without application of sealing material (GapSeal).

Implant	Zimmer		GC	
Fisher Exact Test (*p*-Values)	*Staphylococcus aureus*	*Candida albicans*	*Staphylococcus aureus*	*Candida albicans*
Without sealing material	0.3498	0.1698	0.3034	0.1698
With sealing material	0.2105	0.0698	0.0867	0.0698

* Statistically significant ($p < 0.05$).

Table 5 shows the mean counts of *S. aureus* and *C. albicans* and the influence of different types of connections, abutments, and usage of sealing material on the amount of leaked microbiota. The microbial counts from Table 5 are separately presented in column charts for both GC and Zimmer implants (Figures 10–13). There were no significant differences in leaked counts between different types of connections, abutments and with or without sealing material.

Table 5. Mean counts of Staphylococcus aureus and Candida albicans detected on the internal surface of the implants depending on the abutment fabrication method (original and third-party) and the need for the use of the gap sealing material.

	Staphylococcus aureus	*Candida albicans*
Zimmer	CFU/mL mean +/− SD (median)	CFU/mL mean +/− SD (median)
Negative control original abut.	11.2 +/− 7.9 (11)	1.3 +/− 1.34 (1,5)
Negative control third-party abut.	76 +/− 24.59 (80)	6.2 +/− 3.82 (6)
GapSeal original abut.	5.8 +/− 6.89 (4)	0.3 +/− 0.67 (0)
GapSeal third-party abut.	32 +/− 25.3 (40)	2.8 +/− 4.54 (0)
GC Aadva	CFU/mL mean +/− SD (median)	CFU/mL mean +/− SD (median)
Negative control original abut.	15.2 +/− 6.68 (18)	1.5 +/− 1.58 (1.5)
Negative control third-party abut.	66 +/− 25.03 (60)	8.6 +/− 5.08 (10)
GapSeal original abut.	8 +/− 7.89 (10)	0.3 +/− 0.67 (0)
GapSeal third-party abut.	46 +/− 44.27 (50)	2.2 +/− 3.82 (0)

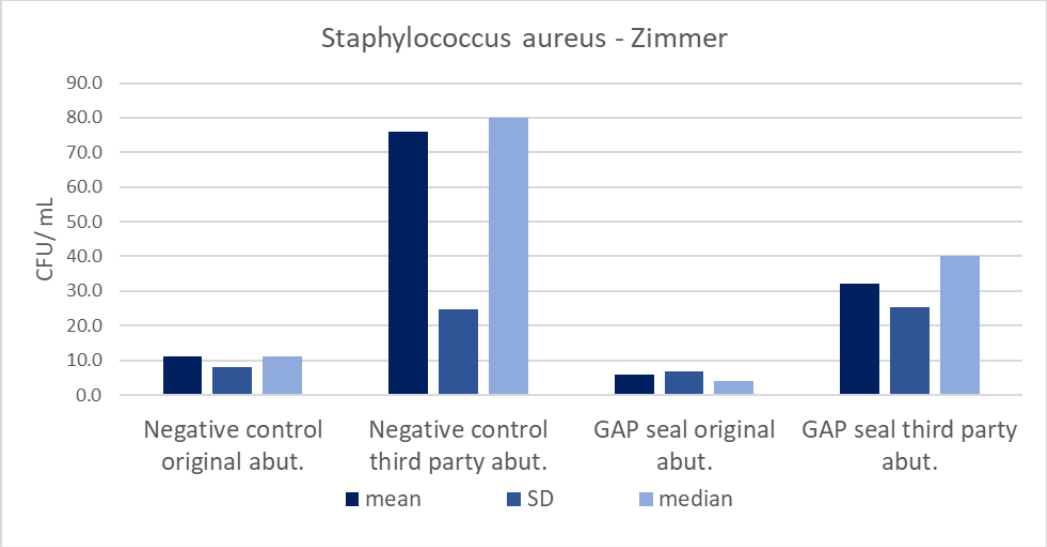

Figure 10. Mean counts of *Staphylococcus aureus* detected on the internal surface of Zimmer implants depending on the abutment fabrication method (original and third-party) and the need for the use of the gap sealing material.

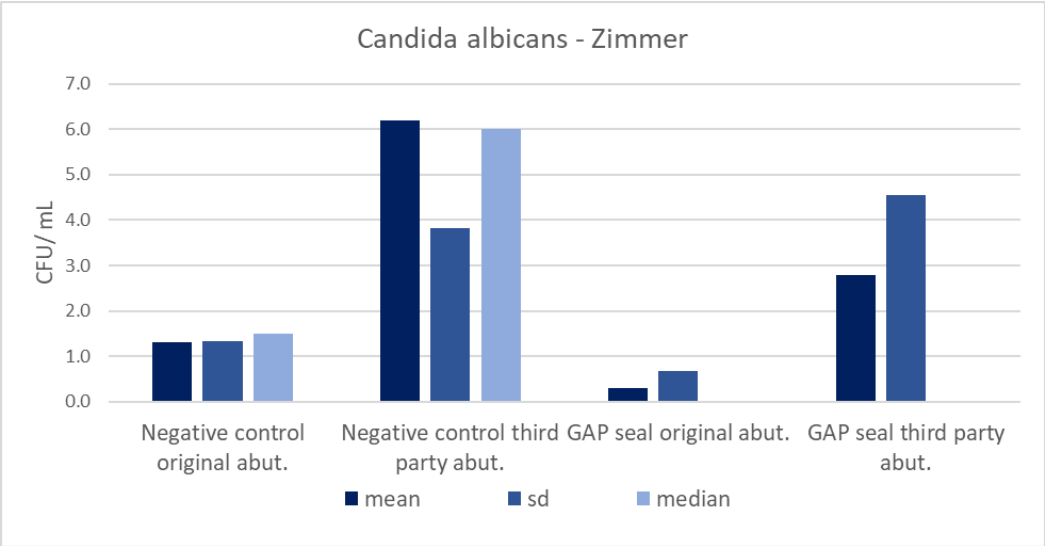

Figure 11. Mean counts of *Candida albicans* detected on the internal surface of Zimmer implants depending on the abutment fabrication method (original and third-party) and the need for the use of the gap sealing material.

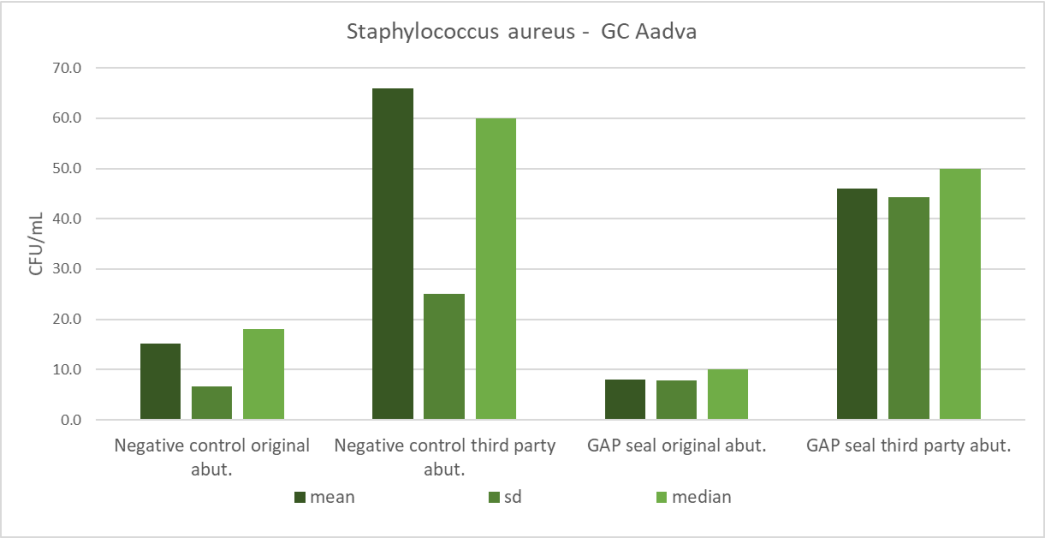

Figure 12. Mean counts of *Staphylococcus aureus* detected on the internal surface of GC implants depending on the abutment fabrication method (original and third-party) and the need for the use of the gap sealing material.

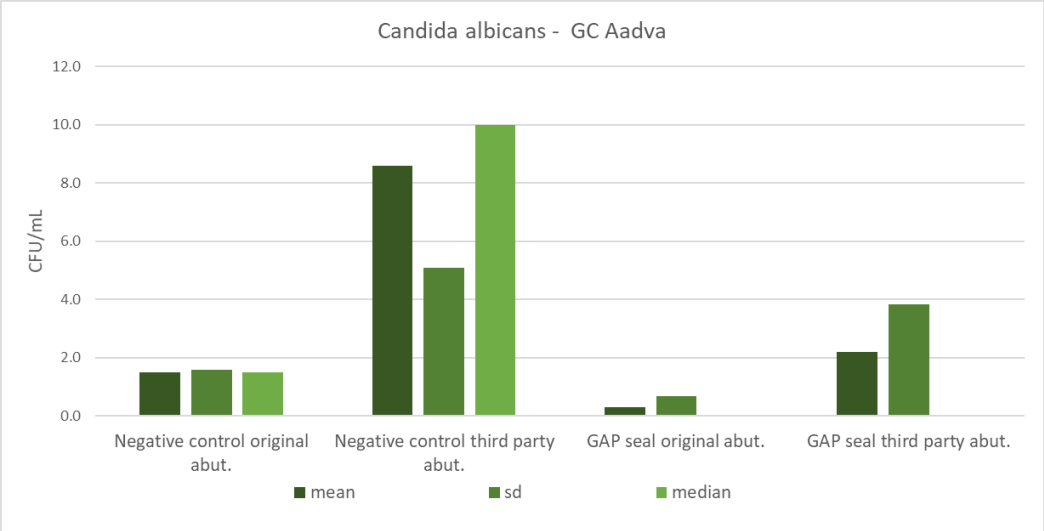

Figure 13. Mean counts of *Candida albicans* detected on the internal surface of GC implants depending on the abutment fabrication method (original and third-party) and the need for the use of the gap sealing material.

