



**Bioceramic cylinders as a scaffold in ectopic bone formation induced by rhBMP6 in autologous blood coagulum**

Nikola ŠTOKOVIĆ, Natalia IVANJKO, Igor ERJAVEC, Ivona MATIĆ JELIĆ, Kristian BAKIĆ, Slobodan VUKIŠEVIĆ

Laboratory for Mineralized Tissues, Scientific Center of Excellence for Reproductive and Regenerative Medicine, University of Zagreb School of Medicine, Zagreb, Croatia

**INTRODUCTION:**In our previous work we have shown that autologous blood coagulum (ABC) is a novel carrier for bone morphogenetic protein 6 (rhBMP6) which is an important molecule in bone regeneration because it possesses osteoinductive properties. Compression resistant matrix (CRM) is used to improve biomechanical properties of the novel autologous bone graft substitute (ABGS). The aim of this work was to elucidate time course of ectopic bone formation following subcutaneous implantation of osteoinductive device containing recombinant human BMP6, ABC and biphasic bioceramic cylinders (80% tricalcium-phosphate/20% hydroxyapatite) as CRM.

**METHODS:**In the process of preparation of the ABGS with osteoinductive properties, 20µg of rhBMP6 was added to 500µL of ABC and coagulated in a syringe containing cylinder (0,1g). Implants were implanted subcutaneously (n=6 per time point) in the axillary region of Sprague Dawley rats and removed on specified time points (7,14,21,35 and 50 days). Histological examination was used to assess the biological events in ectopic bone formation and remodeling. The new ectopic bone was analysed by µCT.

**RESULTS:**On day 7 after implantation endochondral ossification was present outside the cylinder in the peripheral part of implants. On day 14 and day 21 following implantation newly formed bone was present both around the cylinder and in the majority of pores inside the cylinder. Bone marrow was present between the bone trabeculae. Histological findings on day 35 and day 50 were almost similar. Newly formed bone almost completely surrounded the cylinder and was also present in the pores. Bone was uniformly present in the pores regardless of the pore location inside the cylinder and covered the surface of the bioceramics encircling pores with bone. Within the osseous circle, there were few trabeculae and bone marrow with predominance of adipocytes. Ectopic bone formation was confirmed by µCT analyses.

**DISCUSSION & CONCLUSIONS:**In this experiment we investigated time course of ectopic bone formation induced by rhBMP6 which is a part of the aforementioned ABGS. Sequence of biological events as well as amount of newly formed bone were comparable with the findings of our previous experiments in which various bioceramic granules were used as CRM. We have shown that bioceramic cylinders might be used as a CRM in osteoinductive ABGS containing rhBMP6, ABC and CRM. To confirm the possible use of the tested ABGS, it will be tested in larger animals.

**ACKNOWLEDGEMENTS:**This work was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine (Grant Agreement KK01.1.1.01.0008)

**Keywords:** Biologics and growth factors, Biomaterials