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 to 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) applying 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 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 (β-Actin) cells with TRAP and Cathepsin K activity. Additional gene expression markers (RUNX2, OSX) on mRNA-level.

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 filopodia 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. Overall, our data indicate that Duoxa1 plays a crucial role in osteoclastogenesis via attenuating ROS production and TRAF6-mediated signaling.

Disclosure of Interests: None declared

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