In vitro investigation of functionalized pNIPAAM hydrogels with BMP-2 for bone tissue repair application

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Objectives: Thermoresponsive hydrogels attract increasing interest as biomaterial-scaffolds in tissue regeneration. Swollen poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels show generally low cell attachment and a number of complex techniques have been developed in order to overcome this issue. In this study, we report on the biological functionalization of PNIPAAm hydrogels with the bone morphogenetic protein 2 (rhBMP-2) and the in vitro bioactivity study of the functionalized hydrogels performed in the pre-osteoblastic cell line MC3T3-E1. The cellular response is displayed in cell adhesion, proliferation and differentiation. Finally, we explore the potential of BMP-2-functionalized PNIPAAm hydrogels as scaffolds in bone tissue engineering.

Material and Methods: An uncrosslinked terpolymer composed of *N*-isopropylacrylamide (NIPAAm), methacrylic acid (MAA) and 4-benzoylphenyl methacrylate (MABP) was prepared by free radical polymerization. For the attachment of hydrogel layers on glass substrates, a polymer solution was spin coated onto a 4-(3-triethoxysilyl)propoxybenzophenone functionalized surface, followed by the crosslinking under UV-light for 30 min. By applying UV-light, crosslinking of the polymer and the surface attachment were achieved concomitantly. For the covalent immobilization of bioactive rhBMP-2, the hydrogel films were activated by active ester chemistry with *N*-hydroxysuccinimide-2,2,2-trifluoroacetate (TFA-NHS). For the in vitro study we use early passages of the mouse pre-osteoblastic cell line MC3T3-E1. Cell culture experiments on the PNIPAAm material are carried out in a humidified cell incubator at 37 °C in α-MEM with 1% FBS. We investigated cell adhesion by phase contrast microscopy and cell proliferation by means of the PrestoBlue™ viability assay. Calcium biomineralization by MC3T3 cells was determined by the extraction of the Alizarin Red S assay.

Results: Optical microscopy images show a strong initial adhesion of pre-osteoblastic cells one day after seeding on the BMP-2-functionalized PNIPAAm hydrogel coatings compared with the non-functionalized controls. Preliminary cell viability and proliferation data indicate a two-fold increase in cell number after 1 and 3 d on BMP-2-functionalized hydrogels compared to pure hydrogels under culture conditions with only 1% FBS. Covalently immobilized BMP-2 on PNIPAAm hydrogels indicate a four-fold higher biomineralization compared to the control. Our results show that PNIPAAm hydrogel can be modified to selectively guide the attachment and support the growth and osteogenic response of MC3T3 pre-osteoblastic cells.

Conclusions: The strong initial adhesion, proliferation and biomineralization of the pre-osteoblastic cells on the BMP-2-functionalized PNIPAAm hydrogels show a high potential of the material as scaffold for bone tissue repair.