MIMICKING GLUCOCORTICOID-INDUCED OSTEOPOROSIS USING AN IN VITRO TRABECULAR HUMAN BONE MODEL

A. Lang1,2, K. Diesing1, A. Damerau1,2, S. Uzun1, M. Pfefferkorn1,2, T. Gaber1,2, F. Buttgerer1,2, C. H. Lee1, C. H. Chung1, Y. J. Choi2, W. H. Yoo3, J. Y. Kim4, M. S. Lee1.
1Charité-Universitätsmedizin Berlin, Department for Rheumatology and Clinical Immunology, Berlin, Germany; 2German Rheumatism Research Center, Berlin, Germany

Background: The bone matrix consists of inorganic and organic components and a variety of specialized cells such as osteoblasts, osteocytes and osteoclasts. The bone-forming osteoblasts are responsible for the production of organic matrix components; they differentiate later into osteocytes which is accompanied by matrix mineralization. Osteoclasts are multinuclear giant cells, which resorb bone. Healthy bone homeostasis is characterized by a balanced, dynamic and continuous remodeling process. Glucocorticoids (GCs) are commonly used to successfully treat patients with inflammatory rheumatic and other autoimmune diseases. However, long-term treatment with GC can potentially lead to several adverse effects such as the inhibition of osteoblast proliferation and the increase of osteoclastic activity resulting in osteoporosis.

Objectives: Hence, the aim of our project is i) develop an in vitro trabecular human bone model, ii) integrate this bone model into a perfusion system to accelerate mineralization and provide biomechanical stimuli and iii) apply prednisolone to induce osteoporosis. Here we present our initial results describing the successful differentiation of osteoblasts and osteoclasts in a 3D environment, and the accomplished integration of the bone model into a perfusion system.

Methods: In a first step, different cultivation conditions were tested to allow optimal osteogenic or osteoclastic differentiation. To this end, a human bone marrow-derived mesenchymal stromal cells (hMSCs) were treated with osteogenic medium, and b) monocytes (isolated from buffy coats) were differentiated into osteoclasts using following protocol: incubation for 3 days with 25 ng/ml M-CSF followed by an 18-day incubation with M-CSF and 50 ng/ml RANKL. Calcification of hMSCs was evaluated via Alizarin Red S staining. Osteoclasts were identified using immunofluorescence staining observing multinucleated (DAPI) giant (B-Actin) cells with TRAP and Cathepsin K activity. Additional gene expression analyses are currently conducted using qRT-PCR and looking for osteoclast-specific genes. In parallel to the monolayer cultures, cells were transferred on β-tricalcium phosphate (βTCP) – a suitable bone-like scaffold. Furthermore, first experiments in a dynamic bioreactor platform (OSPIN GmbH) were conducted to evaluate the influence of shear stress on the cells and model systems.

Results: We have been able to populate the βTCP scaffold with monocytes, which were differentiated into osteoclasts (morphological changes) without any effect on cellular viability as measured by Live/Dead staining. The morphological changes of those osteoclasts such as formation of podofilin could be demonstrated by scanning electron microscopy. In addition, the cultivation of βTCP populated with hMSCs in a perfusion system showed the upregulation of osteogenic markers (RUNX2, OSX) on mRNA-level.

Conclusion: These first results of our approach to develop an in vitro 3D model for glucocorticoid-induced osteoporosis are promising. Our next step will be the co-cultivation of osteoblasts and osteoclasts under dynamic and optimized cultivation conditions. By combining several cell types, a suitable scaffold and biomechanical stimuli (perfusion), we aim to provide a valid testing platform to study underlying disease mechanisms and for drug development.

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