4.5 Discussion

The presented *in vitro* study tested and compared the gaps of the straight and conical IACs depending on the abutment fabrication method based on bacterial and fungal leakage in static conditions, as well as the antimicrobial efficacy of the sealing material. The null hypothesis was accepted, with findings that the prosthodontic abutment fabrication method was not crucial for successful implant-prosthodontic therapy regarding microbial leakage at the IAC in static conditions. Understanding the pathogenesis of peri-implant diseases, the fabrication method of prosthodontic abutments, and the biomechanical role of IAC is of utmost importance in achieving successful clinical results in implant-prosthodontic therapy.

Considering the finding that bacterial composition of the biofilm formed on dental implants closely resembles that of the neighboring teeth, a switch from peri-implant health to peri-implant mucositis is therefore comparable to gingivitis in terms of bacterial flora (11). The same postulate is applied in transition to peri-implantitis, which is accompanied by anaerobic species that are commonly found in periodontitis (12). The biofilm formed around the dental implants is initially dominated by Gram-positive cocci, but eventually shifts to Gram-negative anaerobic and facultatively anaerobic bacteria, such as *Aggregaticabacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* (13). Moreover, it was observed that peri-implantitis is often associated with opportunistic pathogens

(*Staphylococcus* spp.) and fungal organisms (*Candida* spp.) (14). Significantly higher counts of *S. aureus* and *S. anaerobius* were detected in implants with peri-implantitis when compared to those of healthy implants (15). The oral microbiome has more than 100 fungal species, and *C. albicans* plays an important role in the formation and stabilization of biofilm, consequently enabling the development of peri-implant mucositis and peri-implantitis (16). In addition, *C. albicans* and *S. aureus* are rarely associated with periodontal disease, but possess the ability to attach themselves to titanium surfaces (17). Taking these findings into consideration, it was decided that dental implants in this study would be contaminated with *S. aureus* and *C. albicans*, as they are the most important microorganisms that cause inflammation of the soft and hard tissues around dental implants.

Although the differences in microbial leakage between the original and non-original third-party prosthodontic abutments were not statistically significant, non-original third-party abutments showed a more frequent prevalence of infection through the IAC. This result is in accordance with findings from a study by Alonso-Pérez et al. (18). They concluded that laser-sintered non-original abutment gaps were within the clinically acceptable range of discrepancy. On the other hand, the same authors, in another study, found that original abutments were highly superior to non-original certified abutments in dynamic conditions, but no statistically significant differences were found in static load behavior (19). It was also observed that the use of non-original abutment components with original Astra Tech implants showed significant leakage at the IAC in static conditions when compared to the use of original prosthetic abutments from same manufacturer (20). Since the aforementioned study was also performed in static conditions, it is important to highlight that the results were contrary to the results of this study. From a recent systematic review of in vitro studies by Tallarico et al. (8), it was concluded that the original abutments were superior in terms of marginal accuracy, mechanical outcomes and microleakage in the majority of included studies. Nevertheless, they pointed out that in vitro studies had a high risk of bias, and the outcomes reported in these systematic reviews should be carefully interpreted. According to some authors (21,22), abutment screw closing torque can influence the increased microleakage, and the severity of leakage has an inverse correlation with closing torque. Thus, it is of utmost importance to install the prosthetic abutment to the manufacturer's recommendation. In daily clinical practice, non-original abutments are often selected for financial reasons. Higher leakage values and possible negative mechanical outcomes could be related to many issues that do not allow for exact replication of components, resulting in discrepancies in the dimensions, shape, and design of connecting surfaces. These micromovements at the IAC cause a pumping effect that transports microorganisms from the

exterior to the interior surface of the implant and vice versa, creating a vicious circle that results in ongoing infection. In addition to biological issues, further transition of forces from IAC to the implant itself increases the stress on marginal bone level (20). Precision level and quality control of materials during the manufacturing process are other important factors that must be considered (8).

Further analysis of the results of this study showed that the use of sealing material did not make a statistically significant difference in microleakage at the implant–abutment interface compared to those without sealant. However, GapSeal reduced the amount of leaked microbiota, especially in combination with GC Aadva Standard implants. These improvements were not statistically significant, but gave valuable insights for further studies. A complete hermetic seal at the IAC is not achievable, according to the contemporary literature (9,23,24). The difference between original and third-party abutments regarding microleakage when sealing material is used is inevitably related to internal fit at the IAC. Therefore, it is precisely the marginal accuracy and appropriate design of non-original abutments that play vital roles in the elimination of microleakage. Smojver et al. (9) and Biscoping et al. (25) confirmed that the presence of the sealing agent may be useful in reducing microbial infiltration into the implants. It was concluded that the application of sealing material before abutment connection may reduce the bacterial and fungal populations of the peri-implant, but a complete seal against bacterial infection was not formed at the implant–abutment interface when using different sealing materials (GapSeal, Oxysafe and Flow.sil) (9). Biscoping et al. (25) found that the tested sealing materials (Clorhexamed 1% gel and Berutemp) did not influence the gap at the IAC, but the same materials also decreased the torque necessary for loosening the abutment screws. This finding suggests that sealing agents might contribute to negative mechanical outcomes affecting the reverse torque values. Seloto et al. (26) observed that sealing gel (Loctite 2400) promoted lower vertical misfit values at the IAC and preload maintenance of screw-retained prostheses after mechanical cycling. Furthermore, Yu et al. (27) concluded that the GapSeal material reduced microleakage at the IAC after dynamic loading and reported evident abutment screw thread wear protection in three different implant systems with internal conical connection. It is important to emphasize that dynamic conditions in which that study was conducted contributed to different outcomes and plausible major advantages of sealing material usage when compared to those in static conditions.

Additionally, the presented results did not show a statistically significant difference between original and third-party abutments regarding the type of connection. There is a lack of studies that compare these two types of abutments and the influence of connection type on

microleakage at the same time. Considering the type of connection alone, there are various studies observing the connection type with minimal microleakage. De Sousa et al. (28) observed that the external hexagonal connection was more effective than the Morse Taper connection against microbial infiltration for dual species biofilms. Conversely, Quirynen et al. (29) described that connections with an external six-fold design were more prone to microbial invasion. There is also evidence that implants with an internal hexagonal connection are more resistant to bacterial leakage under dynamic loading (30). The superiority of a conical connection regarding seal performance, gap formation and mechanical stability has also been demonstrated in the literature due to the homogeneous spread of the load (31). Therefore, the aforementioned studies support the results of this study and, although there was no statistically significant difference between a conical connection and straight connection, GC Aadva implants with a conical connection had slightly better results in combination with sealing material regarding microleakage.

Within the limitations of this *in vitro* study, primarily a static testing condition and sample size, interesting scientific results were found. However, a larger sample size is needed in future studies, considering the high standard deviation values in the results, and further extensive clinical research should be conducted to assess the outcomes of this study.

4.6 Conclusions

According to the presented results, the abutment fabrication method had no significant influence on the sealing efficacy of the IAC regarding the leakage of bacteria and fungi. Considering the discussed limitations of this study, third-party custom-made abutments represent a viable solution from a microbiological point of view. It is not mandatory to use sealing material, since there was no statistically significant difference in microleakage relative to the presence of the sealing material regardless of the type of abutments. These findings gave important evidence to support studies that would provide more clinical evidence about the long-term outcomes of custom-made abutments and their sealing efficacy.

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5. Antimicrobial Efficacy and Permeability of Various Sealing Materials in Two Different Types of Implant -Abutment Connections

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5.1 Abstract

Statement of problem: The presence of a microgap along an implant–abutment connection (IAC) is considered the main disadvantage of two-piece implant systems. Its existence may lead to mechanical and biological complications. Different IAC designs have been developed to minimise microleakage through the microgap and to increase the stability of prosthodontic abutments. Furthermore, different sealing materials have appeared on the market to seal the gap at the IAC.

Purpose: The purpose of this study was to evaluate the antimicrobial efficacy and permeability of different materials designed to seal the microgap, and their behaviour in conical and straight types of internal IACs.

Materials and methods: One hundred dental implants with original prosthodontic abutments were divided into two groups of fifty implants according to the type of IAC. Three different sealing materials (GapSeal, Flow.sil, and Oxysafe gel) were applied in the test subgroups. The contamination of implant–abutment assemblies was performed by a joint suspension containing *Candida albicans* and *Staphylococcus aureus*.

