

## Modular Enzyme-responsive Biomaterials for Bone Tissue Engineering

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**INTRODUCTION:** Materials from natural sources were successfully explored in tissue engineering approaches such as cell delivery or tissue regeneration due to their innate biological properties. In contrast to biopolymers synthetic extracellular matrices (ECM) can be specifically designed for example with respect to their mechanical properties, susceptibility to proteolytic activity and presentation of cell adhesion ligands or morphogens<sup>1</sup>. Therefore they represent interesting alternatives for biopolymers in both fundamental biological and clinical applications. Here we present poly(ethylene glycol) (PEG)-based hydrogels that are formed by Factor XIII (FXIII) catalysis. The modular design of this new class of biomaterials enables the formation of matrices containing multiple tethered bioactive molecules and cell-responsive enzymatic substrates in a simple one step reaction. In the context of bone tissue engineering this scheme has been designed to contain building blocks that include enzymatically coupled cell adhesion ligands and the osteogenic growth factor BMP-2.

**METHODS:** Hydrogel networks were formed in TBS (50mM TrisHCl, pH7.6; 50mM CaCl<sub>2</sub>) by stoichiometrically balanced 8-PEG-MMP-Lys and 8-PEG-Gln solutions upon addition of activated FXIII. Cells and bioactive molecules were added to the precursor solution prior to gelation. Adhesion and migration behavior of the mouse preosteoblastic cell line MC3T3-E1 was followed by fluorescence and time-lapse microscopy. Differentiation of MC3T3-E1 and human mesenchymal stem cells in 3D cultures was assessed by determination of alkaline phosphatase activity. Bone healing experiments were performed in critical-size defects of the rat cranium were harvested after five weeks of implantation and analyzed by microcomputed tomography and histology.

**RESULTS:** The enzymatic incorporation of TG-Gln-RGD peptides was almost quantitative and resulted in a dose-dependent spreading of MC3T3-E1 cells in 2D cultures. Cell spreading

and migration in 3D cultures was strongly dependent on the incorporated TG-Gln-RGD concentration but also the matrix responsiveness to matrix metalloproteases. Furthermore 3D-cultured mouse pre-osteoblastic cells and human mesenchymal stem cells differentiated in response to BMP-2 entrapped within the gel or administered to the culture medium. When implanted in rat cranial defects, FXIII crosslinked hydrogels were remodeled by proteolytically invading cells. When BMP was entrapped within the matrix bone formation was induced on the hydrogel tissue interface as has also been observed with chemically crosslinked PEG hydrogels.<sup>2</sup>



*Fig. 1: bone repair in critical size defects Empty matrices were covered by newly formed bone in presence of BMP2 (right). were not healed in absence (left) but*

**DISCUSSION & CONCLUSIONS:** In contrast to materials from natural sources these novel artificial ECMs allow the nearly independent control of properties including matrix stiffness, protease susceptibility and presentation of biological cues. The tailoring of these properties in a wide range enables us to rationally control cell behavior in both in vitro and in vivo contexts. These matrices could be useful tools for experimental cell biology as well as for in vivo applications such as bone tissue regeneration.

**REFERENCES:** <sup>1</sup>Lutolf and Hubbell, Nature Biotechnology, 23 (1): 47-55 (2005) <sup>2</sup>Rizzi S.C., Biomacromolecules. 2006 Nov;7(11):3019-29

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