MECHANICAL AND BIOLOGICAL FEATURES OF HYBRID MATERIAL SCAFFOLDS FOR BONE REGENERATION
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Introduction
Engineering artificial scaffolds that promote cell adhesion and growth in three dimensions is crucial for successful bone tissue engineering. However, the fabrication of three-dimensional (3D) tissue scaffolds with highly precise geometry exhibiting complex micro- and nano-features still remains a challenge. Here, we present our investigations into the suitability of a hybrid organic-inorganic material that can be structured in complex, 3D geometries using Direct fs Laser Writing (DLW) for bone tissue scaffolds. Specifically, we report on the mechanical characteristics of a hybrid material, and the biological response of bone marrow (BM) mesenchymal stem cells (MSCs) on the developed hybrid material scaffolds.

Methods
The composite material was prepared using methacryloxypropyl trimethoxysilane (MAPTMS), 50mole% 2-(dimethylamino) ethyl methacrylate (DMAEMA) and methacrylic acid as the polymerizable monomers, whereas zirconium n-propoxide Zr(OPr)4, and the trimethoxysilane groups of MAPTMS served as the inorganic moieties [Terzaki, 2013]. 3D scaffolds are fabricated layer-by-layer using DLW of the organic-inorganic composite material, a technique based on multi-photon polymerization. Nanomechanical studies, both before and after submersion of the material in cell culture medium, are performed by nanoindentation test instrument applying loads of 1-10,000 μN. For the in vitro study we use passages 1-4 of BM-MSCs isolated from donors posterior iliac aspirates. We investigate cell morphology by confocal fluorescence microscopy and scanning electron microscopy. For the quantification of cell proliferation from different donors we use the PrestoBlue™ assay.

Results
Composite scaffolds with complex geometry were successfully fabricated from a hybrid-50% DMAEMA material by DLW. We obtained hardness (H) values of 0.6 GPa and reduced modulus (E_r) values of 8 GPa after submersion of the hybrid material in cell culture medium. Isolated bone marrow mesenchymal stem cells cultured on the composite scaffolds showed excellent cellular response, independent of the cell donor. A strong cell adhesion onto the 3D scaffolds with spread cell morphology from the first hours of observation and up to several days, together with a proliferation increase after 14 and 21 days validate the biocompatibility of the developed composite material.

Discussion
Our results demonstrate that the 50% DMAEMA hybrid material has appropriate mechanical properties for scaffolds fabrication with DLW, and shows excellent adhesion of BM-MSCs on the developed scaffolds and proliferation increase. These mechanical and biological characteristics of the material, reinforced with our ongoing differentiation studies in vivo, establish the basis for the potential use of this cell-material combination in bone tissue regeneration.

References