Results: It was concluded that the IAC type had no significant influence on microleakage regarding microbial infection. No significant difference was found between the various sealing agents. Only one sealing agent (GapSeal) was found to significantly prevent microleakage.

Conclusions: A complete hermetic seal was not achieved with any of the sealing agents tested in this study.

5.2 Introduction

Continuous improvements in implant design, surgical protocols, and the development of biologically oriented materials have turned implantology into predictable therapy with a high success rate of 90% (1). Two-piece implant systems consist of the fixture or the implant itself and a prosthodontic abutment which is connected to the implant (2). It is precisely the implant–abutment connection (IAC) that is considered the key factor in the long-term stability of the implants and peri-implant tissue health (3). The main disadvantage of two-piece implant systems is a microgap which persists along the IAC even though the prosthetic abutment is fixed to the implant with the abutment screw (4). This gap occurs alongside the abutment fixation screw threads and at the bottom of the screw (5). The value of the microgap varies between 10 and 135 μm, and its existence may lead to biological and mechanical complications (6). Taking into consideration the average dimensions of bacteria—from 0.2 to 1.5 μm in width and from 1 to 10 μm in length—and the aforementioned microgap values, it is obvious that this space between the prosthodontic abutment and the dental implant acts as a reservoir for microorganisms (4). Micromovements of the abutment consequently transport microorganisms through the IAC to the interior surface of the implant system and vice versa, which may lead to infections of peri-implant tissues (7). In the most unfavourable cases, microbial colonisation can lead to peri-implantitis, characterised by rapid peri-implant bone resorption and loss of osseointegration (4). From a mechanical point of view, a microgap permits micromovements and rotation of the abutment, thus reducing the reverse torque value of the abutment screw, which leads to screw loosening and screw fracture in severe cases (8). Different IAC designs have been developed to minimise microleakage through the microgap and to increase the stability of prosthodontic abutments. They are primarily divided into internal and external connection types. External IACs were developed first, but internal types are currently more frequently used. Internal IACs, with a connection feature inferior to the coronal surface of the implant, can be divided into straight or clearance-fit, conical, and mixed (3). Internal connections also exist in various forms, such as hexagon, octagon, cylinder hex, cone screw, and spline (3). According to the contemporary literature, the internal connection type provides better mechanical and biological outcomes, including a microbial seal, than the external connection type (2). Due to the possibility of microbial colonisation and leakage at the IAC, materials that are declared to seal the gap at the IAC have appeared on the market (9). GapSeal (Hager and Werken, Duisburg, Germany) is a sealing material based on a highly viscous silicone matrix with thymol. It has a long-lasting viscosity and can be removed only by ethanol

or by mechanical means; therefore, it should provide prolonged protection against auto- and re-infections at the IAC (7). Thymol has been shown to possess antimicrobial properties such as bacteriostatic activity against most of the Gram-positive and Gram-negative bacteria, and antifungal activity inhibiting *Candida albicans* MTCC 227 biofilm formation (1). Silicones have been used as sealants and adhesives in a broad variety of fields, dental medicine included. They have a low surface tension and thus are capable of wetting various surfaces. Furthermore, their elastic behaviour enables them to absorb movements without tearing away from the adjacent material or tearing themselves apart. Silicones are also biocompatible and resistant to the dynamic conditions found in the oral cavity (11). Another sealing material is Oxysafe Gel (Hager and Werken, Duisburg, Germany), which is used in the treatment of periodontitis and peri-implantitis. It contains patented active oxygen technology with antimicrobial activity, but there is a lack of observed data on sealing efficacy (12). In addition, a sealant based on a polydimethylsiloxane matrix with the addition of thymol is Flow.sil (Bredent GmbH and Co.KG, Senden, Germany). It is declared to ensure reliable sealing through even distribution and thus prevents the harbouring of microorganisms (9).

Since there is no recent literature investigating the efficacy of various sealing materials and comparing their effects in two different types of IACs, the aim of this study was to evaluate, under static conditions, the antimicrobial efficacy and permeability of different materials designed to seal the microgap, and their behaviour in conical and straight types of internal IACs. The null hypothesis was that there would be no difference in antimicrobial efficacy of the various sealing materials in the two different types of IACs.

5.3 Materials and Methods

5.3.1 Study Design

This in vitro pilot study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb (protocol code: 05-PA-30-XII-12/2019 73 on 5th December 2019.). Microbiological preparation, sampling, and processing of samples were performed at the laboratory of the Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb. The methodology was developed based on the protocol used in the recent pilot study by Smojver et al. (2), which has been tested repeatedly for in vitro static conditions.

One hundred dental implants with original prosthodontic abutments were evaluated in this study and divided into two main groups of fifty implants according to the type of IAC. The implants used in this study were Zimmer Tapered Screw-Vent implants (Zimmer Biomet Dental, Palm Beach Gardens, FL, USA) with a straight connection (Figure 5) and GC Aadva Standard implants (GCTech.Europe GmbH, Breckerfeld, Germany) with a conical connection (Figure 6).

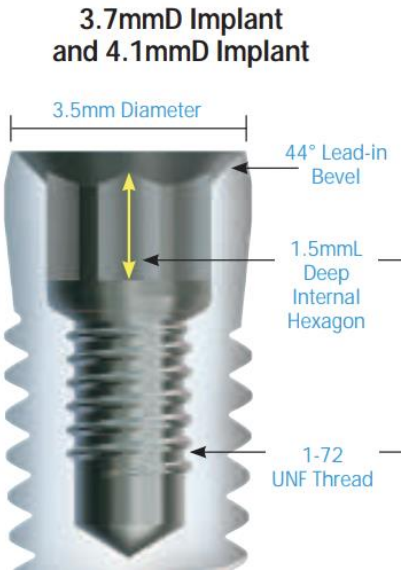


Figure 5. Zimmer Tapered Screw-Vent implant with the straight type of internal IAC. (Reprinted with permission from the manufacturer)

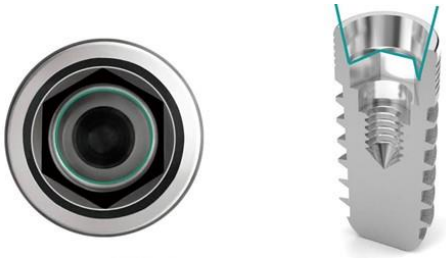


Figure 6. GC Aadva Standard implant with the conical type of internal IAC. (Reprinted with permission from the manufacturer)

Group 1. comprised 50 GC Aadva Standard implants (GCTech.Europe GmbH, Breckerfeld, Germany) of 4.0 mm diameter, connected to the original prosthodontic abutments.

Group 2. comprised 50 Zimmer Tapered Screw-Vent implants (Zimmer Biomet Dental, Palm Beach Gardens, FL, USA) of 4.1 mm diameter, also connected to the original prosthodontic abutments.
In each of the main groups (1 and 2), three subgroups of 10 implants were formed for different sealing materials, as follows:

GapSeal gel (Hager and Werken, Duisburg, Germany);

Oxysafe gel (Hager and Werken, Duisburg, Germany);

Flow.sil (Bredent GmbH and Co.KG, Senden, Germany).

One positive control subgroup of 10 implants with chlorhexidine gel (Curasept ADS 350 gel, Curaden International AG, Kriens, Switzerland) and one negative control subgroup without sealant (10 implants) were also formed in each of the main groups (Figure 7).

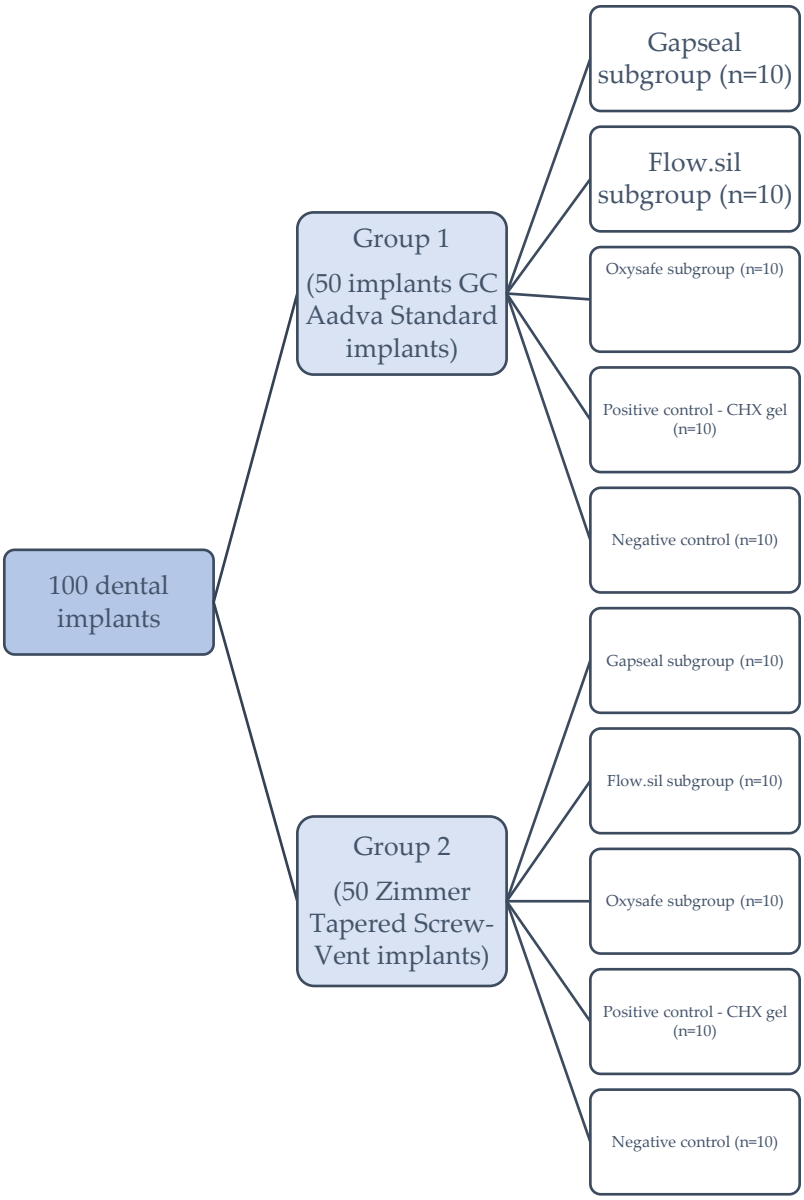


Figure 7. Study design and division of the implants into groups.

