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Porous 3D Printed Bioceramic Scaffolds induce Osteoblastogenesis *in vitro*

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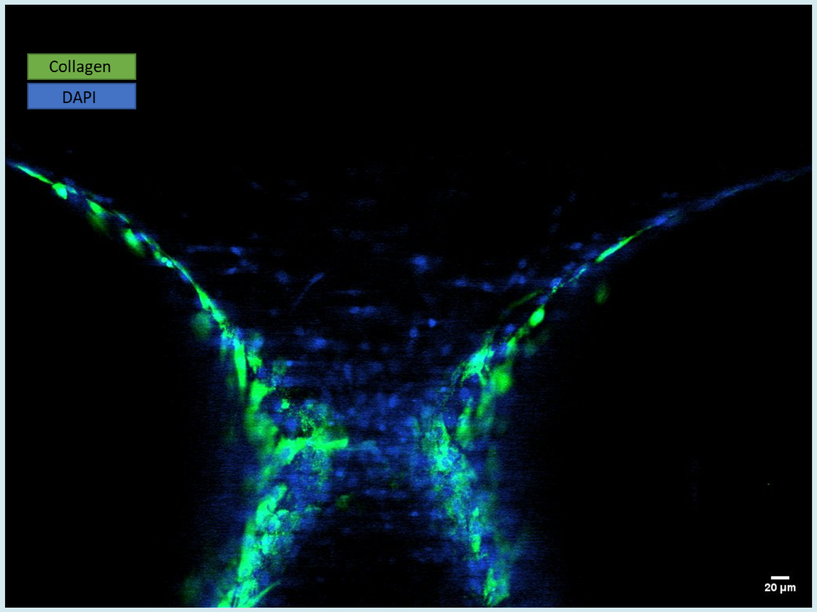
INTRODUCTION

Recently published studies[1,2] have shown great bone remodeling properties of the 3D printed bioceramic implant, P3D Bone. CT-imaging and histological analyses clearly visualized that the bioceramic implant was resorbed and replaced by de novo bone over a 6-month period.
Although these are interesting finds, the study does not investigate whether the development of new bone relies on osteoclast presence and signaling or if the implant material and structure alone is sufficient to support new bone formation. Several studies have shown that osteoclasts secrete signaling molecules which promotes osteoblastogenesis and subsequently bone formation[3]. One could therefore assume that the remodeling of the P3D Bone implant is reliant on the presence of osteoclasts near the implant to activate residing osteoblasts.
This study aimed to examine whether the 3D printed bioceramic material alone is sufficient to differentiate mesenchymal stem cells into osteoblasts and whether these osteoblasts are able to produce extra cellular matrix.

Cell culturing on a **3D printed porous β-tricalcium phosphate** scaffold is sufficient to **induce osteogenesis** without the presence of traditional inducers in human mesenchymal stem cells.



Collagenous extra cellular matrix produced by osteoblasts cultured on P3D Scaffolds for 17 days.

Picture was taken at a place on the scaffold where two edges meet. Cells are stained with DAPI and evenly distributed over the scaffold. The green color signifies the prescence of collagen, indicating that the seeded hMSCs have differentiated to collagen-producing osteoblast.
Cells were fixated and labelled with Collagen antibody, Alexa488 conjugated secondary antibody and DAPI stain. Image acquired using LS confocal microscopy and processed in FIJI.
DAPI: BLue, Collagen: Green

Image acquisition was performed at the Danish Molecular Biomedical Imaging Center (DaMBIC, University of Southern Denmark), supported by the Novo Nordisk Foundation (NNF) (grant agreement number NNF18SA0032928)

