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Improved cell seeding results in improved mineralization

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INTRODUCTION: Cell seeding onto a 3D scaffold is the first step for engineering a bone tissue-like structure. An efficient and homogenous method to seed cells is needed to provide a reproducible amount of attached cells with a uniform distribution. Even though dynamic seeding methods have been reported to be superior to static ones because of their more homogeneous cell distribution using kinetic forces [1], many laboratories still apply cells by pipetting them on top of their scaffolds. With this study, we present an improved cell seeding strategy that results in an improved amount of attached cells and significantly affects the amount and distribution of mineralized extracellular matrix (ECM) deposited by human mesenchymal stem cells (hMSCs) on porous silk fibroin (SF) scaffolds.

METHODS: Scaffolds (height: 3 mm; diameter: 5 mm) were manufactured as reported previously [2]. Static seeding involved pipetting a cell suspension (1×10^6 cells/20 μ L) onto pre-wetted scaffolds and incubation for 90 min at 37°C. In the dynamic seeding process, scaffolds were incubated with a cell suspension (1×10^6 cells/4 mL) in 50-mL tubes placed on an orbital shaker for either 2 h, 4 h or 6 h in an incubator at 37°C. Cell number was determined after seeding by DNA quantification. Cell distribution was assessed by H&E staining on histological sections. For culture, the constructs were transferred into a bioreactor with osteogenic cell culture medium. After 3 weeks, microcomputed tomography (μ CT) imaging was used to visualize and quantify mineralized ECM.

RESULTS: DNA quantification showed significantly higher cell numbers using the dynamic seeding approach for 4 h and 6 h (Tab. 1).

Table 1: DNA and mineralized ECM quantification of cell-seeded scaffolds

Seeding Method	Static	Dynamic 4 h	Dynamic 6 h
DNA [ng]	23 \pm 3	44 \pm 5	58 \pm 4
ECM/DNA [mm ³ / μ g]	0,2 \pm 0,2	21,3 \pm 4,7	32,4 \pm 14,4

Dynamic seeding resulted in a more homogenous cell distribution throughout the bulk of the scaffold and increased ECM mineralization (Tab. 1). In general, the mineralized ECM was more homogeneously distributed both vertically and horizontally as compared to the static seeding approach (Fig. 1).

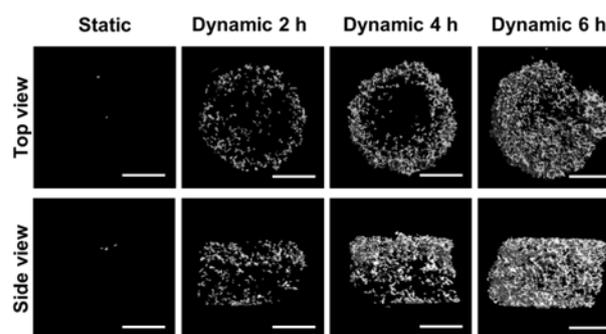


Fig. 1: μ CT images of mineralized ECM produced after 3 weeks by hMSCs seeded on SF scaffolds with a static or dynamic seeding method using an orbital shaker for 2 h, 4 h or 6 h. Scale bar: 2 mm.

DISCUSSION & CONCLUSIONS: In this study, we demonstrated that the use of a dynamic seeding method using an orbital shaker leads to significantly higher numbers of cells inside a SF scaffold, which in turn results in increased bone-like tissue formation. Our results not only imply that the initial cell seeding density significantly affects tissue formation but also indicate that homogenous cell-seeding using a dynamic method supports uniform tissue development. The application of this simple seeding technique can go beyond bone tissue engineering and be used for seeding similar porous scaffolds with hMSCs.

REFERENCES: ¹P. Thevenot et al (2008) *Tissue Engineering Part C, Methods* **14** (4), 319-331. ²S. Hofmann et al (2007) *Biomaterials* **28**, 1152-62.

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