5.3.2. Implant–Abutment Assembly Preparation

Every dental implant was removed from its commercial package and placed in a sterile stainless-steel clamp using sterile forceps (Henry Schein, Melville, NY, USA). All the instruments used in this study were sterilised in Euroklav 23 VS+ (Melag, Berlin, Germany). Implants were placed into the clamp in a strictly vertical position (Figure 8) which enabled a firm rotational movement during the tightening of the prosthodontic abutments.

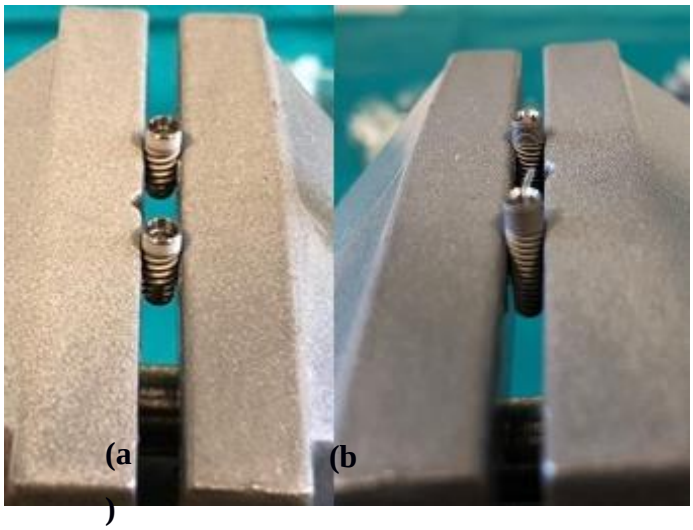


Figure 8. (**a**) GC implants (conical IAC type) in a sterile clamp; (**b**) Zimmer implants (straight IAC type) in a sterile clamp.

Sterile brain heart infusion (BHI) broth (0.3 µL) (calf brains (12.5 g/L), beef heart infusion solids (5.0 g/L), proteose peptone (10.0 g/L), D-glucose (2.0 g/L), sodium chloride (5.0 g/L), and disodium hydrogen phosphate (2.5 g/L) at a pH 7.4 ± 0.2 and 25 °C) was added to the internal surface of the implants using a sterile micropipette (Merck KGaA, Darmstadt, Germany) to serve as a non-selective nutrient medium in case of microbial penetration. The sealing material was then applied to the internal surface of the implants in the test subgroups (GapSeal, Oxysafe, or Flow.sil) depending on the subgroup (Figure 9). Application of the sealing materials was performed strictly according to the recommendations from the manufacturers. Chlorhexidine gel is considered an effective antimicrobial agent used in different fields of dentistry and therefore was applied in the positive control subgroup (14). The negative control subgroup did not receive any material.

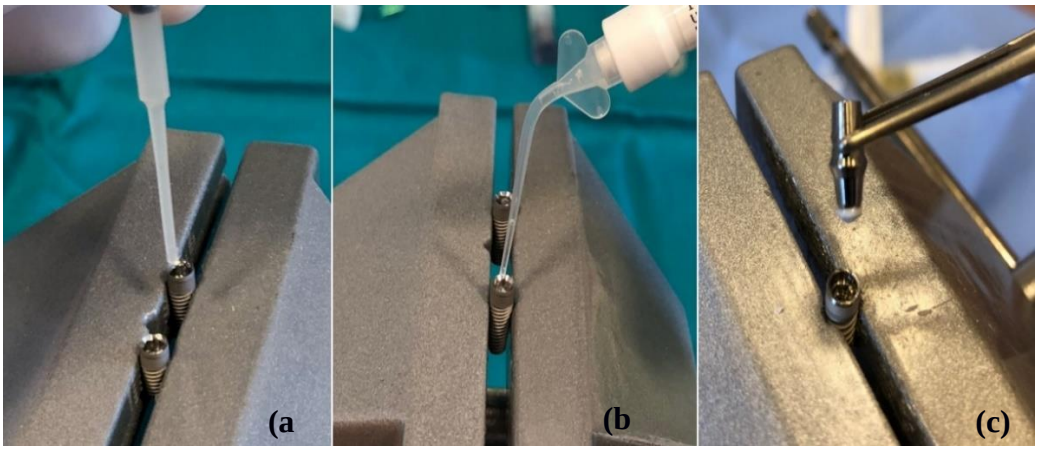


Figure 9. Application of the sealing materials. (**a**) GapSeal; (**b**) Oxysafe; (**c**) Flow.sil.

Original prosthodontic abutments were removed from their commercial packaging under sterile conditions and placed into the implants (Figure 10) using the values recommended by the manufacturers (30 N/cm for Zimmer Tapered Screw-Vent and 20 N/cm for GC Aadva Standard implants).

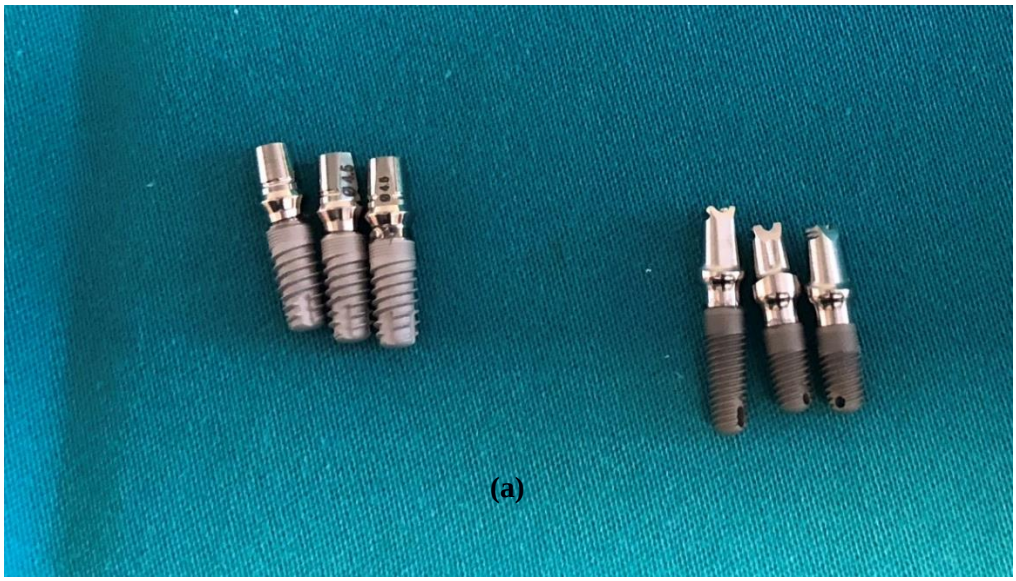


Figure 10. (**a**) GC implants with original prosthodontic abutments; (**b**) Zimmer implants with original prosthodontic abutments.

5.*3.3 Contamination Procedure*

Candida albicans and *Staphylococcus aureus* strains were isolated from a clinical sample at the Clinical Hospital Centre Zagreb. Separated isolation of the fungal and bacterial strains was performed in Columbia Agar for 72 h. Suspensions were prepared using thioglycolate broth, following their mixture into a single, joint suspension. The suspension density was set to 600 nm using an optical densitometer (Densimat, Biomerieux, Marcyl'Etoile, France). This value is equivalent to 1×10^8 colony-forming units per millilitre (CFU/mL).

Contamination of implant–abutment assemblies was performed by immersing them in 300 μL of joint microbial suspension (containing *C. albicans* and *S. aureus* at a density of 0.5 McFarland) for 14 days under aerobic conditions. The incubation temperature was set at 35 °C. The microbial suspension covered the neck of the implant and cervical part of the abutment, but the access hole for the abutment screw remained above the suspension level to eliminate the possible impact of penetration of the suspension along the fixation screw and thus false positive contamination.

After the incubation period, the implant–abutment assemblies were removed from Eppendorf tubes using sterile forceps. External contamination was prevented by their immersion in 70% ethanol for 2 min following drying of the samples with a sterile gauze and placement in a sterile stainless-steel clamp. Then, disassembly in a strictly vertical position was performed. The internal surface of the implants was sampled using paper points (Absorbent points, DENTSPLY Maillefer, Tulsa, OK, USA) which were placed into 0.5 mL of sterile phosphate-buffered saline (PBS) in Eppendorf tubes. The tubes containing paper points and PBS were inserted into a vortex mixer (Corning® LSE™ vortex mixer, Corning, NY, USA) for 60 s to suspend fungal and bacterial cells.

