Photosensitive gelatine-methacrylamide as engineered extracellular matrix for three-dimensional cell culture

K Hölzl[1,2], L. Atanasova[3], M Markovic[1,2], P Gruber[1,2], J Van Hoorick[4], S Van Vlierberghe[4], P Dubruel[4], R Liska[2,5], A Ovsianikov[1,2]

[1] Institute of Materials Science and Technology, Technical University Vienna,[2]Austrian Cluster for Tissue Regeneration, [3]Institute of Chemical Engineering, Technical University Vienna, [4] Polymer Chemistry and Biomaterials Group, Department of Organic and Macromolecular Chemistry, Ghent University, Belgium, [5]Institute of Applied Synthetic Chemistry, Technical University Vienna, Austria.

INTRODUCTION: In order to meet the need to repair or replace damaged tissue, the fabrication of functional tissues remains a major aim for tissue engineering and regenerative medicine. Methacrylamide-modified gelatin (gelMOD) [1], is an enzymatically degradable hydrogel derived from collagen. Due to the introduced methacrylamide groups it becomes photocrosslinkable with tunable mechanical properties. As it is already well known that cells respond to their environment [2], gelMOD might represent a good material to study the influence of substrate properties on cells.

METHODS: Human bone-marrow derived mesenchymal stem cells (hMSC) were encapsulated in 10 wt% of gelMOD with 0.6 mM of photoinitiator LiTPO-L. The cells were resuspended in the gelMOD solution at a cell density of 10^7 cells/mL. The hydrogel was then polymerized for 10 minutes using UV-A light (365 nm, 4 mW/cm^2). Afterwards, the cells were cultured in basal or osteogenic medium for 17 days. Metabolic activity and viability was monitored using a Presto Blue assay and live-dead staining.

RESULTS: The cells survived the process of encapsulation and remained viable after 17 days in culture. There was no significant difference in metabolic activity between the cells cultured in basal- and osteogenic medium. Furthermore, no significant difference in metabolic activity was present between day 0 and day 17. Live-dead staining indicated a round morphology of the cells after 0 and 7 days, but a more stretched appearance was present after 17 days. Moreover, the hydrogel pellets, which were stimulated with osteogenic medium were milky in contrast to the pellets cultured in basal medium. This indicates a strong presence of calcium deposits in the stimulated samples. Gene expression analysis will support our data to characterize the influence of gel properties on the cells.

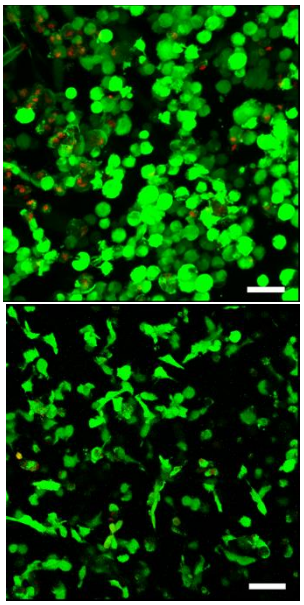

Fig. 1: live dead staining of hMSC cells inside the hydrogel: the day after encapsulation (left), after 17 days in basal medium (right); scale bar: 50 µm

DISCUSSION & CONCLUSIONS: As the cells showed consistent metabolic activity, the material is suitable for long-time cell culture for hMSC cells. As stretching of the cells and mineralization occurred, gelMOD might also be a good material to mimic the microenvironment of bone.

By varying the degree of crosslinking of gelMOD hydrogel, the mechanical properties can easily be tuned. As a consequence, gelMOD is a promising material for studying the effect of substrate stiffness on stem cell fate.

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ACKNOWLEDGMENTS: The authors acknowledge the financial support of the European Research Council (Starting Grant-307701, A.O.). We would like to acknowledge support of Dr. Irina Druzhinina (Institute of Chemical Engineering, Technical University Vienna).

DISCLOSURES: We declare no potential conflicts of interest relevant to this work.