CO-POLYMERIC BIOMATERIALS FOR BONE TISSUE ENGINEERING

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Introduction
Biodegradable biomaterial scaffolds are useful tools to conduct tissue development. At the same time biomaterials have an impact on the host immune response. The induced immune response is essential since it can facilitate the healing process. It is therefore important to predict and promote the proper immune response after implantation [1]. The aim of the present study is to synthesize and characterize chitosan-*grafted*-poly(ε-caprolactone) copolymers (CS-*g*-PCL) with PCL contents of 20 wt% and 50 wt% and evaluate (i) their immunomodulatory potential by analyzing the differentiation of primary bone marrow derived macrophages (BMDMs) cultured on copolymeric films, (ii) the osteogenic differentiation potential of pre-osteoblastic cells on copolymeric films, and (iii) the angiogenic potential of human umbilical vein endothelial cells cultured on copolymeric materials.

Materials and Methods
Copolymeric material specimens were synthesized and characterized by scanning electron microscopy (SEM), NMR, FTIR [2]. Cell culture experiments were performed with primary bone marrow derived macrophages (BMDMs), pre-osteoblastic cells MC3T3-E1, and human umbilical vein endothelial cells (HUVECs). Cell viability and proliferation was quantified by means of the PrestoBlue assay. Cell morphology on the copolymers was visualized by SEM and fluorescence confocal microscopy. The osteogenic response was evaluated in vitro by measurement of the alkaline phosphatase activity, collagen production in the ECM, calcium biomineralization by alizarin red staining, and osteogenic gene expression by PCR [3]. For the assessment of the in vitro angiogenic response we quantified the production of Platelet Derived Growth Factor (PDGF BB), a characteristic marker of endothelial "tip cell", which leads the angiogenic sprouting, as well as the expression of angiogenesis-related genes DLL4, VEGFR2, ANGPT2, SPROUTY2, PDGFBB an MMP2 by means of semi-quantitative PCR.

Results and Discussion
We have successfully synthesized novel CS-*g*-PCL copolymers and prepared thin films on glass substrates. *In vitro* experiments of BMDM onto CS-*g*-PCL films have shown a strong cell attachment and good cell proliferation after 7 days in cell culture. Our data from the cytokines secretion detection by ELISA show that the CS-*g*-PCL copolymer significantly decreases the secretion of the inducible levels of pro-inflammatory cytokines IL-12/23 by 31±6%, and thus possesses anti-inflammatory ability. Moreover, this anti-inflammatory action is correlated with the increased chitosan content of the copolymer. In addition, the CS-g-PCL copolymer significantly enhances the production of arginase 1 (Arg1), the hallmark of M2 polarized macrophages, as shown by semiquantitative RT-PCR analysis.

Conclusions
We demonstrate an enhanced osteogenic response of pre-osteoblastic cells on CS-*g*-PCL copolymers and a pronounced angiogenic differentiation potential of human umbilical vein endothelial cells, supporting their potential use as scaffolding materials in vascularized bone tissue engineering.

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References
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