Samples from the Eppendorf tube contents were transferred to 5% blood agar with an incubation period of 48 h and a temperature of 37 °C. Identification and quantification of the resulting colonies (Figure 11) were verified using a MALDI Biotyper (Bruker Daltonics, Hamburg, Germany). Microbial contamination (CFU/mL) was counted for each sample, and the obtained results underwent further statistical analysis.

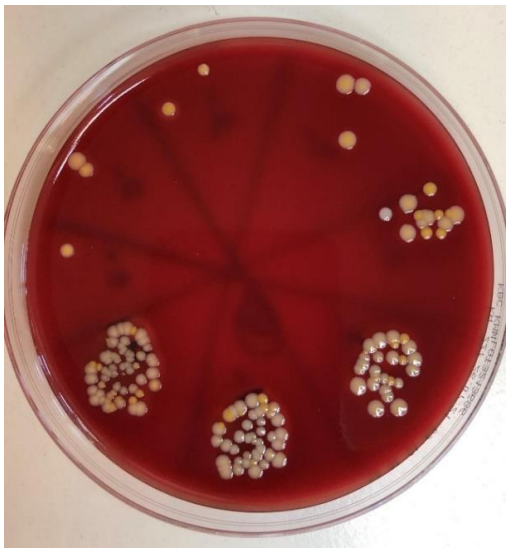


Figure 11. The 5% blood agar with colonies of *S. aureus* and *C.albicans* ready for CFU/mL analysis.

5.3.4 Statistical Analysis

Fisher's exact test was used to perform statistical analysis using MedCalc software version 20.014 (MedCalc Software Ltd., Ostend, Belgium). The traditional level of statistical significance was set at $p < 0.05$.

5.4 Results

In order to quantitatively describe the samples used in this study, the results were determined based on the frequency of microbial leakage for descriptive purposes. A positive result was signified by the presence of *C. albicans* or *S. aureus*, while the complete absence of these microorganisms gave a negative result.

According to the frequencies of bacterial and fungal microleakage for both straight (Table 1) and conical (Table 2) types of connections, all sealing materials were compared to positive and negative controls regarding *S. aureus* (Table 3) and *C. albicans* (Table 4) infection using Fisher's exact test. The IAC type had no influence on the internal fit regarding *S. aureus* infection, and there was no statistically significant improvement with the use of different sealing materials in comparison with the control subgroups since the *p*-values were above the level of significance ($p > 0.05$). GapSeal was the only sealing agent that was significantly more efficient compared to the negative control subgroup ($p = 0.008$) (Table 3). The same conclusion could be drawn regarding *C. albicans* infection (Table 4).

There was no statistically significant improvement with any sealing material, except GapSeal, compared to the negative control subgroup (p = 0.000). The IAC type had no influence on microleakage according to the p-values of Fisher's exact test.

Table 1. Frequencies of bacterial and fungal microleakage (Zimmer Tapered Screw-Vent implants).

	Flow.sil	**OXYSAFE**	**GapSeal**	**Positive Control (CHX)**	**Negative Control**
S.aureus	80.00%	80.00%	50.00%	70.00%	80.00%
C. albicans	70.00%	60.00%	20.00%	50.00%	60.00%

Table 2. Frequencies of bacterial and fungal microleakage (GC Aadva Standard implants).

	Flow.sil	**OXYSAFE**	**GapSeal**	**Positive Control (CHX)**	**Negative Control**
S.aureus	90.00%	90.00%	60.00%	80.00%	90.00%
C. albicans	70.00%	60.00%	20.00%	50.00%	60.00%

Table 3. Comparison of Fisher's exact test values for microleakage between sealing materials and control subgroups regarding *S. aureus* infection.

Connection Type	Subgroup	Fisher Exact Test (*p* Values)		
		Flow.sil	**Oxysafe**	**GapSeal**
straight and conical	positive (CHX)	H0 not rejected (0.465)	H0 not rejected (0.465)	H0 not rejected (0.320)
	negative (no seal)	H0 not rejected (0.356)	H0 not rejected (0.605)	**H0 rejected (0.008) ***
straight	positive (CHX)	H0 not rejected (1.000)	H0 not rejected (1.000)	H0 not rejected (0.650)
	negative (no seal)	H0 not rejected (1.000)	H0 not rejected (1.000)	H0 not rejected (0.350)
conical	positive (CHX)	H0 not rejected (0.605)	H0 not rejected (0.605)	H0 not rejected (0.628)
	negative (no seal)	H0 not rejected (1.000)	H0 not rejected (1.000)	H0 not rejected (0.303)

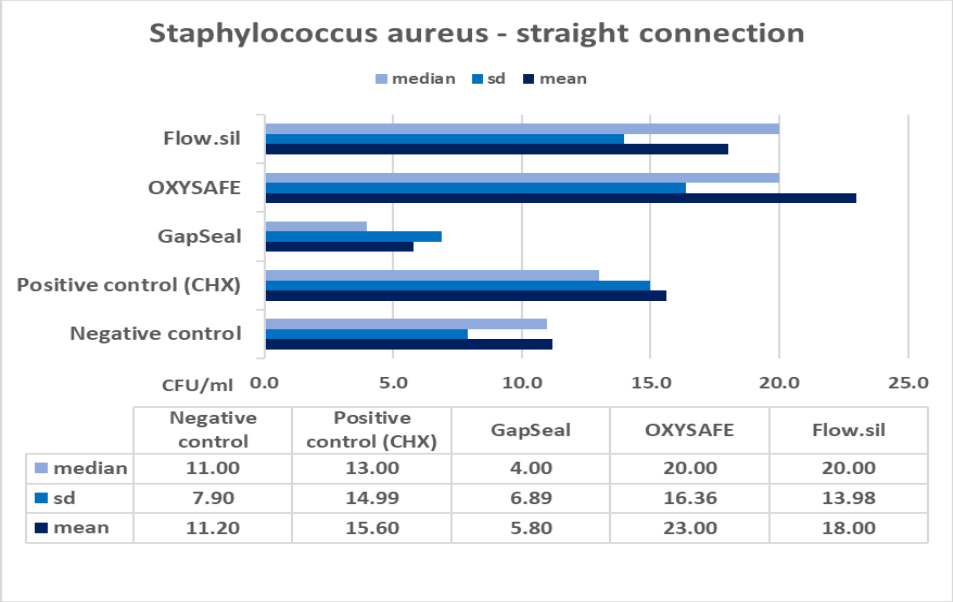
* Statistically significant ($p < 0.05$).

Table 4. Comparison of Fisher's exact test values for microleakage between sealing materials and control subgroups regarding *C. albicans* infection.

Connection Type	Subgroup	Fisher Exact Test (*p* Values)		
		Flow.sil	**Oxysafe**	**GapSeal**
straight and conical	positive (CHX)	H0 not rejected (0.333)	H0 not rejected (0.751)	H0 not rejected (0.096)
	negative (no seal)	H0 not rejected (0.235)	H0 not rejected (0.065)	**H0 rejected (0.000) ***
straight	positive (CHX)	H0 not rejected (0.650)	H0 not rejected (1.000)	H0 not rejected (0.350)
	negative (no seal)	H0 not rejected (1.000)	H0 not rejected (1.000)	H0 not rejected (0.170)
conical	positive (CHX)	H0 not rejected (0.650)	H0 not rejected (1.000)	H0 not rejected (0.350)
	negative (no seal)	H0 not rejected (1.000)	H0 not rejected (1.000)	H0 not rejected (0.170)

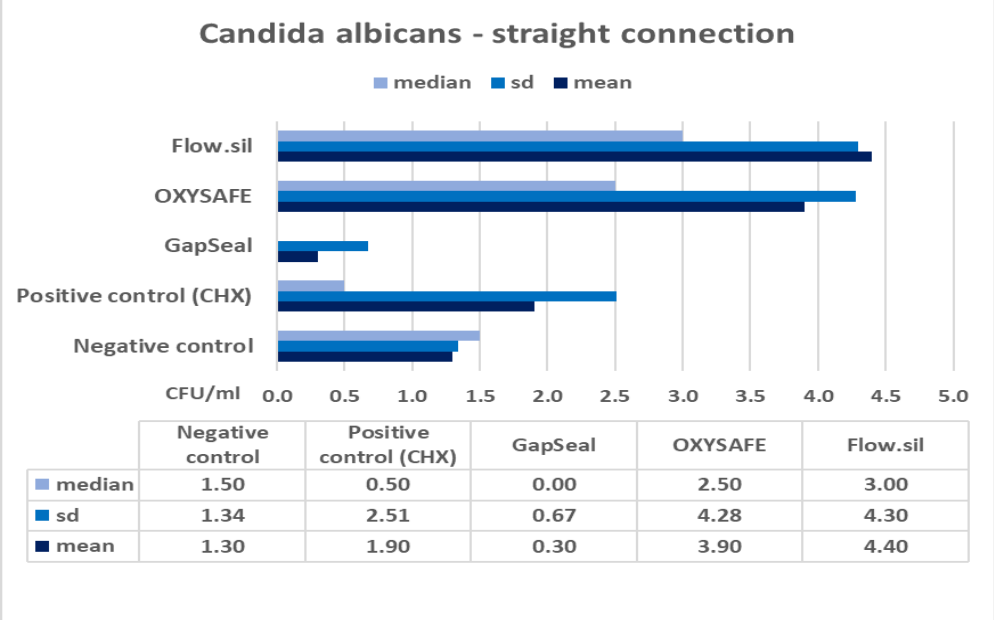
* Statistically significant ($p < 0.05$).

