Bio-interactive 3D PEG hydrogels for phenotypic modulation of SMCs during bladder tissue remodeling

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Introduction: The formation of scar tissue is one of the major issues when replacing or regenerating bladder tissue. As functional contractile smooth muscle cells (SMCs) de-differentiate into a synthetic proliferative phenotype, also referred to as myofibroblast, excessive amounts of collagen are produced and the normal compliant bladder alters into a poorly compliant tissue unable to sustain the low pressures of the normal bladder. Little is known concerning why this de-differentiation occurs or the signals involved. We hypothesize that synthetic bioactive PEG hydrogels modified with integrin binding peptides and MMP-degradable sites could help providing the right signals for smooth muscle cell differentiation during tissue remodeling and enhance the understanding of the complex signaling occurring within the extra cellular matrix (ECM). As smooth muscle cells originate from mesenchyme during bladder development, and mesenchymal stem cells (MSCs) are thought to have a superior regenerative capacity, we sought to examine MSC potency of differentiation into functional SMCs in an in vitro model. The goal of this study was to investigate the influence of geometry on cell phenotypes by comparing integrin- and cell specific protein expressions of SMCs and MSCs in 2D and 3D environment.

Methods:
Hydrogel preparation: Poly (ethylene glycol)-vinyl sulfone (PEG-VS) was synthesised, and PEG hydrogel formation was performed as previously described (Lutolf MP, Biomacromolec. 2003; 4(3): 713-22). In brief, the cell adhesion peptide C-RGDSP was reacted with the PEG-VS macromere by Michael Type addition. A matrix metalloproteinase (MMP) sensitive cross linker was used to form the gel and to provide degradation sites. Primary human SMCs were isolated from bladder tissue received at open surgery. Human MSCs isolated from bone marrow were received as a generous gift from the Pierre Charbord lab, Université François-Rabelais, France. SMCs or MSCs were mixed with the gel precursor and encapsulated within the gel at a density of 300 000cells per 200µl gel precursor. Cells were maintained in culture within gels for up to 4 weeks. Medium was changed two times weekly.
Cell phenotype characterization: Cell viability and spreading was assessed by LIVE/DEAD staining and fluorescence microscopy following standard protocols. Phenotypical alterations were investigated by flowcytometry on cells labelled with antibodies against a panel of integrin subunits and cell specific proteins, and cell proliferation was determined by Alamar Blue assay. The MMPs secreted by cells were determined and quantified by zymography.

Results/Discussion: The SMCs acquired an elongated morphology already 2-3 days post seeding, and by day 7 had created networks through out the hydrogels. The MSCs took longer time to spread and did not form networks to the same extent as the SMCs. However, despite less spreading, MSCs maintained high viability over a 3-week period. The MSCs showed significant up-regulations of smooth muscle α-actin and myosin (Figure 1 (A)), and $\alpha 1$ and $\beta 1$, when transferred from 2D to 3D suggesting a differentiation towards a SMC-like phenotype. Also SMCs when transferred from 2D to 3D up-regulated the smooth muscle specific proteins (Figure 1 (B)). The cell adhesion peptide incorporated within the gels, RGD, have binding sites predominantly for $\alpha 5\beta 1$, and $\alpha v\beta 3$ integrins. The up-regulation of αv and $\beta 3$ in the MSCs, and αv in SMCs could hence be explained by the binding sites available in the RGD sequence. However, both cell types down-regulated $\alpha 5$, and the SMCs also $\beta 1$. A down-regulation of $\alpha 5\beta 1$ imply a less synthetic phenotype. The proliferative capacity of MSCs in 3D was rather sparse compared to 2D, while the SMCs were almost as invasive within 3D gels as in 2D. Both cell types expressed MMP-1 and MMP-2, both of which have the ability to cleave the cross-linker and degrade the gel. The MSCs in 3D were expressing less MMP-1 and more MMP-2 compared to 2D, while the SMCs were doing the opposite. On going work is focused on examining the ECM proteins secreted by these cells to further determine the character of the cells and the newly synthesised ECM.

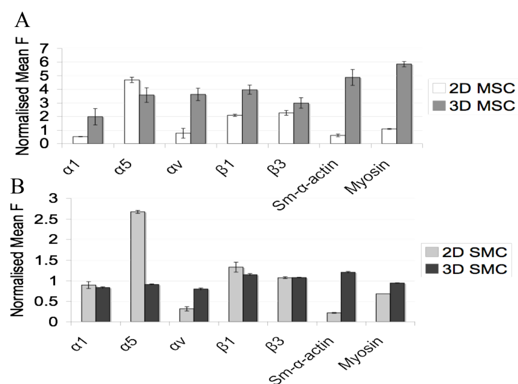

Figure 1. Integrin- and SMC-specific protein expressions of MSCs (A) and SMCs (B) cultured within PEG hydrogels.

Conclusions: We demonstrate that MSCs differentiate towards a quiescent smooth muscle cell-like phenotype when cultured within 3D PEG hydrogels, significantly up-regulating the smooth muscle specific proteins alpha actin and myosin, and expressing more MMP-2 than in 2D. Further, our data suggest that the primary SMC phenotype in vitro alters towards a less synthetic phenotype when changing the geometry of the matrix from 2D to 3D. This study addresses the importance of 3D geometry on cell fate, and proposes synthetic instructive biomaterials as a mean to direct cell functions and circumvent scar tissue formation during bladder reconstruction.