Bioactive Surface Gradients to Control Cell Adhesion

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Statement of Purpose: Incorporating discrete cell adhesion motifs onto substrates in a defined orientation with increasing concentration offers a robust strategy for measuring ligand dependent cell response. We have designed a versatile process to produce gradients of surface-bound biomolecules that elicit specific responses through well-characterized receptor-ligand interactions. This approach provides a tool to investigate the dependence of cell function on ligand density. For instance, the adhesive Arg-Gly-Asp (RGD) tripeptide sequence is found in a variety of ECM proteins and is recognized by a number of integrins [1]. The synthesis, characterization and preliminary biological assessment of bioactive films possessing gradients of RGD peptides will be described.

Methods: Our approach to the fabrication of bioactive surface gradients has been to develop a "universal substrate" to which various species can be attached (Figure 1). The first step is the generation of a surface energy gradient on a dimethylsilyl octyl self-assembled monolayer (SAM). This process has proven to be very reproducible and yields an increasing amount of terminal acid groups whose spatial concentrations were quantified previously [2]. The surface energy gradient was further derivatized with a difunctional linker to yield a substrate possessing an increasing amount of alkyne groups in one direction. The resulting propargyl gradient surface can be functionalized with any azo-derivatized species using "click chemistry" [3]. An azo-functionalized Gly-Arg-Gly-Asp-Ser (GRDGS) peptide was synthesized using solid phase peptide synthesis methods. RGD density gradients were blocked sequentially with 1 % BSA for 1 h and 5 % Pluronic F68 for 16 h prior to cell seeding. A10 smooth muscle cells were plated on gradient substrates for 4 h in 10 % fetal bovine serum, followed by fixation and fluorescent staining for analysis.

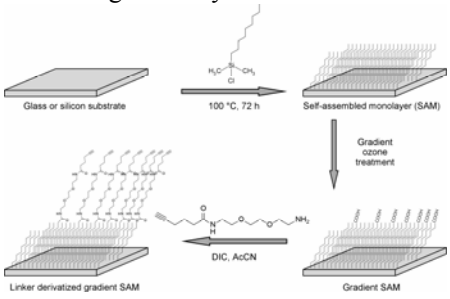

Figure 1. Fabrication of "universal substrate" with density gradient of alkyne species for ligand attachment.

Results / Discussion: The advancing water contact angle decreased monotonically from approximately 105˚ to 10˚ along the gradient SAM. Precise characterization of the spatial concentration and distribution of tethered species on the gradient requires multiple techniques and these efforts are ongoing. We quantified the number of adherent cells and cell spreading with increasing RGD peptide density on 40 mm gradients (Figure 2). In preliminary studies we observed that the number of adherent cells remained at background levels before increasing to a maximum cell density, while the projected cell area increased until reaching saturation. A likely explanation is that a threshold number or critical density of integrin linkages is required to support stable adhesion and initiate cell spreading. Images of cells stained for vinculin containing focal adhesions taken at intervals along the gradient also indicate possible surface dependent changes in cell shape and migratory behavior (Figure 3). A systematic study of focal adhesion number, size, anisotropy and distribution as a function of surface peptide density is ongoing, and detailed analyses of extracellular matrix production are also planned.

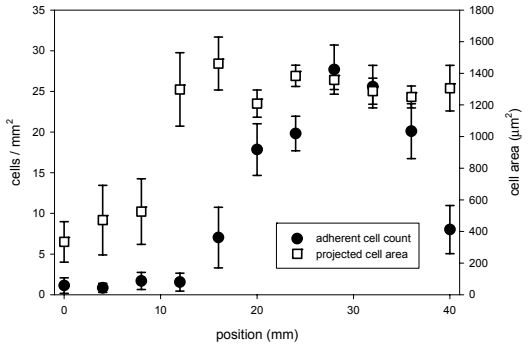

Figure 2. Cell adhesion and spreading on RGD density gradient. Error bars represent standard error of the mean and are the estimate of standard uncertainties.

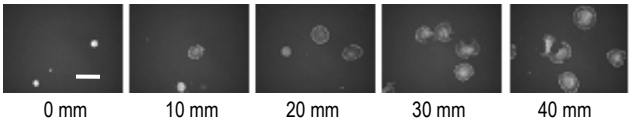

Figure 3. A10 cells immunostained for vinculin indicate changes in cell spreading, number, and polarization with RGD density. Bar 100 μm.

Conclusions: A truly "universal substrate" for bioactive species functionalization provides a versatile tool to probe biological hypotheses where ligand concentration and orientation are important. Peptide functionalized gradient substrates have shown the ability to control cell adhesion and therefore provide a tool for screening surface directed cell function. Furthermore, this work extends the NIST combinatorial approach for materials characterization to an investigation of cell-material interactions.

References: [1] Ruoslahti, E. Ann. Rev. Cell Dev. Biol. 1996; 12: 697-715. [2] Roberson, S. V.; Fahey, A. J.; Sehgal, A.; Karim, A. Appl. Surf. Sci. 2002; 200: 150-164. [3] Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Angew. Chem. Int. Ed. 2001; 40: 2004-2021.

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