The column charts below (Figures 1–4) show the mean counts of *S. aureus* and *C. albicans* detected on the internal surface of the implants depending on the IAC type, and the influence of using different sealing materials on microbial leakage.



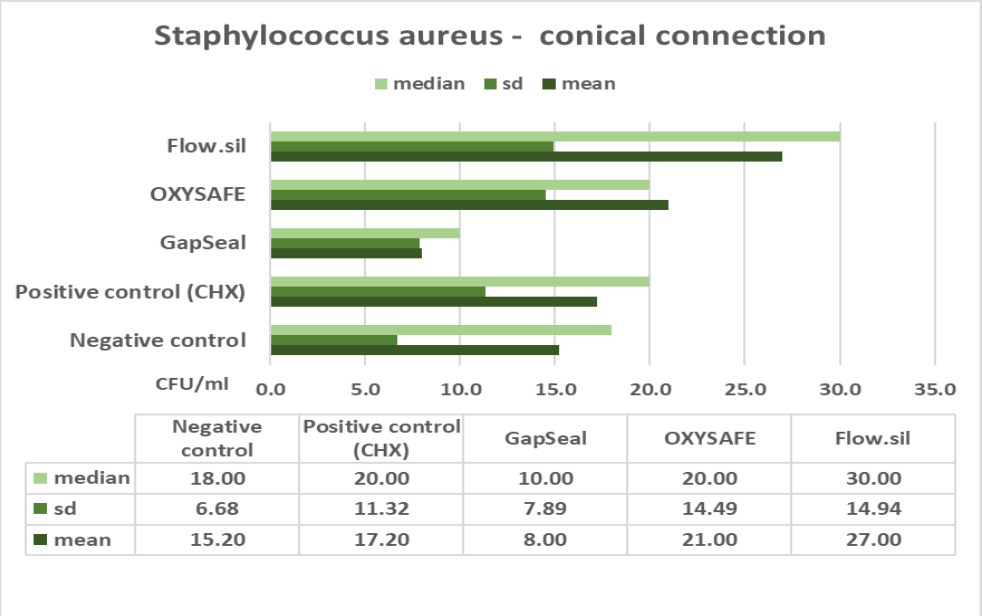
	Negative control	Positive control (CHX)	GapSeal	OXYSAFE	Flow.sil
median	11.00	13.00	4.00	20.00	20.00
sd	7.90	14.99	6.89	16.36	13.98
mean	11.20	15.60	5.80	23.00	18.00

Figure 1. Mean counts of *S. aureus* detected on the internal surface of Zimmer implants and impact of using different sealing materials.



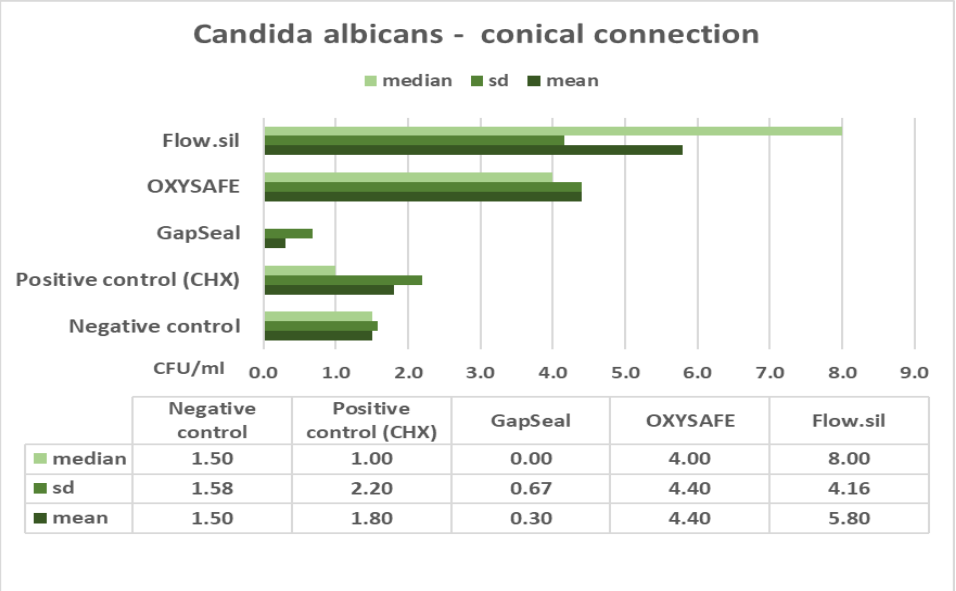
	Negative control	Positive control (CHX)	GapSeal	OXYSAFE	Flow.sil
median	1.50	0.50	0.00	2.50	3.00
sd	1.34	2.51	0.67	4.28	4.30
mean	1.30	1.90	0.30	3.90	4.40

Figure 2. Mean counts of *C. albicans* detected on the internal surface of Zimmer implants and impact of using different sealing materials.



	Negative control	Positive control (CHX)	GapSeal	OXYSAFE	Flow.sil
median	18.00	20.00	10.00	20.00	30.00
sd	6.68	11.32	7.89	14.49	14.94
mean	15.20	17.20	8.00	21.00	27.00

Figure 3. Mean counts of *S. aureus* detected on the internal surface of GC implants and impact of using different sealing materials.



	Negative control	Positive control (CHX)	GapSeal	OXYSAFE	Flow.sil
median	1.50	1.00	0.00	4.00	8.00
sd	1.58	2.20	0.67	4.40	4.16
mean	1.50	1.80	0.30	4.40	5.80

Figure 4. Mean counts of *C. albicans* detected on the internal surface of GC implants and impact of using different sealing materials.

5.5 Discussion

In the presented study, two different types of IACs were compared in terms of their sealing efficacy, as well as the antimicrobial efficacy and permeability of different materials designed to seal the microgap. The null hypothesis was accepted regarding the IAC type, with no significant difference in microleakage between straight and conical types of connections (Tables 3 and 4). The results also show that a complete seal against microbial infection was not achieved at the IAC despite the use of different sealing materials (Figures 1–4). Consequently, no significant difference was found between the various sealing agents designed to prevent microleakage. Only one sealing agent (GapSeal) was found to significantly prevent microleakage, especially against *Candida* spp. infection (Table 4). Taking into consideration the pathogenesis of peri-implant diseases, which is largely determined by the constant microbial microflow through the IAC, it is essential to become thoroughly acquainted with different IAC types and the biomechanical features of existing sealing materials.

The bacterial composition of the biofilm is comparable between dental implants and neighbouring teeth, with a vast variety of oral microorganisms accumulating on implant surfaces (15). Early colonisers are most commonly Gram-positive cocci with the ability to create the preconditions for later colonisation by Gram-negative anaerobic and facultatively anaerobic bacteria (16). The "red complex" bacteria (*Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*), *Aggregaticabacter actinomycetemcomitans*, and *Fusobacterium nucleatum* are the main pathogens associated with late colonisation of periodontal and peri-implant sites. However, there is strong evidence in numerous studies that the peri-implant microbiome is distinct from the periodontal microbiome, especially in the later stages of the disease (16–18). The microorganisms identified in peri-implantitis that are not commonly detected in periodontitis include *Staphylococcus* spp., *Enterobacter aerogenes, Helicobacter pylori, Pseudomonas* spp., and *Candida* spp. (19). It can be concluded that peri-implantitis is often associated with opportunistic pathogens. *C. albicans* is one of the many fungal species present in the oral microbiome as a commensal and a major pathogen in oral and systemic candidiasis (20). It also plays an important role in biofilm arrangement (1). As a part of colonising bacterial microbiota, *S. aureus* is commonly associated with failed dental implants (19). According to in vitro studies, it also has a strong affinity to titanium surfaces, which contributes to its role in peri-implant pathology (21). Due to these data, a decision was made to contaminate the implant–abutment assemblies with a joint microbial suspension containing *C. albicans* and *S. aureus*.

