Interlinked PEG-4MAL Microgels for Rapid Immune Cell Migration

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Statement of Purpose: We engineered a polyethylene glycol maleimide (PEG-MAL) granular hydrogel that provides a porous synthetic scaffold while maintaining many of the advantageous properties of conventional bulk PEG hydrogels. Synthetic hydrogels, such as PEG and its derivatives, have become widely used for regenerative medicine due to their plug-and-play design and excellent biocompatibility. Conventional bulk PEG hydrogels are limited by the nanoscale crosslinking network preventing rapid cell motility. Using granular hydrogels interlinked by guest-host interactions, we designed a scaffold for rapid cell migration through the induced interstitial spaces. Furthermore, these granular hydrogels demonstrate shear-thinning behavior and viscoelasticity. This enables injectability without the destruction of the scaffold and cells within. The granular hydrogels are interlinked with reversible, non-covalent guest-host interactions, providing self-healing capabilities to the overall scaffold. Based on these studies, interlinked granular hydrogels show potential as an injectable scaffold that can be used to observe and control immune cell migration.

Methods: Interlinked granular PEG hydrogels were generated by close-packing two species of PEG-MAL microgels functionalized with guest/host molecules. Four-arm PEG-MAL macromer was reacted with adamantane-thiol (Ada) (guest) or mono-6-mercapto-β-cyclodextrin (β-CD) (host) at pH 5.6. The precursors were combined with PEG-dithiol crosslinker and AlexaFluor-488 (β-CD) or 568-maleimide (Ada) and emulsified in mineral oil with SPAN80 to form microgels. To form interlinked networks, equal amounts of the Ada and β-CD species of microgels were mixed and packed by centrifugation. Microgel size distribution and presence of guest-host interactions were characterized by optical microscopy and quantified by ImageJ. Strain amplitude, cyclic strain, frequency sweeps and viscous flow sweeps were performed on an Anton Paar 706 rheometer. Cell viability and migration were performed with THP-1 monocytes and imaged by confocal microscopy.

Results: We generated a PEG-MAL microgel matrix with interlinked guest-host molecules (Figure 1A) and an open interstitium. We demonstrated control over the size of the microgels by varying vortexing time and quantified the correlating size of the interstitial space. This control over size allows for the tuning of the scaffold pore size. We confirmed the reversible guest-host interactions between Ada and β-CD by binding soluble β-CD conjugated with Alexa-Fluor 488 to microgels with and without Ada conjugated to the PEG-MAL. After washing to remove excess soluble β-CD, the samples were imaged for the presence β-CD on the microgels. We conducted rheology to observe any differences in mechanical properties between guest-host and PEG-MAL (without guest/host) microgels. Both species of microgels demonstrated shear thinning, self-healing, and viscoelastic behavior. The guest-host microgels were able to undergo several cycles of high strain and maintain the original elastic storage modulus, whereas PEG-MAL microgels dissociated. A transwell invasion study was performed to observe the motility of THP-1 monocytes through the interlinked microgel matrix over 24 hours. A thin compact layer of guest-host hydrogel was formed in the bottom of the transwell insert and cells were placed on top. Interlinked microgels functionalized with RGD showed increased cell invasion in comparison to unfunctionalized and bulk PEG-MAL controls.

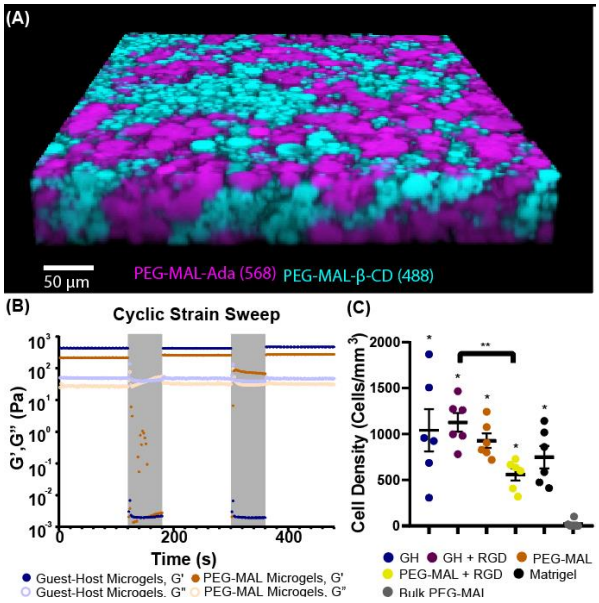

Figure 1. (A) Confocal microscopy z-stack volume rendering of PEG-MAL-Ada and PEG-MAL-β-CD microgels. (B) Cyclic strain between low (1%) and high (500%) strain (shaded) of storage (G') and loss modulus (G"). (C) Cell invastion within gel matrix after a 24 hour transwell migration assay.

Conclusions: Here we report the development of a polyethylene glycol (PEG) hydrogel scaffold stabilized with guest-host interactions between microgels. The granular system provided by packing microspheres together created an open interstitium that enabled substantially faster cell invasion than conventional bulk PEG hydrogels. Furthermore, the granular system showed shear-thinning capabilities that suggest the guest-host microgels would be injectable. Due to batch emulsion techniques, these microgels can be manufactured in large quantities using off-the-shelf components, making it available to adopt in most lab settings. Future investigations include fine-tuning the guest-host molecular interactions occurring within the material system and characterizing the *in vivo* response to implanted guest-host microgels. Interlinked microgels provide a modular scaffold while preserving the engineering toolbox of PEG-MAL.