Adhesive Peptides Modified with Long Spacers Encourage Human Corneal Epithelial Cell Attachment and Spreading

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Statement of Purpose: A synthetic biomimetic basement membrane should incorporate multiple biochemical cues to best approximate the *in vivo* environment. Our goal is to design a synthetic hydrogel platform that supports the addition of multiple biochemical cues, including adhesive ligands and growth factors, without impacting the bulk mechanical properties of the gel. The addition of high concentrations of monoacrylated ligands will change the modulus of a material[1]. We investigated methods that decreased the required concentration of monoacrylated ligands to be incorporated to the bulk material. One approach is to fabricate monoacrylated ligands with a spacer to increase the distance between ligand and bulk material which would allow peptides and growth factors to be more accessible to the cell and require lower concentrations in the final material. We synthesized ligands composed of flexible PEG spacers of varying length and the adhesive ligand RGD to explore the role of spacer length in cell attachment and spreading.

Methods: The adhesive ligand RGD was synthesized using standard Fmoc solid phase synthesis techniques. While still on resin, the peptide was manually coupled to a monodisperse heterofunctional Fmoc-NH-PEG_n-COOH spacer (n=5, 11, or 27). The primary amine terminus of the PEG-peptide was functionalized into an acrylamide. Peptides were purified with HPLC and characterized with mass spectrometry. RGD peptides were also modified with an acryloyl-PEG_{77}–N-hydroxysuccinimide spacer as described elsewhere[2]. These PEG-peptide conjugates were incorporated into the bulk of a photopolymerized poly (ethylene glycol) diacrylate (MW3400) hydrogel at concentrations varying from 0.2 to 2mM. Telomerase-immortalized human corneal epithelial (hTCEpi) cells were plated on planar hydrogels, allowed to attach for 24 hours, then fixed, stained for nulcei and actin and imaged. Images were analyzed for number of cells attached and projected spread area.

Results: Modifying the PEG spacer length between RGD and the bulk hydrogel influences both hTCEpi cell attachment and spreading. Cells exhibited a rounded morphology and fewer cells attached to hydrogels with RGD peptides modified with no spacer, PEG_5 or PEG_{11} spacer. However, hydrogels that were functionalized with a longer spacer length of PEG_{27} or PEG_{77}, showed a positive relationship between RGD concentration and cell number; as the concentration of RGD increased, the number of cells attached to the surface increased significantly. Furthermore, at the highest concentration of RGD for the longer PEG spacer lengths, there was almost a two fold increase in projected cell area compared to shorter PEG spacer lengths.

Conclusions: We have developed a novel method to control the presentation of ligands by systematically changing the PEG spacer length between the ligand and bulk hydrogel. With these materials we have shown that there is a minimum linker length that supports cell attachment and spreading at low concentrations. In addition, cell spreading and attachment is similar on surfaces modified with ligands attached to PEG_{27} spacers or PEG_{77} spacers. The mechanism for the dependence on spacer length is unknown. We hypothesize that the flexibility of the longer chain supports rearrangement of the bioactive motifs. An alternative explanation is that the longer PEG chain increases relative RGD concentration that is available to interact with cells due to extension from the hydrogel.

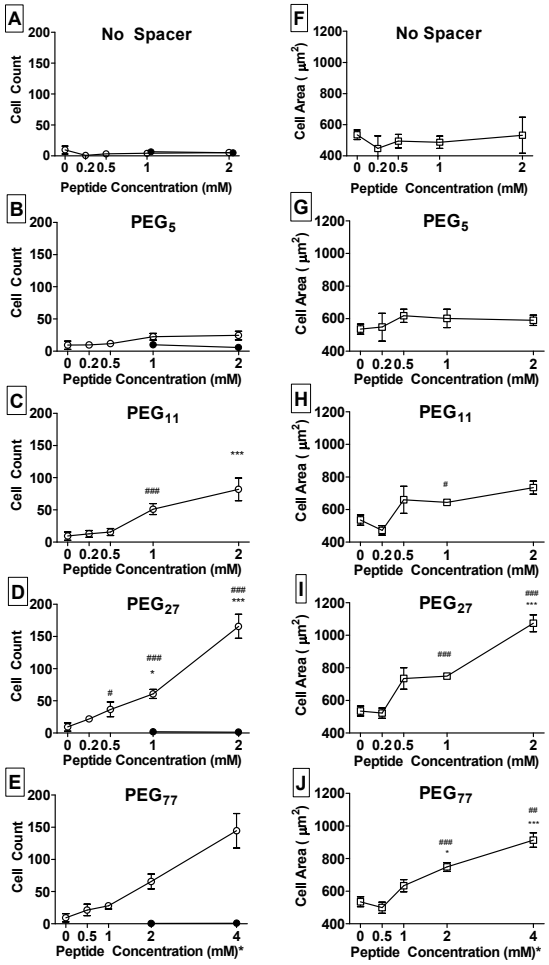

Figure 1. A-E) hTCEpi cell number per surface for a given spacer length. F-J) hTCEpi projected cell area for a given PEG spacer length after 24 hours.

References:

1. Beamish, JA. J Biomed Mater Res. 2010; 92A:441-450
2. Hern, DL. J Biomed Mater Res. 1998; 39:266-276.