The presented results showed that the IAC type had no significant influence on microleakage. It is crucial to identify the most important factors and conditions in which the study was conducted in order to draw a viable conclusion regarding the relationship between the IAC type and microleakage. Tsuruta et al. (22) observed that the amount of microleakage was significantly smaller in Nobel BioCare implants with an internal conical connection than those with an internal parallel connection type, especially after more than 1000 cycles of tensile and compressive loading. These results are not in accordance with those obtained in this study, but the most important factor that needs to be taken into consideration is the dynamic conditions in which the study was conducted. On the other hand, a static *in vitro* study by Gherlone et al. (23) also demonstrated significantly less microleakage with an internal conical connection in comparison with other internal connections (hexagonal and Morse locking taper). Only 30% of implants with a conical IAC were contaminated with the *Escherichia coli* suspension, whereas the other control internal IAC types were 100% contaminated. In a similar *in vitro* study to the presented one, Discepoli et al. (24) evaluated microleakage at five different IAC types. No sealing materials were used, but microbial leakage of *S. aureus* was independent of the IAC type. They also concluded that there was a tendency toward a better sealing efficacy against *S. aureus* for internal conical and hexagonal connections. In a recent review by Bittencourt et al. (25), it was concluded that microleakage in the Morse conical connection was lower when compared with the internal and external hexagon connections. According to the literature, perfect sealing at the IAC has not been provided by any implant system, and a complete hermetic seal is not yet achievable (26). Ardakani et al. (27) observed that microleakage through the IAC occurs in all implant systems, with special emphasis on torquing abutments to 20 N/cm to minimise microbial leakage. This statement is analogously supported by the results of this study in which a complete seal was not achieved either with different IAC types or with the use of sealing materials. It is crucial to point out that microleakage at the IAC is dependent on the torque applied to the system. Larrucea et al. (28) observed no microbial leakage when a 20 and 30 N/cm torque was applied to internal conical connection models infected with *Porphyromonas gingivalis*. However, the contemporary view is that conical and mixed IAC systems behave better regarding the microgap dimensions and consequential amount of leaked microbiota (26,29).

Further analysis of the results showed no statistically significant difference between the various sealing materials used to prevent microleakage at the IAC (Figures 1–4). GapSeal was the only material that was significantly more efficient compared to the negative control subgroup. Analysing the descriptive statistics, it was clear that the presence of media at the IAC reduces

leakage, especially with the use of GapSeal (Tables 1 and 2). The improvements made by the sealing agents can be explained either by having antimicrobial properties or a pure mechanical sealing ability. GapSeal has been found to reduce microleakage at the IAC after dynamic loading in different implant systems with an internal conical connection (30). Seloto et al. (31) concluded that Loctite 2400 sealing gel contributed to the sealing efficacy of the IAC by decreasing vertical misfit values. It is worth mentioning that the implants used in that study had an external hexagonal connection, and thus the results cannot be directly compared with those of the present study. In a recent study by Smojver et al. (9), it was observed that the presence of GapSeal material significantly reduced microleakage at the IAC. These results are in accordance with the results obtained by Nayak et al. (32), who saw the least growth of *Enterococci* when the GapSeal sealing agent was used. The above-stated results accord well with those of the presented study and provide solid proof of GapSeal's usefulness. On the other hand, Mohammadi et al. (33) found that Atridox significantly delayed bacterial microleakage when compared to other materials, including GapSeal. In addition, it was concluded that a complete hermetic seal against microbial infection is not achievable despite the possible reduction in microbiota found at the IAC.

Finally, since there is no literature investigating the efficacy of various sealing materials and comparing their effects in two different types of IACs, valuable results were attained. The sample size of 100 dental implants was sufficient to analyse the differences between the two types of IACs and the effects of various sealing materials on microleakage. However, due to the presence of three different sealing agents, a larger sample size would be better. It is of great importance to re-emphasise that this was an *in vitro* study and further clinical research is necessary to evaluate the obtained results.

5.6 Conclusions

Based on the results of this study, it can be concluded that the IAC type has no significant influence on microleakage regarding microbial infection. Additionally, GapSeal significantly reduces microleakage, especially against Candida spp. infection. Despite the plausible benefits of GapSeal application, a complete hermetic seal was not achieved with any of the sealing agents used in this study. Further clinical research with longer follow-up periods should be conducted to evaluate the effects of using different sealing materials at various IAC types.

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6. GENERAL DISCUSSION

In terms of bacterial and fungal infection, the concept of a complete hermetic seal at the implant-abutment interface was not supported by the results of this series of studies (Chapter 3, Table 1; Chapter 4, Table 5; Chapter 5, Tables 3 and 4). These findings are consistent with those of multiple other studies (45,66,67). However, the presence of a medium at the IAI (such as a gel) decreases microleakage, either due to its antimicrobial properties or its physical properties. In the negative control groups (in all three studies) where no sealants were used, the leakage is mostly a result of the lack of completely accurate fit between the implant and prosthodontic abutment.

According to Duarte et al. (57), despite this fact, the importance of screw tightening increases over time because it influences increased microleakage. Microleakage may depend on the tightening technique, and the leakage values were found to be dependent on the closing torque. The amount of closing torque has an inverse relationship with the severity of leakage (60). Zitzman and Berglundh (68) concluded in 2007 that the incidence of peri-implant mucositis was up to 50% and peri-implantitis 12–43% of cases. The peri-implant biofilm composition changes from gram-positive cocci to gram-negative anaerobic and facultatively anaerobic bacteria, including *Aggregaticabacter actinomycetemcomitans, Bacteroides forsythus Porphyromonas gingivalis, Fusobacterium nucleatum and Prevotella intermedia* (68). Furthermore, several observational studies have demonstrated that peri-implantitis is more frequently associated with opportunistic pathogens (*Staphylococcus aureus and Pseudomonas aeruginosa*) (10,11) and fungal organisms (*Candida albicans, Candida boidinii, Penicillium sp., Rhadotorula sarycesis, and Pachaces*) (12,13). In the studies conducted by Reynaud et al. (69) and Dahlen et al. (70), *Candida spp*. colonies were discovered in periodontal pockets and periodontitis cases, but also in failed implants. *Candida albicans* is a major pathogen in oral and systemic candidiasis and a major fungus in 20–40% of healthy individuals' oral cavities, thus is considered as a commensal (71). In view of the aforementioned findings and due to the paucity of research on the efficacy of different types of sealants in preventing the leakage of this type of fungus, in the presented series of studies, it was decided to use *Candida albicans* for contamination of the inner surface of dental implants. Depending on the geometry of the IAI, several *in vitro* and *in vivo* studies (45,50,51,72) have evaluated the capability of different bacteria to penetrate an implant through the microgap formed between the implant and prosthodontic abutment. According to Quirynen et al. (72), external hexagonal connections are more susceptible to microbial invasion. Jansen et al. (45) found that internal hexagonal connection implants were more resistant to bacterial microleakage in dynamic conditions.

Koutouzis et al. (51) found that internal Morse taper IAI type has minimal microbial colonization after incubation in *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* solutions. Based on these studies, it can be concluded that all types of dental implants and prosthodontic abutments leak microorganisms through the micro - gap at their interface. Due to these findings, it was decided to contaminate the implant–abutment assemblies with a *C. albicans*- and *S. aureus*-containing microbial suspension.

Immune mechanisms eliminate the biofilm created by different microbial species on the external surface of dental implants, whereas internal colonization can persist and result in an unpleasant, malodorous taste as well as tissue damage and periodontal infections (53,56).

To prevent microbial infiltrations in mentioned regions, it was recommended to use silicon sealing material and chlorhexidine varnish separately at the cervical portion of the dental implant (57). However, this was ineffective for more than 5 weeks, indicating that they were unable to prevent microleakage. Nayak et al. (60) concluded that leakage was reduced due to the low viscosity of GapSeal gel used in their study, which allowed it to flow easily across the IAI, resulting in a better seal. This is consistent with the results from the presented series of studies and those obtained by Podhorsky et al. (73), who also utilized GapSeal. Infections caused by *Staphylococcus aureus* responded similarly to chlorhexidine gluconate (CHX), whereas infections caused by *Candida albicans* responded better (Chapter 3, Table 3). Duarte et al. (66) demonstrated that a combination of chlorhexidine gluconate and tymol, one of the primary components of GapSeal, could have the possibility to decrease the microbial count in the oral cavity for a 6–9-week period, while leaving 40 % of IAIs intact.

The gaps between the straight and conical IAIs based on fungal and bacterial leakage under static conditions as well as the antimicrobial efficacy of the GapSeal material (Chapter 4) were compared and evaluated. Acceptance of the null hypothesis was based on the findings that the fabrication method of the prosthodontic abutment was not essential for successful implant-prosthodontic therapy regarding microleakage at the IAI under static conditions. To achieve successful clinical results in implant-prosthodontic therapy, factors such as the pathogenesis of peri-implant mucositis and peri-implantitis, the prosthodontic abutments fabrication method, and the biomechanical role of the IAI need to be considered during treatment planning and execution. Although the differences in microleakage between original and non-original third-party abutments were not statistically significant, non-original third-party prosthodontic abutments demonstrated a higher incidence of infection via the IAI (Chapter 4, Table 3). This result is relatable to that from a study by Alonso-Pérez et al. (74). They claimed that values of

laser-sintered non-original abutment microgaps were clinically admissible. In contrast, in a different study (75) the same authors claimed that original abutments were vastly superior to non-original certified abutments under dynamic conditions, but there were no significant differences in static conditions. There is also an evidence that the use of non-original abutment components with original Astra Tech implants resulted in significantly higher microbial leakage at the IAI under static conditions compared to the use of original prosthodontic abutments from the same manufacturer (76). Given that the aforementioned study was also conducted under static conditions, it is crucial to note that the results contradict those of this study. Tallarico et al. (77) published a systematic review of *in vitro* studies and concluded that the original abutments were superior in terms of their mechanical outcomes, such as marginal accuracy with consequential lower microleakage values. However, they noted that *in vitro* studies had a high risk of bias and that the results given in these systematic reviews should be interpreted with caution. According to some authors (57,60), the increased microleakage can be affected by the abutment screw's closing torque, and the severity of microleakage has an inverse correlation with prosthodontic abutment closing torque. Therefore, it is important to install the prosthodontic abutment according to the manufacturer's instructions. In everyday clinical practice, non-original abutments are frequently chosen for economic reasons. Discrepancies in the dimensions, shape, and design of connecting surfaces may contribute to higher microleakage values and the potential for negative mechanical outcomes because of the impossibility of exact component replication. These micromovements at the IAI result in a pumping effect transporting microorganisms from the exterior to the interior surface of the dental implant and vice versa, resulting in a persistent, ongoing infection. In addition to biological concerns, the continued transfer of forces from the IAI to the dental implant itself increases the stress at the marginal bone (76). Other important factors that must be considered (77) are the level of precision and control of the quality of materials used in the manufacturing process.

