

A Three-Dimensional Hydrogel Matrix for Human Mesenchymal Stem Cell and Urothelial Cell Growth and Differentiation

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INTRODUCTION: There is a critical need for bladder tissue replacements due to malfunction or loss of tissue following bladder diseases or malformations. Numerous studies that have focused on using cell delivery vehicles or acellular scaffolds to improve bladder regeneration have shown the re-establishment of normal bladder urothelium while the success in restoring functional bladder smooth muscle cells (SMC) remains scarce¹. Both scaffold delivered SMCs as well as host SMCs migrating into an acellular scaffold in vivo have a tendency to switch on a synthetic proliferative phenotype instead of the required quiescent contractile one, eventually resulting in non compliant fibrous scar tissue. We hypothesise that providing the right stimuli, to human mesenchymal stem cells (MSC) will result in their subsequent differentiation into quiescent smooth muscle cells. A biodegradable poly (ethylene glycol) (PEG) hydrogel provides an excellent scaffold for investigating basic biology, as well as the incorporation and delivery of biological signals for tissue regeneration and repair. In this initial study we investigated the optimal gel properties for the growth of MSCs in, and urothelial cells (UCs) on a PEG hydrogel, respectively.

METHODS: Poly (ethylene glycol)-vinyl sulfone (PEG-VS) was synthesised as previously described². The cell adhesion peptide C-RGDSP was reacted with the PEG-VS macromere by Michael Type addition. A matrix metalloproteinase (MMP) sensitive crosslinker (GPQG↓IWGQ) was used to form the gel and to provide degradation sites. To find the optimal gel properties for cell attachment and spreading, the amounts of RGDSP, PEG and crosslinker were altered. The RGDSP was varied between 100-350 μ M while keeping the amounts of cross links and percentage of PEG constant. The RGD was then kept constant at 200 μ M while varying the amount of PEG from 5.5-7.5% (w/v), and the crosslinker from $r = 1.0$ -2.8 where r equals the molar ratio of crosslinker to available PEG, respectively. Human mesenchymal stem cells (Cambrex) were mixed with the gel precursor and polymerised into the gel at a density of 30 000cells per 30 μ l gel precursor. Primary human urothelial cells were seeded on top of gels,

15000cells per 30 μ l gel, after 1h of swelling in medium. Cell attachment and spreading was explored by assessment of cell morphology through bright field and fluorescence microscopy. Cells were fluorescently labelled following standard protocols.

RESULTS: The optimal RGD content for both MSCs and UCs was found to range between 200-250 μ M. At RGD contents below 150 μ M cells rounded up alone (MSCs) or in aggregates (UCs). Gels with 7.0-7.5% PEG, and an amount of crosslinker ranging between $r = 1.4$ -1.7 were found to be preferential for cell spreading and growth. These optimal gel properties correspond to rather loose, elastic gels. In more cross linked i.e. stiffer gels the cells were unable to degrade the gel in time to make room for attachment and spreading, and in looser, less cross linked gels the cells were less able to attach, and gels difficult to handle.

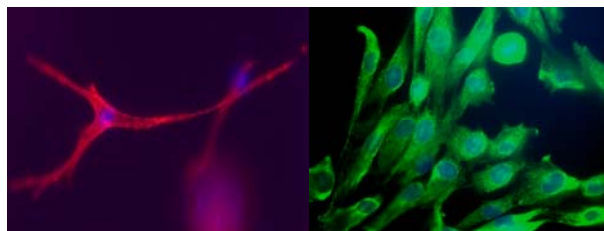


Fig. 1: Mesenchymal stem cells spreading within a three dimensional PEG hydrogel matrix (Phalloidin, DAPI staining)(left). Urothelial cells cultured on top of a PEG hydrogel retain their typical cuboidal shape (pancytokeratin staining AE1/AE3 (FITC), DAPI) (right).

DISCUSSION & CONCLUSIONS: A three dimensional PEG hydrogel scaffold has been modified for the growth of human MSCs and UCs. Current work is focused on investigating gene expressions by qPCR to determine cell phenotypes.

REFERENCES: ¹ A. Atala (2004) *Am J Transpl* 4(suppl. 6):58-73, ² M. Lutolf and J.A. Hubbell (2003) *Biomacromolecules* 4(3):713-22.

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