HUMAN SPONGY BONE EXPLANT – A USEFUL *EX VIVO* MODEL FOR IMPLANT OSSEOINTEGRATION TESTING

AGATA PRZEKORA*, PAULINA KAZIMIERCZAK, MICHAL WOJCIK

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, MEDICAL UNIVERSITY OF LUBLIN, POLAND
*E-MAIL: AGATA.PRZEKORA@UMLUB.PL

[ENGINEERING OF BIOMATERIALS 163 (2021) 12]

Introduction

Permanent bone implants should exhibit some crucial features, enabling their good osseointegration (formation of a direct connection with the host bone tissue) after their implantation into the organism [1]. It may be assumed that implant that shows good bioactivity and osteoconductivity (ability to promote osteoblast adhesion, proliferation, and osteogenic differentiation) under *in vitro* conditions, should also provide good osseointegration *in vivo*. Nevertheless, according to available literature, the only method to confirm implant osseointegration is to perform *in vivo* animal tests. On the other hand, the use of animal models at preliminary stage is against the principles of the '3Rs', aiming to Replace, Reduce and Refine the use of animals wherever possible. The aim of the study was to determine the osseointegration process using *ex vivo* explant model. For this purpose, human spongy bone explant was drilled and filled with chitosan/curdlan/HA biomaterial followed by its long-term culture under *in vitro* conditions. In this research, chitosan/curdlan/HA biomaterial was used as a model bone implant due to its high biocompatibility and osteoconductivity that was demonstrated under *in vitro* conditions in our previous studies [2,3].

Materials and Methods

Femoral head of the patient undergoing total hip replacement surgery was used in the study after obtaining informed consent and approval of the Bioethics Committee of Medical University of Lublin (no. KE-0254/74/2020, 30 April 2020). The spongy bone was cut into small pieces (approx. 8 mm x 8 mm and 5 mm in height) that were drilled to obtain the 3 mm-diameter defects. The defects were then filled with the chitosan/curdlan/HA biomaterial and the explants were subjected to long-term culture under *in vitro* conditions: 25 days in a complete culture medium followed by 21 days in the complete culture medium supplemented with 50 μg/ml L-ascorbic acid and 10 mM β-glycerophosphate to induce bone extracellular matrix (ECM) synthesis by the osteoblasts [4]. After 46-day culture of the bone explant, its viability was confirmed by fluorescent staining using calcein-AM (green fluorescence of viable cells) and propidium iodide (red fluorescence of nuclei of dead cells). Osseointegration process was determined by evaluation of osteoblast growth between the biomaterial surface and the host bone by SEM and confocal laser scanning microscopy (CLSM). Moreover, newly deposited ECM (collagen, fibronectin) within the bone-biomaterial connection was assessed using immunofluorescence and CLSM observation.

Results and Discussion

This study presents for the first time *ex vivo* determination of osseointegration process using human spongy bone explant that was drilled and filled with the biomaterial followed by its long-term culture under *in vitro* conditions. Performed experiments clearly proved that human bone explant may stay alive for a long period of time (at least for approx. 50 days). Live/Dead staining revealed that surface of the bone explant was covered by viable osteoblasts that outgrew from the tissue. Furthermore, Live/Dead staining showed also viable cells at the bone-biomaterial interface, proving material osseointegration with the bone explant. Formation of the direct connection between the bone explant and biomaterial was also visualized by SEM (FIG. 1) [4].

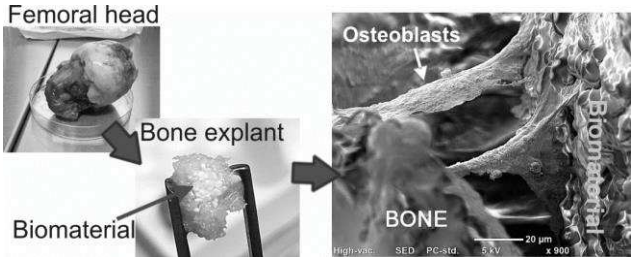

FIG. 1. Schematic representation of the main concept of the study and SEM micrograph presenting the sheets of osteoblasts formed between the bone and biomaterial [4].

Moreover, osteoblasts were demonstrated to have the ability to produce bone ECM (type I collagen, fibronectin) at the bone-implant interface, which was proven by immunofluorescence and CLSM observation (FIG. 2).

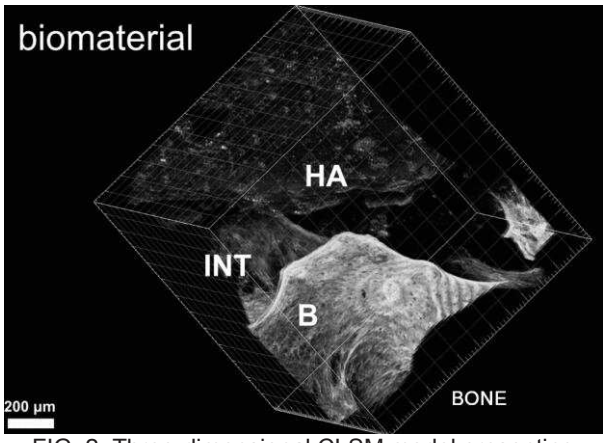

FIG. 2. Three-dimensional CLSM model presenting osseointegration and ECM deposition between the bone tissue and the biomaterial (B – bone tissue, HA – hydroxyapatite granule of the biomaterial, INT – osteoblasts and ECM at bone-biomaterial interface) [4].

Conclusions

Within this study it was demonstrated that *ex vivo* bone explant, which is a heterogeneous tissue containing many different cell types, may serve as an excellent model to test biomaterial osseointegration during preliminary studies, reducing animal tests which is compatible with the principles of '3Rs', aiming to Replace, Reduce and Refine the use of animals wherever possible.

Acknowledgments

The research was supported by the Ministry of Education and Science in Poland within the statutory activity of the Medical University of Lublin (DS3/2021 project).

References

[1] S. Parithimarkalaignan, T. V. Padmanabhan, J. Indian Prosthodont. Soc. 13 (2013) 2–6.
[2] A. Przekora, K. Palka *et al.*, Mater. Sci. Eng. C. 58 (2016) 891-899.
[3] A. Przekora, M. Vandrovcova *et al.*, Biomed. Mater. 12 (2017) DOI:10.1088/1748-605X/aa56f9.
[4] A. Przekora, P. Kazimierczak, M. Wojcik, Mater. Sci. Eng. C 119 (2021) DOI: 10.1016/j.msec.2020.111612