Proteoglycan sequestration provides spatially modulated mesenchymal stem cell proliferation

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Introduction: Proteoglycans are present within the extracellular matrix and on the cell surface of most mammalian cell types. Within the extracellular environment, proteoglycans regulate cell behavior (e.g. adhesion, proliferation, and differentiation) by non-covalently interacting with diverse proteins (e.g. soluble signaling molecules, receptors, and glycoproteins). We have developed substrates that mimic extracellular proteoglycan sequestration to allow for material-mediated regulation of stem cell behavior. Our approach uses covalently immobilized peptide sequences to non-covalently sequester proteoglycans present during cell culture. Here we report the use of this strategy to achieve non-covalent sequestration of proteoglycans onto otherwise bio-inert self-assembled monolayers (SAMs) of alkanethiolates on gold. Moreover, we demonstrate that spatially patterning sites of proteoglycan sequestration on the substrate provides spatially controlled up-regulation of mesenchymal stem cell (MSC) proliferation. We envision that this approach may ultimately lead to materials that provide spatial control over up- or down-regulation of diverse stem cell behaviors by spatially patterning regions that promote or resist proteoglycan binding.

Methods: Peptides Arg-Gly-Asp-Ser-Pro (RGDSP) and Lys-Arg-Thr-Gly-Gln-Tyr-Lys-Leu (KRTGQYKL) were synthesized using a standard Fmoc solid-phase peptide synthesis protocol. Tri(ethylene glycol)undecanethiol was synthesized using a standard protocol. Carboxylate-terminated hexa(ethylene glycol)undecanethiol was from Prochimia (Sopot, Poland). SAMs were formed by incubating gold-coated substrates overnight in an ethanolic solution containing alkanethiols. Peptides were immobilized onto SAMs by first incubating SAMs in an aqueous solution of N-hydroxysuccinimide and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, followed by incubation of SAMs in a 1x phosphate-buffered saline solution (pH 7.4) containing peptide.

Results: We used polarization-modulated infrared reflectance-absorbance spectroscopy (PM-IRRAS) to characterize proteoglycan binding onto SAMs. Specifically, we characterized KRTGQYKL SAMs incubated in FBS or FBS treated with heparin lyase I, as well as SAM presenting TYRKKGLQ, a non-functional peptide, incubated in FBS. Our results (Fig. 1A) demonstrated peaks indicative of proteoglycan binding on KRTGQYKL SAMs incubated in FBS, which were absent in spectra collected from KRTGQYKL SAMs incubated in FBS treated with heparin lyase I or TYRKKGLQ SAMs incubated in FBS. This result demonstrates that proteoglycans bind specifically to KRTGQYKL SAMs, and that heparin mediates binding of proteoglycans onto KRTGQYKL SAMs. We next characterized the influence of sequestered proteoglycans on MSC proliferation. Our results (Fig. 1B) demonstrated that MSC number was significantly greater within regions of a SAM presenting RGDSP and KRTGQYKL when compared to regions presenting RGDSP and TYRKKGLQ. This result indicated that proteoglycans sequestered from FBS increase MSC proliferation. Importantly, this increase in proliferation was observed during culture in medium supplemented with 10% FBS, demonstrating that MSC proliferation can be increased without the introduction of additional growth factors. Interestingly, MSCs cultured on SAMs presenting RGDSP alongside KRTGQYKL or TYRKKGLQ in medium supplemented with FBS and PD173074, an inhibitor of FGF receptors 1 and 3, demonstrated no difference in cell number (Fig. 1C), indicating that an FGF family member is responsible for the increased MSC proliferation observed on KRTGQYKL SAMs.

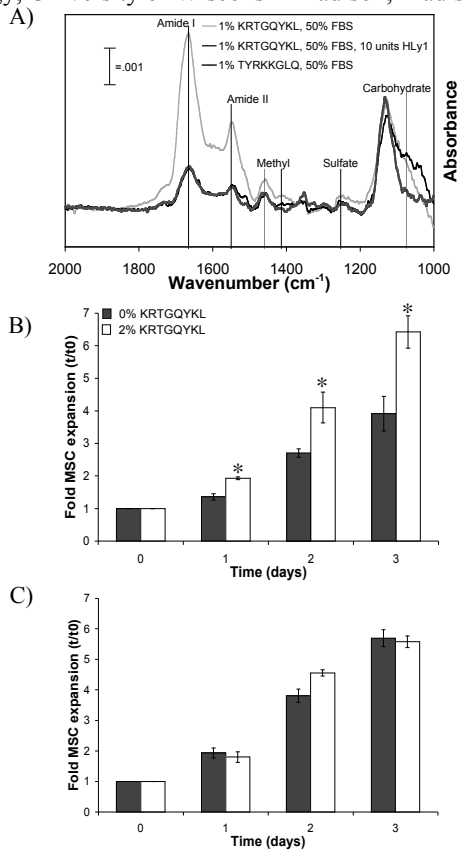

Figure 1: (A) PM-IRRAS spectra of KRTGQYKL SAMs incubated in FBS (—), FBS treated with heparin lyase I (—), or TYRKKGLQ SAMs incubated in FBS (—○—). (B) MSC proliferation on proteoglycan binding SAMs in medium supplemented with (A) 10% FBS, (B) 10% FBS and 200 nM PD173074, an inhibitor of FGF receptor 1 and 3 signaling. * represents significant difference compared to control ($p < 0.05$)

Conclusion: Our results demonstrate that sequestration of proteoglycans from serum enhances MSC proliferation in the absence of any additional growth factor supplements. Moreover, our results suggest that this approach may ultimately promote proteoglycan-mediated regulation over other therapeutically relevant MSC behaviors, such as differentiation.