

Time-lapsed monitoring of tissue engineered bone

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INTRODUCTION: In in vitro tissue engineering of bone, most analysis methods are destructive (biochemical assays, histology, FTIR) and do not allow time-lapsed monitoring of individual samples. We demonstrate the feasibility of in situ monitoring for the development of bone tissue, engineered from human mesenchymal stem cells (hMSC) by the integral use of time lapsed, nondestructive micro-computed tomography (μ CT). This is an important step towards monitoring controlled bioreactors.

METHODS: 3D porous silk fibroin (SF) scaffolds were produced by isolating silk fibroin from cocoons and molding them in a salt leaching process (diameter: 5mm, thickness: 1mm). Human mesenchymal stem cells were isolated from blood marrow and characterized regarding their ability to differentiate into osteogenic and chondrogenic lineages and regarding the expression of an antigen pattern typical for hMSC. After expansion of the hMSC, P2 cells were seeded onto the SF scaffolds and placed in custom-made culture containers allowing tissue incubation and non-destructive time-lapsed μ CT monitoring under sterile conditions. Osteogenic medium (containing dexamethasone and BMP-2) was supplied and exchanged 3 times per week. For μ CT monitoring the constructs were imaged 9 times during 44 days of culture with a resolution of 36 μ m. Biochemical assays on DNA content, alkaline phosphatase activity and calcium deposition were employed to check for an effect of the multiple irradiations on tissue development.

RESULTS: Integral imaging of the constructs resulted in movie-like illustrations of the forming bone (Fig. 1) and provided dynamic sample-to-sample comparison of samples' morphological parameters. The image data indicated an exponential increase of bone formation with a stable degree of bone mineralization. Interestingly, while the amount of bone volume varied considerably at the end of the observation period for the different samples, the degree of mineralization and specific bone surface

converged to a set value for all samples, indicating that although integral bone volume can change, the quality of the existing bone is consistent throughout the construct and between the samples. Tissue formation was unaffected by μ CT irradiation, as detailed using biochemical assays (DNA, ALP, calcium).

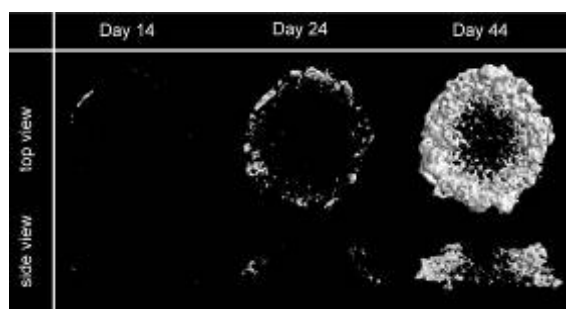


Fig. 1: Time-lapsed μ CT images demonstrating bone-like tissue formation. Full top view and side view of the center part of the disc-shaped construct are displayed.

Table 1. Comparing tissue engineered morphology at day 44 to human biopsy data of trabecular bone.

	in vitro engineered	human biopsies[1]
BV/TV [%]	10	14
BS/BV [mm^{-1}]	26	20
Tb.Th [mm]	0.15	0.15
Tb.Sp [mm]	0.94	0.74
SMI [1]	2.6	1.5

DISCUSSION & CONCLUSIONS: This study demonstrated the feasibility of in situ imaging and quantification of tissue engineered bone over 44 days using human stem cells for the first time. In future, this will allow monitoring and control of bioreactors for optimal tissue differentiation and growth.

REFERENCES: ¹ T. Hildebrand et al (1999) *J Bone Miner Res*, **14**:1167-74.

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