The use of sealing material had no statistically significant effect on microbial leakage at the IAI when compared to those without sealing material (Chapter 4, Table 4). However, GapSeal decreased the amount of leaked microorganisms, particularly with GC Aadva Standard implants (Chapter 4, Table 4). These enhancements were not statistically significant, but they provided valuable information for future research. According to contemporary literature (78-80), a complete hermetic seal at the IAI cannot be achieved. Internal fit at the IAI is definitely linked to the difference between original and third-party prosthodontic abutments regarding microbial

leakage when sealing material is utilized. Therefore, the elimination of microleakage is dependent on the marginal accuracy and appropriate design of non-original third-party abutments. Biscoping et al. (81) concluded that the presence of a sealing material may reduce microbial infiltration into implants, and this was also confirmed in the presented study (Chapter 3). The application of sealing material prior to abutment fixation may reduce peri-implant microbiota populations, but a complete seal against infection was not formed at the IAI despite the use of different sealing materials (GapSeal, Oxysafe, and Flow.sil) (78). Biscoping et al. (81) discovered that the tested sealing agents (Clorhexamed 1% gel and Berutemp) had no effect on the gap at the IAI but decreased the torque required to loose the prosthodontic abutment screws. This finding suggests that sealing materials may contribute to adverse mechanical outcomes that affect the reverse torque values. Seloto et al. (82) found that the sealing material (Loctite 2400) decreased vertical misfit values at the IAI and promoted maintenance of preload in screw-retained prostheses after dynamic loading. In addition, Yu et al. (83) claim that GapSeal gel reduces microbial leakage at the IAI level after dynamic loading in three different dental implant systems with an internal conical connection. It is worth noticing that the study was carried out in dynamic conditions, which certainly contributed to different results and possible major advantages of using sealing materials when compared to static conditions of the presented series of studies. In addition, the presented results did not reveal a statistically significant difference in microleakage between original and non-original third-party prosthodontic abutments regarding the different type of IAI (connection type). There is an insufficient number of studies simultaneously comparing these two types of prosthodontic abutments and the effect of IAI type on microleakage.

Thus, the studies mentioned above support the findings of the presented study, and although there was no statistically significant difference between a conical and straight type of IAI, GC Aadva implants with a conical IAI had slightly better microleakage results when used in conjunction with a sealing material.

The sealing efficacy, antimicrobial efficacy, and permeability of various microgap-sealing materials between two different types of IAIs and the original prosthetic abutments were compared (Chapter 5). Regarding IAI type, the null hypothesis was accepted, as there was no significant difference in microleakage between straight and conical connections (Chapter 5, Tables 3 and 4). Despite the use of various sealing materials, the IAI did not achieve a complete seal against microbial infection, as demonstrated by the results (Chapter 5, Figures 1–4).

Therefore, there was no discernible difference between the various sealing agents designed to prevent microleakage. GapSeal was the only sealing material preventing microleakage significantly, particularly against *Candida spp*. infection (Chapter 5, Table 4). Considering the pathogenesis of peri-implant diseases, it is essential to become well-versed in the various IAI types and biomechanical characteristics of existing sealing materials. The results presented indicate that the type of IAI had no significant effect on microleakage. To draw a valid conclusion regarding the relationship between IAI type and microleakage, it is essential to identify the most important factors and conditions under which the presented series of studies were conducted. Tsuruta et al. (84) found a significantly lower amount of microleakage in implants with an internal conical connection compared to those with an internal straight connection type, particularly after more than 1000 cycles of compression tests and tensile loadings. These results differ from those of the presented study, but the dynamic conditions under which the study was conducted are the most important factor to consider. In contrast, a static *in vitro* study conducted by Gherlone et al. (85) showed significantly less microbial leakage with an internal conical IAI than with other internal connections (Morse taper and hexagonal type). Only 30% of dental implants with a conical IAI were contaminated with the *Escherichia coli* suspension, while hexagonal and Morse taper IAI types were contaminated up to 100%. In an *in vitro* study similar to the one presented, Discepoli et al. (86) evaluated microbial leakage at five distinct IAI types. *S. aureus* microbial leakage was independent of the IAI type, without the use of any sealing agents in the study. In addition, they determined that internal conical and hexagonal connections tended to have higher sealing effectiveness against *S. aureus*. Bittencourt et al. (87) concluded in a recent review that Morse conical connection have had lower microbial leakage values than the internal and external hexagon connections. According to the literature, no implant system has provided perfect sealing at the IAI, and a complete hermetic seal is not yet achievable (88). This statement is supported by Ardakani et al. (89), who found that microbial leakage through the IAI occurs in all dental implant systems, with a particular emphasis on tightening abutments to 20 N/cm to prevent microbial leakage as much as possible. These statements are analogously supported by the findings of these series of studies, which demonstrated that a complete seal could not be achieved with either the different IAI types or sealing materials. It is essential to note that microleakage at the IAI is a function of the torque applied to the system. Larrucea et al. (90) found no microleakage when 20 and 30 N/cm of torque were applied to *Porphyromonas gingivalis*-infected internal conical connection models. Modern opinion, however, holds that conical and mixed IAIs perform better in terms of microgap dimensions and resulting microleakage (88, 91).

According to additional analysis (Chapter 5, Figures 1–4), there was no statistically significant difference between the various sealing materials used to prevent microleakage at the IAI. GapSeal was the only material that significantly outperformed the subgroup of negative controls. The presence of media at the IAI clearly reduces microleakage, particularly when GapSeal is employed (Chapter 5, Tables 1 and 2). The improvements made by the sealing agents can be attributed to either their antimicrobial properties or their mechanical sealing ability. Application of GapSeal has been found to reduce microleakage at the IAI in implant systems with an internal conical connection after dynamic loading (83). These findings are consistent with those of Nayak et al. (60), who observed the least amount of *Enterococci* growth when using the GapSeal sealing agent, which is consistent with the results of the presented study and provides solid evidence of GapSeal's utility. Mohammadi et al. (92) discovered that Atridox significantly postponed bacterial microleakage compared to other sealing agents, such as GapSeal. Nevertheless, it was still determined that a complete hermetic seal against microbial infection is not possible, despite the possibility of a reduction in microbiota at the IAI.

Finally, since there is no literature comparing the effectiveness of various sealing materials and their effects in two distinct types of IAIs, the results obtained are valuable. The sample size of 100 dental implants was adequate for analyzing the differences between the two types of IAIs and the effects of different sealing materials on microleakage. It is crucial to emphasize that these were *in vitro* studies, and that additional clinical research is required to evaluate the results.

7. CONCLUSIONS

Within the limitations of the present study, the following can be concluded:

1. There is no difference in the antimicrobial effect and permeability for microorganisms that cause peri-implant diseases regarding different types of sealing material compared to the negative control group.

2. There is no difference in the sealing ability of different sealing materials regarding the type of platform or the geometry of the implant connection with prosthetic abutments compared to the negative control group.

3. Results show that there is no statistically significant difference regarding the microbial leakage between the original and third-party custom-made abutments, regardless of the use of GapSeal as sealing material.

8. LITERATURE

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9. CURRICULUM VITAE

Igor Smojver, DMD, Specialist in Oral Surgery, graduated from the School of Dental Medicine, University of Zagreb in 2009. During his studies, he was a demonstrator at the Department of Endodontics and restorative dentistry and a volunteer at the Department of Oral Surgery. Specialist training performed at the Department of Oral Surgery Universitiy Hospital Centre Zagreb and passed the specialist exam in 2016. In 2017 he enrolled postgraduate doctoral study of dental medicine in Zagreb, under the mentorship of Prof. Dragana Gabrić and Prof. Ana Budimir from the field of dental implantology. He is author and co-author of 13 scientific papers, 7 of which are in the CC database, 39 poster presentations at international congresses and 1 chapter in the book. He regularly participates in professional courses training in the country and abroad, occasionally as a lecturer. He is a member of the Croatian Dental Chamber Medicine (HKDM), Croatian Medical Association (HLZ), Croatian Society for Oral Surgery (HDOK), Croatian Society for Dental Implantology (HDDI). He places special emphasis on his daily work to microsurgical operative techniques, procedures on soft tissues and implant-prosthetic rehabilitation in the aesthetic zone, while in scientific work he is focused on prevention and treatment of peri-implantitis.

List of publications:

Katalinić I, **Smojver I**, Morelato L, Vuletić M, Budimir A, Gabrić D. Evaluation of the Photoactivation Effect of 3% Hydrogen Peroxide in the Disinfection of Dental Implants: In Vitro Study. Biomedicines. 2023; 11(4):1002. (**JCR Q2**)

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