

Enzymatically formed, Modular designed Biomaterials

M. Ehrbar¹, M.P. Lütolf², A. Sala¹, S.C. Rizzi^{1,3}, J.A. Hubbell², F.E. Weber¹

¹ Bioengineering, Department of Cranio-Maxillofacial Surgery, University Hospital Zurich, Zurich Switzerland

² Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland

³ Present address: School of Life Sciences, University of Technology (QUT), Brisbane, Australia

INTRODUCTION: Naturally occurring materials were successfully explored in Tissue engineering approaches¹. However synthetic hydrogels that can be designed for specific applications with respect to mechanical properties, susceptibility to proteolytic activity and biological cues might be good alternatives for cell delivery and tissue regeneration. Here we present poly(ethylene glycol) (PEG)-based hydrogels that are biomimetic in both their synthesis and degradation. This new class of biomaterials enables the formation of matrices and the tethering of multiple engineered bioactive molecules in a simple one step reaction, which is catalyzed by the action of the activated transglutaminase enzyme factor XIIIa. The modular designed of the system allows the almost independent and flexible variation of physical and biological properties by using different building blocks.

METHODS: Hydrogel networks were formed in TBS (50mM TrisHCl, pH7.6; 50mM CaCl₂) upon addition of activated FXIII to PEG precursor solutions, which consisted of a stoichiometrically balanced mix of 8arm-PEGs that are functionalized with either Lys-domaine or TG-domaine (two known substrate domains for FXIII)². Cells and/or bioactive molecules were added to the precursor solution prior to gelation. Coupling efficiency and release of RGD-peptides and vascular endothelial growth factor (VEGF) molecules that were modified with a TG-domain were assessed by HPLC and ELISA respectively^{2,3}. Adhesion and migration behavior of cells was followed by fluorescence and time-lapse microscopy. Matrices containing tethered VEGF were grafted on top of the chick chorioallantoic membrane (CAM) at embryonic day 9 and bioactivity was determined by morphometric analysis.

RESULTS: We show that bioactive molecules, such as peptides and proteins, with concentrations up to 100µM can be quantitatively and site specifically tethered to the forming hydrogel, without altering the

network's macroscopic properties. The incorporation of the integrin ligand RGD supported cell adhesion on 2D substrates in a dose dependent manner and was indispensable for cell spreading and migration in a 3D environment. The migration behaviour of cells in 3D culture could be modulated by varying polymer concentration and substrate's susceptibility to proteolytic degradation. Incorporated vascular endothelial growth factor (VEGF) was shown to be released upon cell-derived proteolytic degradation of the gels and, due to the precise control over the conformation of the immobilized morphogen, completely retained its bioactivity.

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

REFERENCES:

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