Design of multidimensional culture systems for probing the role of integrin binding in fibroblast activation

Megan E Smithmyer, April M Kloxin.
University of Delaware.

Statement of Purpose: Fibrosis is implicated in 45% of deaths in the developed world.[1] Because fibroblast activation and persistence are recognized as key steps in the progression of fibrosis, a number of biomaterials have been used to study what extracellular factors influence fibroblast function and fate. In particular, synthetic hydrogels have emerged as an important and useful subset of biomaterials for understanding how fibroblast phenotype is affected by matrix modulus,[3,4] and integrin binding, usually by including either synthetic peptides or by conjugation of whole proteins.[5,6] While there are benefits to either method, where whole proteins are more mimetic of the in vivo environment while peptides can be included for 3D culture without disrupting the hydrogel network; it is unclear how these two methods differ in their effects on fibroblast phenotype. Towards answering this question, we present work investigating the effects of integrin binding to whole proteins or to related peptide mimics on fibroblast adhesion and activation using well-defined synthetic hydrogels for multidimensional (2D, 2.5D, 3D) culture. These studies provide insight into the design of culture models for studying fibrosis, as well as the role of integrin binding and polarization on fibroblast activation.

Methods: 8-arm PEG was functionalized with norbornene (NB) end groups using published protocols.[6] Solid phase peptide synthesis was used to synthesize a cell-degradable peptide crosslink GCRDVPMS↓MRGGDRCG, a collagen mimic CG(POG)$_3$POGFOGER(POG)$_4$G (GFOGER, $\alpha_2\beta_1$ and $\alpha_1\beta_1$), a fibronectin mimic CGKSSPHSRN(G)$_{10}$RGDS (PHSRN, $\alpha_5\beta_1$), and a pendant amine peptide CGGGK for coupling of whole proteins. The identities of the peptides were confirmed using mass spectrometry. Hydrogels were formed by mixing PEG-NB with crosslink, an integrin binding peptide or CGGGK, and LAP in PBS then exposing the mixture to UV light. For comparison of cell response to whole proteins versus peptide mimics, proteins were conjugated to the surface using Sulfo-SANPAH. Human pulmonary fibroblasts (CCL151) were cultured on top (2D culture) or within (2.5D or 3D culture) the materials in Ham's F12K media with penicillin, streptomycin, fungizone and 10% fetal bovine serum. Cells were immunostained to assess activation (alpha smooth muscle actin, collagen I), imaged using confocal microscopy, and analyzed using Volocity.

Results:

Fibroblasts were cultured on hydrogels functionalized with fibronectin or collagen or peptide mimics (PHSRN and GFOGER). Fibroblasts were found to adopt similar morphology on gels functionalized with fibronectin or PHSRN, but expressed slightly more alpha smooth muscle actin (αSMA) on the peptide, indicating increased activation. On GFOGER, fibroblasts were found to form activated clusters, as shown in Figure 1A, whereas no clustering was observed on collagen. The level of expression of αSMA differed between the two conditions and was found to be dependent on the concentration of collagen, where the higher collagen concentration lead to higher αSMA expression comparable to αSMA expression on GFOGER. Additionally, fibroblasts were cultured *within* hydrogels containing the GFOGER peptide to better understand how cell polarization affects the formation of focal adhesions, and ultimately, αSMA expression. Fibroblasts were cultured in traditional 2D culture on gels, as a 'sandwich' between hydrogel layers (2.5D Figure 1B), and in 3D culture within gels. The cells were stained for vinculin and the position of vinculin within the cell body was correlated to αSMA.

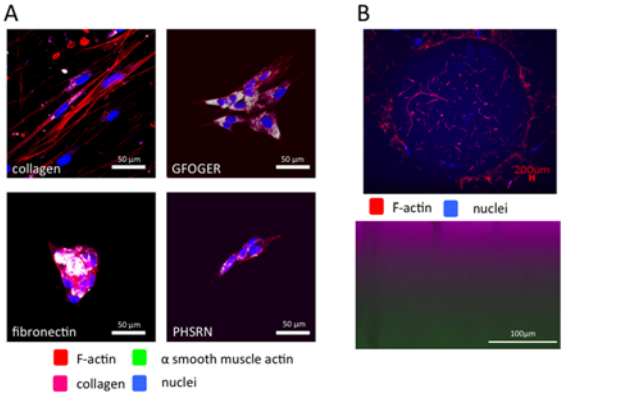

Figure 1. (A) Fibroblast response to matrices with either whole proteins or peptides. (B) Layered hydrogels were formed and used for 2.5D culture of fibroblasts (here, GFOGER functionalized hydrogel). AlexaFluor 647 maleimide and AlexaFluor 488 labeled CGRGDS were used to fluorescently label gel layers which were imaged using confocal microscopy.

Conclusions: The differences we observed in αSMA between our protein and peptide conditions indicate that the proteins and peptides were not entirely equivalent at the concentrations first used. The discovery that increasing the concentration of collagen in our hydrogel system increases the level of αSMA expression indicates that there may be differences in the level of integrin binding in those two conditions, which are being further investigated. Further, these findings motivate the need for careful selection and evaluation of peptide mimics and culture geometry when designing matrices for cell culture models. Taken together, this work contributes to our understanding of how the extracellular environment affects fibroblast activation toward the development of improved disease models for studying fibrosis.

References:

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