Bioactive Hydrogels based on Collagen-Mimetic Proteins

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Statement of Purpose: Recent advances in adult mesenchymal stem cell (MSC) research have shown these cells to be a promising patient-derived cell source that are not only capable of extensive proliferation but also of differentiation into a range of cell types.[1, 2] Although significant advances in the use of MSCs for functional regeneration have been achieved, a number of obstacles remain before MSC-based engineered tissues can be considered viable clinical alternatives. In particular, rational design of scaffolds to elicit desired MSC lineage progression is problematic due to our incomplete understanding of MSC responses to extracellular matrix (ECM)-mediated stimuli, including matrix stiffness and biochemical cue identity. In the present work, we begin to address this challenge by probing MSC responses to collagen-based biochemical motifs using novel hybrid scaffolds. These hydrogels were generated by covalently crosslinking diacrylate-derivatized poly(ethylene glycol) (PEGDA) and a novel collagen-mimetic protein, termed Scl2-1. Scl2-1 is unique in that it contains the GXY repeats and stable triple helix of native collagen but lacks collagen's array of cell adhesion, cytokine binding, and enzymatic degradation sites. Thus, Scl2-1 provides a "blank-slate" into which desired collagen adhesion sequences can be programmed by site-directed mutagenesis while maintaining the triple helical context natively associated with these motifs. The current work explores the impact of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrin binding on focal adhesion kinase (FAK) and mitogen activated protein kinase (MAPK) signaling and associated MSC differentiation using modified Scl2-1.

Methods:

Expression and Mutagenesis of DCs. Scl2-2 and Scl2-3 (Scl2-1-based proteins containing $\alpha_1\beta_1/\alpha_2\beta_1$ and $\alpha_1\beta_1$ binding motifs, respectively) were generated using site-directed mutagenesis of the plasmid encoding for Scl2-1, were recombinantly expressed in *E.coli* BL21, and were purified by affinity chromatography.[3] ***Scl2 Conjugation to a PEG Linker***. DCs were functionalized with photoreactive crosslink sites according to a protocol adapted from Sebra et al.[4] Briefly, DCs were reacted with acrylate-PEG-hydroxysuccinimide (Ac-PEG-NHS, M_W 2000) at a 1:1 molar ratio of NHS:lysine in 50 mM bicarbonate buffer (pH 8.5). Excess Ac-PEG-NHS and other reaction byproducts were removed via dialysis and functionalization was confirmed with FTIR spectroscopy. ***Fabrication of PEGDA-Scl2 Hydrogels***. PEGDA-Scl2 gels were fabricated by combining 10 wt % PEGDA (10 kDa) with photoinitiator (Irgacure 2959), 1 mg/mL of Ac-PEG-Scl2, AC-PEG-Scl2-2, or AC-PEG-Scl2-3, and $1x10^6$ MSCs/mL. The solutions were then crosslinked via 90 s exposure to 365 nm UV light (UV-Transilluminator, ~6 mW/cm^2). ***Construct Culture and Analysis***. Constructs were cultured in DMEM supplemented with 10% heat-inactivated FBS and antibiotics/anti-mycotics. Following 7 days total culture time, samples were harvested and MAPK signaling and MSC differentiation were analyzed by competitive ELISA and/or histology.

Results: MSCs were encapsulated in PEGDA-Scl2 hydrogels containing various combinations of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrin binding motifs (Scl2-2 - $\alpha_1\beta_1/\alpha_2\beta_1$ site; Scl2-3 - $\alpha_1\beta_1$ site). In fabricating these gels, a weight ratio of PEGDA to Scl2 of 100:1 was used, implying that the elastic modulus, mesh size, and degradation rate of each hydrogel network would be dominated by PEGDA. Gels were cultured in media containing 10% FBS for 7 days. Over this time period, no alterations in gel volume or modulus were observed. Combined, these experimental conditions ensured that Scl2 identity was the primary design variable across gels. As expected, levels of active pFAK were significantly higher in the Scl2-2 and Scl2-3 gels than in the Scl2-1 negative control. In addition, the fraction of cells expressing myocardin (an early marker of smooth muscle cell differentiation) was substantially increased by the presence of Scl2-2 while expression of runx2 (an early osteoblast marker) was not (**Fig. 1**). Further examination of associated MAPK signaling suggested that pERK1/2 and p38 signaling played critical roles in the MSC fate decisions between smooth muscle and osteoblast lineages in these hydrogels.

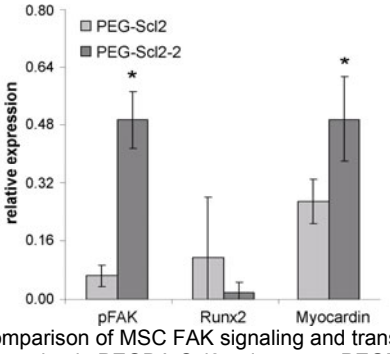

Fig 1. Comparison of MSC FAK signaling and transcription factor expression in PEGDA-Scl2 gels versus PEGDA-Scl2-2 gels. *, significant difference, $p < 0.05$.

Conclusions: The present results demonstrate our ability to achieve a controlled 3D environment that can be used to probe MSC responses to highly defined collagen-based stimuli combinations while removing the influence of the array of additional signals provided by native collagen and while maintaining collagen's triple helical context. This controlled hydrogel platform enables more precise examination of the signaling underlying observed cell responses and should significantly enhance our understanding of the processes associated with MSC lineage progression.

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