Caged RGD-presenting Hydrogels for the Dynamic, In vivo Display of Adhesion Ligands

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Statement of Purpose: Cells rely on time-dependent binding and activation by the ECM to initiate downstream signal transduction [1]. It is unknown whether adhesion to a ligand is required throughout various cell processes, or only during a specified time period ("temporal threshold"). While most synthetic ECM platforms only present static bioadhesive ligand densities or are limited to model substrates [2,3], we have developed a UV-labile caged-RGD system that offers the flexibility of light-activated spatiotemporal control of ligand presentation [4]. Upon UV irradiation, the "caging" group, tethered adjacent to the aspartic acid residue, is cleaved, unmasking the cyclic RGD peptide. With this technology, the effects of temporal presentation on cell processes can be elucidated. In this study, we engineered hydrogels presenting caged RGD adhesive peptides and demonstrate that in vivo activation of this ligand modulates inflammation.

Methods: We used poly(ethylene glycol) (PEG) hydrogels (Laysan Bio) crosslinked using acrylate-based photo-chemistry [5]. The caged cyclo(RGDfk) peptide has been described [4]. Functionalized PEGDA-conjugates were created using acryl-PEG-SVA (Laysan Bio) via NHS ester chemistry with primary amines on RGDS and caged-RGD. Adhesive peptides were tethered onto the surfaces of bulk PEGDA hydrogels by coating with acrylated-PEG conjugates and crosslinking using TEMED/ammonium persulfate. After stringent washing, triggerable nature of these adhesive peptides was examined in vitro using NIH3T3 fibroblasts. Functionalized hydrogels were implanted subcutaneously into the backs (2 hydrogels per animal) of the mice and exposed UV light at Day 0, 7, and 14. Mice were sacrificed at 1 and 3 weeks. Explanted samples were analyzed by H&E staining.

Results: Control experiments showed that the photo-labile caging group is not compromised during the free radical cross-linking reaction that tethers the adhesive peptide to the PEG-hydrogel. Upon exposure to UV light, the "uncaged" RGD exhibited an 8-fold increase in NIH3T3 cell adhesion (at 24 h post-seeding) compared to the no UV light exposure, "caged" RGD, mutated RGD peptides, and unfunctionalized controls (Fig. 1). For the in vivo study, hydrogels presenting caged and static RGD were implanted subcutaneously and exposed to UV at different post-implantation times. At the one week endpoint, the "uncaged" RGD (Day 0 Expose) has equivalent fibrous capsule thickness as the static RGD control, and exhibits a 2-fold increase in capsule thickness compared to "caged" RGD (No Expose) and non-adhesive PEGDA controls (Fig 2). At the three week endpoint, in the Day 7 Exposure condition, fibrous capsule thickness is attenuated compared to the Day 0 Exposure conditions, yet still greater in thickness than the No Exposure condition, indicating that it is possible to activate the cage transdermally after 7 days (Fig 3).

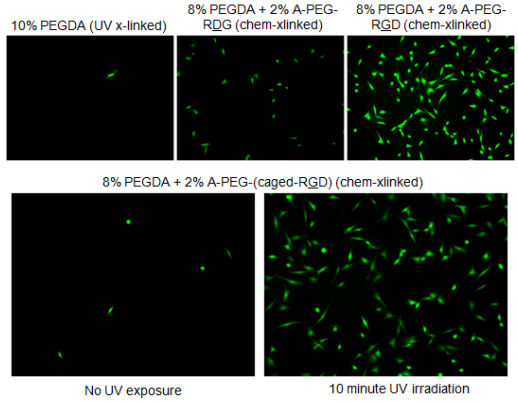

Figure 1. Calcein AM / Ethidum Homodimer II (Live/Dead) Stain. UV irradiated caged-RGD exhibits an 8-fold increase in cell adhesion vs. non-UV control.

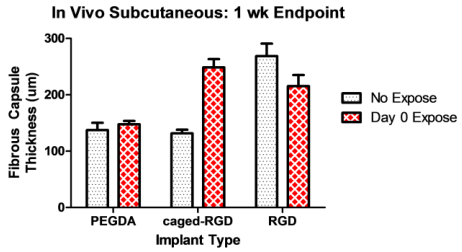

Figure 2. Fibrous capsule thickness increased significantly with the activation of caged-RGD (>100um). Data is consistent with PEGDA and RGD-surface controls.

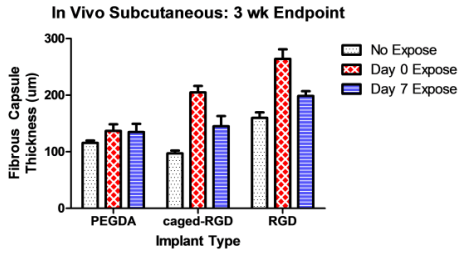

Figure 3. Fibrous capsule thickness increases on caged-surface condition with "Day 0 Expose" vs. "No Expose." On "Day 7 Expose," there is attenuation of fibrous capsule thickness presumably caused by limited adhesion during acute phase immune response.

Conclusions: In vivo data demonstrates the first successful in vivo characterization of dynamic adhesive ligands by attenuating fibrous capsule formation and represents a promising strategy to both limit adhesion of acute phase primary inflammatory responders and promote downstream tissue integration.

References: [1] Rowley et al., *JBMR*, 60, 217-23 (2002); [2] Okano et al., *Biomaterials*,16 :297-303 (1995) ; [3] Yeo et al, *JACS*, 125 :14995-5 (2003) ; [4] Petersen et al., *Angew Chem.*, 47:3192-95 (2008); [5] Phelps et al., PNAS, 107; 3323-8 (2009)

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