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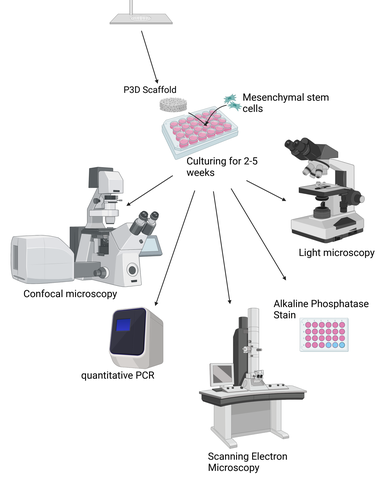
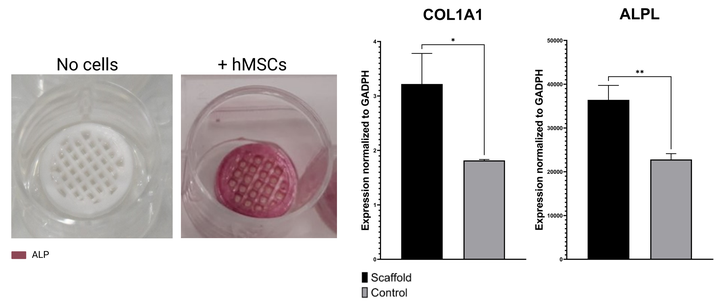
METHODS

To study if in vivo bone formation on P3D Bone implants rely on osteoclast prensence we produced small implants with dimensions suitable for in vitro use (hereafter referred to as P3D Scaffolds). The P3D Scaffolds were produced using the same proprietary technology and material as the P3D Bone implant resulting in an in vitro system that accurately mimics our P3D Bone implants[4].
The P3D Scaffolds were 3D printed with a physiological relevant macroporous size between 600 and 800um.
TERT4 immortalized mesenchymal stem cells were seeded and cultured on P3D Scaffolds for two to five weeks in standard maintenance medium and subsequenbtly analyzed using qPCR, SEM, ALP stain and confocal microscopy to evaluate the osteogenic programme and extracellular matrix production on different levels.



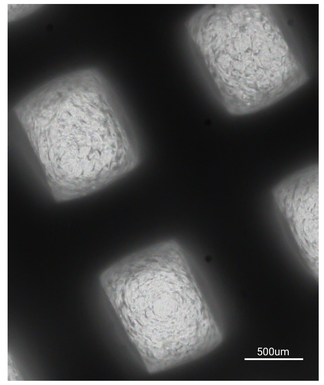
RESULTS

Expression of osteoblast marker genes and production of collagen in response to culturing on P3D Scaffolds.

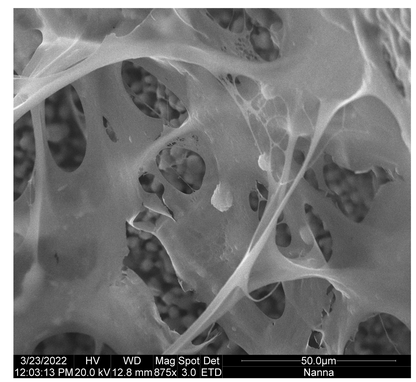


Qualitative evaluation of osteoblast differentiation 20 days after seeding of hMSCs on the P3D Scaffold. Left side: An empty P3D Scaffold as control. Right side: ALP signal from hMSCs cultured on P3D Scaffolds indicative of hMSCs differentiation into osteoblasts.

Quantitative evaluation of osteogenic marker genes, COL1A1 and ALPL, expression after 21 days of culture in standard maintnance medium . Controls were cultured on traditional cell culture plates and run in parallel. Marker gene expression was normalized to the expression of the housekeeping gene GADPH. Errorbars represent SEM, *:p<0.05, **:p<0.01



Micrograph of hMSCs cultured on P3D Scaffolds for 35 days. Standard maintanance medium, 37°C and 5% CO2. The picture was taken immediately after medium change. Acquired using Nikon Eclipse TS2 and 4x objective.



Collagen can be seen to cover the porous surface of the scaffold, and cells are clearly distinguised within the collagen layer.
After 35 days culturing on P3D Scaffolds, cells were fixated in 4% paraformaldehyde, treated with 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol and finally dryed in HMDS. Images are acquired using SEM.

CONCLUSION

The presented data suggests that the bone formation occurring after implantation of the 3D printed bioceramic implant P3D Bone does not solely rely on the osteoclastic signaling to promote osteoblastogenesis. The data further suggests that the bioceramic material and structure itself functions as an inducer of the osteogenic gene program, subsequent extracellular matrix production and ultimately de novo bone formation at the implant site.

References

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