

## CO-CULTURE OF OSTEOBLASTS AND OSTEOCLASTS ON RESORB- ABLE MINERALISED COLLAGEN SCAFFOLDS: ESTABLISHMENT OF AN *IN VITRO* MODEL OF BONE REMODELING

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**INTRODUCTION:** Bone tissue is characterized by permanent reconstruction (remodeling), triggered by osteoblasts and osteoclasts. To study this process we developed an *in vitro* model that includes osteoblasts - which are responsible for bone regeneration - and osteoclasts which cause bone degradation. Since the biopolymer collagen I and the mineral hydroxyapatite (HAP) are the main components of bone matrix *in vivo*, both cell types are co-cultured on scaffolds of mineralized collagen. By that the main characteristics of bone (synthesis, breakdown, mineralized matrix) are brought together to form a representative model. Our first studies using this model treat the influences of osteocalcin presence and mechanical stimulation on the remodeling process.

**METHODS:** Mineralized collagen I scaffolds have been prepared after a biomimetic process<sup>[1]</sup>. The bone cells have been seeded onto two-dimensional (2D) membranes ("tapes") or sucked into three-dimensional (3D) porous sponges, both made from mineralized collagen I. The pores of the 3D scaffolds showed diameters up to 200  $\mu\text{m}$  which is sufficient for the ingrowth of cells. Murine osteoblastic cells (ST2, obtained from DSMC) were seeded 24 hours before human monocytes (purified from peripheral blood buffy coats), which differentiated to osteoclasts during culture. The cells were cocultivated in a perfusion cell culture system for up to 6 weeks. The cell culture medium contained dexamethasone, 1,25-dihydroxy vitamin D<sub>3</sub>, M-CSF (macrophage colony stimulating factor), RANK-L (receptor activator of NF- $\kappa$ B ligand) and bovine and human serum<sup>[2]</sup>.

Osteocalcin was added to 2D tapes by incubating the scaffolds in medium containing 10  $\mu\text{g}/\text{mL}$  osteocalcin, using the strong interaction of this protein with hydroxyapatite<sup>[3]</sup>.

Mechanical stimulation was carried out indirectly by ultrasound (intensity of 2  $\text{W}/\text{cm}^2$ ; frequency of 1

MHz; exposure time of 20 min per day) coupling of the culture medium.

Morphology, growth and differentiation state of osteoblasts and monocytes/osteoclasts were examined with different microscopical methods like SEM and LSM and quantitative RT-PCR.

**RESULTS:** Co-cultures of osteoblasts and osteoclasts showed typical differentiation over culture periods of 6 weeks. Both 2D and 3D scaffolds have been seeded with the cells. A uniform colonisation of the porous sponges could be obtained by a pressure gradient which permits cell seeding at 4-5 mm depth in the 3D sponges. SEM as well as LSM using fluorescent labelling revealed the morphology and the differentiation state of osteoblasts and monocytes and moreover also the genesis of osteoclasts. Furthermore, typical osteoblast marker like alkaline phosphatase (ALP) and osteoclast marker like tartrate-resistant acid phosphatase (TRAP) could be measured (Fig. 2). Newly synthesised collagen could also be observed using SEM and indirect immunofluorescence.

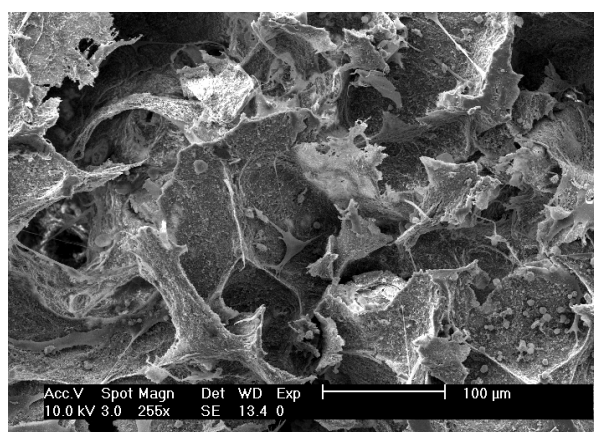


Fig. 1: co-culture of murine osteoblasts (ST2) and human monocytes on 3D-sponge, made from mineralized collagen I, after 5 d in dynamic culture, SEM

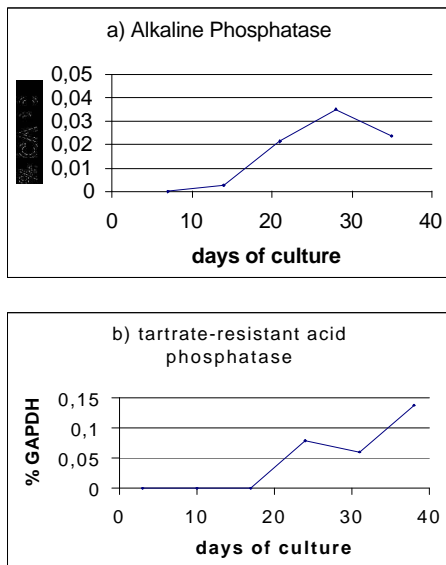


Fig. 2: Expression of mRNA of a) ALP and b) TRAP in co-cultures of osteoblasts and osteoclasts over 6 weeks.

Additionally, a system for mechanical stimulation of the *in vitro* model, based on ultrasound, was tested. Therefore, it is now possible to stimulate the cells during perfusion culture.

The functionalization of the scaffolds with osteocalcin resulted in dramatic changes of gene expression.

#### DISCUSSION & CONCLUSION:

Using a coculture of osteoblasts and osteoclasts on a bonelike synthetic extracellular matrix, it is possible to establish an *in vitro* model for the remodeling of bone. An additionally mechanical stimulation serves to approach the biological example.

#### REFERENCES:

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