Developing Grafted Poly(γ-propargyl L-glutamate) as a Platform to Present Nano-Clustered Extracellular Cues

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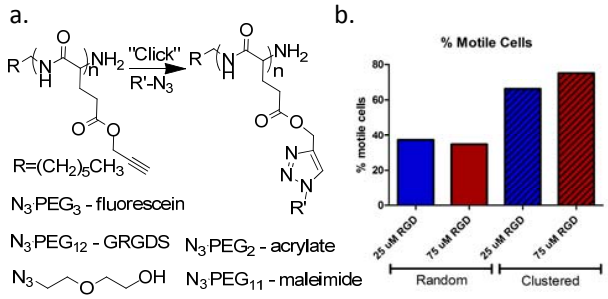
Statement of Purpose: Signal integration and cross talk of transmembrane proteins such as integrins, growth factors, and even cell surface proteases in response to extracellular cues are widely recognized as essential to regulating cellular response but poorly understood.[1] In vivo, nanoscale co-localization of these signaling factors via mechanisms such as ordered binding to extracellular matrix molecules and cell membrane lipid rafts or tetraspanin networks have been suggested as key modulators of cell surface topography and the resulting intracellular signaling landscape. However, efforts to more completely characterize the agency of these interactions have been hindered by challenges in 1) definitively characterizing cell surface co-localization and 2) independently modulating nanoscale clustering to prove correlated phenotypes. Biomaterial techniques such as those based on nanolithography have been deployed to begin understanding the effects of colocalization by monitoring cell response to nano-patterned adhesion molecules on stiff 2D cell culture surfaces.[2] The goal of this project is to move towards developing a 3D polymeric hydrogel screening platform to better characterize how nanoclustering of bio cues such as adhesion peptides and growth factors influence cellular phenotype. Specifically we introduce poly(γ-propargyl L-glutamate) (PPLG) as an synthetically tractable, readily characterized, highly modular tool to deploy nano-clustered tethered ligands in established Michael-type and UV polymerized poly(ethylene glycol) (PEG) hydrogels.
Methods: PPLG was synthesized as in the literature through NCA polymerization[3] and functionalized with PEG_{12}-RGD (as a representative cell adhesive ligand), PEG_3-fluorescein (to quantify PPLG incorporation), PEG_2-acrylate or PEG_{11}-maleimide (as crosslinkers), and PEG_2-OH (to enhance water solubility). The grafted PPLG was introduced into both UV polymerized and michael-type addition polyethylene glycol hydrogels. The intermediate and final PPLG molecules and PPLG-containing gels were characterized by 1H-NMR, GPC, and microscopy for composition, molecular weight, stability in water, and swelling behavior. hTERT immortalized mesenchymal stem cells (htMSCs) were labeled with cell tracker green (Invitrogen) and seeded on the PEG-PPLG-RGD hydrogels. Percent motile cells was quantitatively observed by time lapse microscopy.
Results: We successfully synthesized PPLG-based polypeptide having, on average,150 alkyne side chains and grafted with various function groups through copper catalyzed 1,3 cycloaddition. Because the polydispersity index of the synthesized PPLG is low (~1.2) and the grafting efficiency is both high and largely independent of a grafted group's functionality, we are able to create PPLG backbones with clustered RGD functionality by substituting the PPLG backbones with different ratios of each grafted group. Incorporation of mM concentrations of acrylate grafted PPLG into bulk PEG hydrogels was verified by grafting PPLG with, on average, 4 PEG2-acrylate and 0.25 PEG3-fluorescein per polypeptide. Plate reader quantification of fluorescein fluorescence in the swollen gel and supernatant suggests greater than 95% incorporation of functionalized PPLG into the hydrogel and confocal microscopy suggests homogeneous PPLG incorporation. Additional studies gelling either maleimide or acylate functionalized PPLG with only 4 arm PEG thiol further support the ability of grafted PPLG to structurally incorporate into PEG hydrogels.



Schematic 1a. Grafting PPLG with azide sidechains.
Fig. 1b. Percent Motile hTERT MSCs on PPLG doped PEG hydrogel with clustered RGD ligands

The ability of PPLG to present nano-clustered bio-functional cues was demonstrated through cell response to PEG hydrogels made cell adhesive by PPLG that present RGD ligands either in a clustered (average 6.5 RGD molecules per PPLG (N6)) and nearly random manner (average 1.3 RGD molecules per PPLG (N1)). As expected, based on published cell response to RGD tethered to glass,[4] cells did not adhere to hydrogels without RGD and were more motile on gels presenting clustered RDG than random RGD. This differential cell response confirms PPLG-based clustered presentation.
Conclusions: Grafted PPLG has been shown to readily incorporate into UV polymerized and Michael-type addition polyethylene glycol hydrogels. Increased cell motility on surfaces presenting PPLG-based nano-clustered RGD supports the potential to use PPLG to deliver defines nanoclusters of cell cues. Ongoing and future work seek to monitor cell response to a panel of adhesive peptides and tethered growth factors nano-clustered in 2D and 3D PEG based hydrogels. Such a screening platform would help better characterize signaling responses to nano-clustered cues and, potentially, direct the design of novel therapeutics able to more specifically modulate cell response.

References:

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