Light-triggered in vivo Activation of Adhesive Peptides Regulates Cell Adhesion and Vascularization of Biomaterials

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Statement of Purpose: Cell adhesion to the extracellular matrix (ECM) provides biochemical signaling, mechanical support and mechanotransduction. Misregulated cell-ECM interactions have been implicated in many pathological conditions such as tumorigenesis and abnormal vascular development. The engineering of synthetic materials for regenerative medicine will likewise need precise spatiotemporal control of ligand presentation in order to achieve desired effects. Recent technologies exploit physical stimuli such as light, temperature or electric fields to regulate biomaterial-cell interactions in vitro. However, there are presently no technologies to trigger ligand presentation in vivo. The objective of this project is to engineer a material able to spatially and temporally control the in vivo presentation of a bioligand using a synthetic cell-adhesive cyclic RGD peptide with a protecting group ('cage') as well as investigating how spatiotemporal bioligand activation in vivo regulates cell adhesion and vascularization of the material.

Methods: DMNPB-caged c[RGDfC] peptide was synthesized from a TCP-L-Gly-Fmoc resin (Intavis Peptides) and sequentially coupling Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Cys(trt)-OH, Fmoc-D-Phe-OH and Fmoc-L-Asp)DMNPB-OH. Scrambled c[RDGfC] peptide was synthesized in a similar fashion. Either DMNPB-caged c[RGDfC] or c[RDGfC] were functionalized onto a 4-arm PEG-MAL (20kDa, Laysan Bio) macromer. Functionalized macromers were cross-linked with MMP-cleavable peptide GCRDVPMSMRGG-RCG. For vascularization studies in vivo, rhVEGF$_{165}$ was pre-functionalized to the PEG-MAL macromer to a final concentration of 10 µg/mL.

In vivo studies: Hydrogels were implanted subcutaneously in 6-10 week old Balb/c mice. A custom-built UV exposure system was used for transdermal irradiation. ***Cell Adhesion:*** To study cell adhesion to the implanted biomaterials, hydrogels were UV-exposed before implantation, immediately after implantation or implanted without UV exposure. After 24 hours, mice were euthanized and hydrogels explanted, stained with DAPI and imaged. ***Patterning:*** For transdermal patterning studies, an exposure mask with a 0.9 mm hole was placed over the implanted hydrogel and UV-exposed. After 24 hours, mice were euthanized and hydrogels explanted, stained with DAPI and imaged. ***Vascularization:*** For vascularization, hydrogels were polymerized within the subcutaneous pockets. At day 14, mice were euthanized, hydrogels explanted and stained with antibodies against CD31 and α-smooth muscle actin.

Results: Hydrogels were implanted subcutaneously, irradiated transdermally with UV light to 'uncage' and activate the ligand followed by explanting after 24 hours. Hydrogels containing the c[RGDfC] ligand and UV-exposed prior to as well as immediately after implantation show no difference in cell adhesion and have equivalent levels compared to a static RGD control. Hydrogels containing the scrambled c[RDGfC] ligand exhibited minimal cell attachment regardless of irradiation conditions and were comparable to static scrambled RDG controls (Figure 1A). Additionally, when hydrogels having c[RGDfC] were irradiated through a photomask, cell adhesion was only noted in the area of irradiation indicating spatial control over ligand activity (Figure 1B,C). To assess the functionality of the caged compound, vascularization was assessed at 14 days post implantation. Hydrogels with c[RGDfC] showed in-growth of vasculature when irradiated at days 0 and 7 while no vasculature was noted when hydrogels were not UV-exposed. Additionally, no vasculature was noted in any irradiation conditions with hydrogels containing the scrambled c[RDGfC] ligand (Figure 1D).

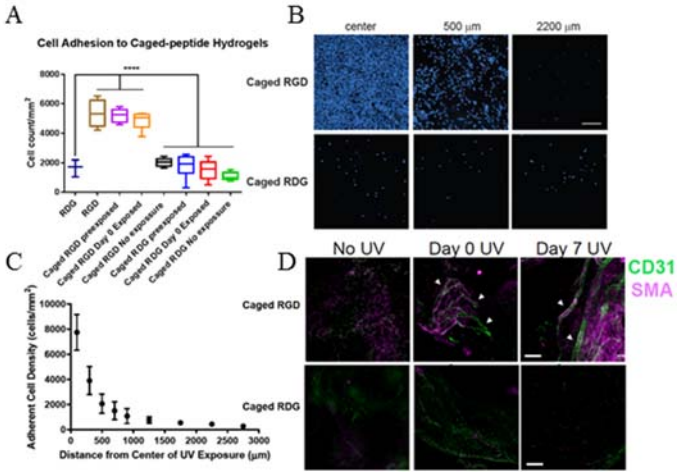

Figure 1: A) *Quantification of cell adhesion to hydrogels explanted after 24 hours p<0.0001* **B)** *Representative images of spatial control of cell adhesion (scale bar, 200 µm) with associated* **C)** *Quantification* **D)** *Representative images of vasculature infiltrating hydrogels. White arrows point where SMA wrap around CD31 staining indicating mature vessels. (scale bar, 100 µm)*

Conclusions: We demonstrate the ability to spatially control the activation of a bioligand in vivo resulting in differential effects on cell adhesion. Interestingly, activation of the ligand after 7 days results in similar vascular infiltration at 14 days compared to activation at day 0 indicating the ability to retain physiological activity of the ligand. This basis of the work demonstrates that triggered presentation of bioligands can be utilized to direct tissue reparative responses associated with biomaterials.

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