Developing 3D bone scaffolds for in vitro bone resorption

Developing 3D bone scaffolds for *in vitro* bone resorption

S.J.A. Remmers[a], M.C.M. Sleddens[a], K. Ito[a,b], S. Hofmann[a,c]
a Orthopaedic Biomechanics, Department of Biomedical Engineering and the Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands
b Department of Orthopaedics, UMC Utrecht, Utrecht, The Netherlands
c Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

Introduction

In vitro models have been designed to study the processes underlying bone resorption and formation, but commonly use animal cells or cell lines in 2D culture, which may not be fully representative of human bone remodeling[1]. To more closely mimic human bone remodeling, we aim to develop a system in which human primary osteoblasts and osteoclasts can be cultured on decellularized trabecular bone plugs that allows quantification of bone resorption and formation using micro computed-tomography (μCT). The aim of the present study is to obtain a suitable scaffold enabling *in vitro* bone resorption. To achieve that, we investigated various preparation protocols to i) extract bone scaffolds from porcine femurs, ii) remove bone marrow and cells and iii) sterilize the scaffold in a way that still enables monocytes to attach.

Materials and Methods

Trabecular bone plugs were prepared from porcine femurs using a 4 mm trephine drill. Trabecular bone plugs were treated with various combinations of defatting[2] and cell removal cocktails[3-7]. The plugs were sterilized by autoclaving or peracetic acid-ethanol sterilization. The plugs were scanned using μCT both prior to and after the treatments to quantify total bone volume and changes as a result of the treatment. Afterwards, they were decalcified, paraffin embedded, and used for histology. Finally, monocytes were seeded onto new decellularized and sterilized plugs using a static or dynamic method, and relative cell attachment and distribution was visualized using an MTT assay.

Results

Earlier experiments have shown that it is essential yet challenging to prepare sterile and decellularized 3D mineralized constructs from bone, and to culture osteoclasts on them that resorb quantifiable amounts of tissue. Histology showed that a defatting step is necessary to remove bone marrow and bone lining cells from the plugs (Fig. 1A+B). Osteocyte removal was achieved using all methods, with defatting and incubating with triton x-100, NH_4OH and benzonase[6] resulting in the best decellularization. Both sterilization methods resulted in sterile scaffolds, with autoclaving being less elaborate.. μCT (Fig. 1C) showed a consistent but statistically not significant trend of decreased total bone volume after the treatments. The MTT assay showed that both static seeding and dynamic seeding led to monocyte attachment (Fig. 1D). More cells seemed to attach using the static conditions. While dynamic seeding seems to hold potential for anchored cells, static seeding seems to be more reproducible for monocytes. Different sterilization techniques did not lead to a different outcome.

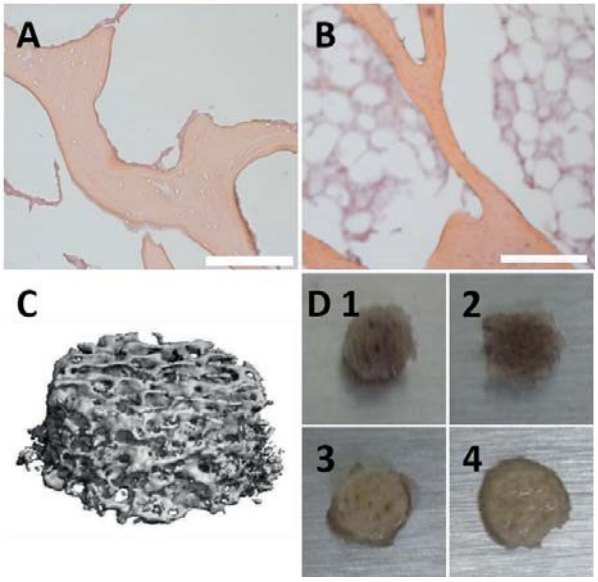

Fig. 1: A+B) H&E staining of bone plugs defatted and decellularized (A) or not (B). Orange: trabeculae. Purple dots in trabeculae: osteocytes. White areas in trabeculae: empty osteocyte lacunae. C) Trabecular bone plug scanned using μCT. D) MTT assay of static (1+2) and dynamic (3+4) seeding of cells (1+2) or vector. Purple color indicates presence of cells. Scale bars are 200 μm. Bone plugs are 4 mm in diameter.

Discussion and conclusion

Our results indicated that the preferred method for decellularization, sterilization and seeding is as follows: First defatting[2], then incubation in Triton X-100, NH_4OH and benzonase[6] followed by autoclaving and static cell seeding. Monocyte attachment to a scaffold is the first step towards *in vitro* bone resorption. In a next step we need to show that the monocytes can actually differentiate into osteoclasts in 3D on bone scaffolds, and that they can resorb quantifiable amounts of bone. Only then can we monitor *in vitro* osteoclastic bone resorption in 3D[8].

References

[1] Edmondson et al. *Assay Drug Dev Technol*. (2014)
[2] Scheffler et al. *Cell and Tissue Banking* (2005)
[3] Marcos-Campos et al. *Biomaterials* (2012)
[4] Smith et al. *Acta Biomaterialia* (2014)
[5] Sadr et al. *Biomaterials* (2012)
[6] Chen et al. *J Bone Miner Res* (2007)
[7] Chen et al. *ACS Appl Mater Inter* (2015)
[8] Hagenmüller et al. *Ann Biomed Eng* (2007)

Acknowledgements

This project was supported by the European Union's Seventh Framework Programme (FP/2007-2013) / grant agreement No. 336043.