Control of Mesenchymal Stem Cell Motility in 3-D Synthetic Biomaterials

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Introduction

Though widely unappreciated, marrow-derived stem cell migration is a critical event in natural wound healing. During normal and injury-prompted tissue repair, mesenchymal stem cells (MSCs) can be induced to leave native bone marrow by chemoattractants, proteolyze the basal lamina to enter the blood stream, and, finally, adhere to foreign matrix at the wound site. In these scenarios, MSCs are required to migrate while encountering a diverse set of extracellular cues, including, but not limited to, changes in matrix stiffness, adhesivity, proteolytic sensitivity, porosity, and mitogen gradients. Therefore, studying MSC migration phenotypes *in vitro* poses particular challenges for biomaterial design. Here, we present a synthetic poly(ethylene glycol) (PEG) system in which adhesivity, matrix stiffness, porosity, degradability and growth factor presentation can be independently tuned. We propose to use this highly tunable system to analyze distinct biophysical features of the 3-D ECM on MSC migration. While no model system will be able to recapitulate MSC migration scenarios for every environment in the body, we hypothesize this synthetic environment in particular will contain enough flexibility of design to be applicable to a wide variety of ECM microenvironments.

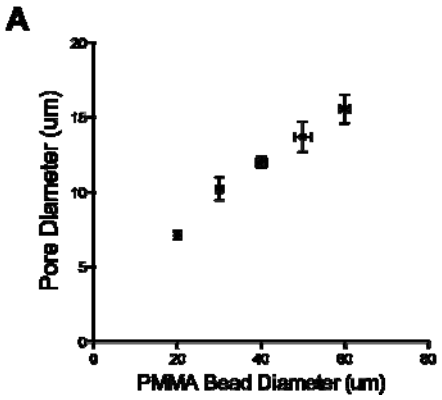
Materials and Methods

To create hydrogels with a macroporous internal structure, we have adapted and modified a previously published method [1]. Briefly, poly(methyl methacrylate) (PMMA) microparticles are forced into an ordered array within a PDMS gasket with defined dimensions via high-speed rotation. A polymer solution based on PEG dimethacrylate (PEGDMA) is then poured around the ordered PMMA beads and polymerized via UV-light for 3 min. Post-polymerization, the PMMA beads are leached away from the hydrogels using regular changes of tetrahydrofuran over three days, leaving a PEG-based hydrogel with internal macropores. The concentration of the crosslinker PEGDMA (from 10-34%) and a hetero-bifunctional PEG-methacrylate (PEGMA) (from 1-12.5 mM) are varied to control the bulk mechanical properties and the cell-adhesive ligand density, respectively. To facilitate cell adhesion and migration within these hydrogels, we coupled the adhesive peptide sequence GRGDSP using nitrophenyl chloroformate activation of the hydroxyl end of PEGMA, post-polymerization.

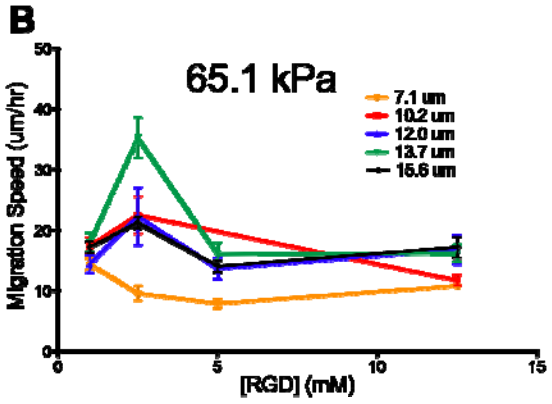
Immortalized marrow derived stem cells (hTERT MSCs) were seeded into the porous hydrogels via light centrifugation. MSC migration was then tracked via a humidified confocal microscope and Imaris software (Bitplane) using isosurface tracking domains.

Results

Using confocal microscopy and a fluorescein-modified PEGMA, we have shown that the interconnected pore sizes in these hydrogels can be tightly controlled by varying the diameter of the PMMA beads (Fig. A). By varying the concentration of the crosslinker PEGDMA from 10-34%, we can control the bulk mechanical properties of these gels (tested by nanoindentation) from tens to hundreds of kPa (data not shown). These pore sizes and stiffnesses are also independently tuneable from the ligand density within these gels, which was verified by UV-vis spectroscopy (not shown).



Finally, it appears that these three independently tuneable biomaterial properties all have distinct control of MSC migration in 3-D. A biphasic dependence on RGD concentration emerges in the higher pore sizes (see the 13.7 um condition, Fig B.). However, this biphasic response disappears when gel stiffness increases (not shown).



Conclusions

We have seen that the pore size, compliance, and adhesivity of these PEG-based hydrogels can be independently tuned, and each of these factors seems to impact MSC migration in our 3-D synthetic matrices. In addition, we are currently working on novel chemistries to incorporate tethered EGF and degradation sites as additional independent parameters to direct motility. Finally, once a relationship between these biophysical parameters of the ECM and MSC migration is defined, we will begin to parse out the signalling molecules responsible for this response.

References

1. Stachowiak, A.N., et al., Advanced Materials, 2005. **17**(4): p. 399-403.