# Versatile Gradient Substrates for 'Click' Biofunctionalization

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**Statement of Purpose:** Biomimetic surfaces are engineered to present ligands for specific receptors, thereby controlling cell-material interactions to elicit a desired response. In order to facilitate research on biomimetic and tissue engineered medical products, we have developed a novel and versatile method for fabricating continuously variable concentration gradients of surface conjugated biomolecules.

**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). First a surface energy gradient is generated on a dimethylsilyl octyl self-assembled monolayer (SAM). This reproducible and tunable process yields an increasing amount of terminal acid groups proportional to UV exposure time [1]. These functional groups were further derivatized with a difunctional linker to yield an increasing amount of alkyne groups. The resulting gradient surface can be functionalized with any azide-derivatized species using "click chemistry" cycloaddition[2]. This scheme is particularly amenable to peptide applications because azides are easily added during synthesis, and neither azides nor alkynes occur naturally in biological systems. Further advantages of this immobilization scheme include the high conversion rate and controlled orientation. An azide-Arg-Gly-Asp (RGD) peptide was synthesized using solid phase peptide synthesis methods and coupled to alkyne gradient substrates. RGD density gradients were blocked sequentially with 5 % Pluronic F68 for 16 h and 1 % BSA for 1 h prior to seeding A-10 smooth muscle cells (SMC) for 6 h in 2 % fetal bovine serum. Cells were fixed and fluorescently labeled for analysis.

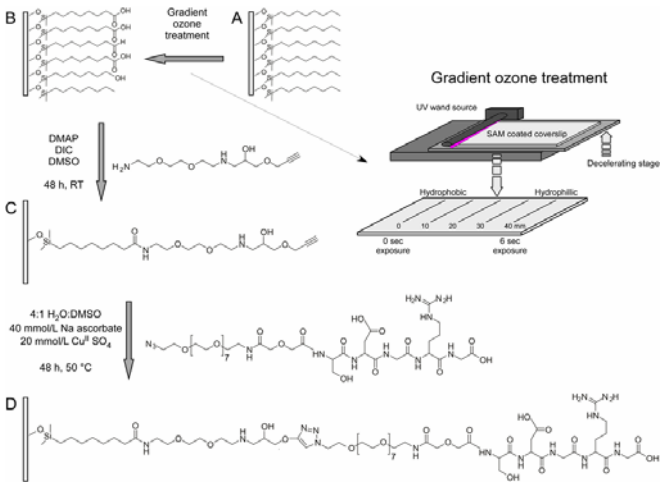


**Figure 1.** Fabrication of a versatile gradient substrate for biofunctionalization and subsequent RGD peptide immobilization by click chemistry. (A) SAMs are subjected to variable UV-ozone treatment to generate a (B) continuously variable carboxyl density gradient. A difunctional linker converts the acid species into an (C) alkyne gradient. (D) An RGD azide-peptide is covalently immobilized into the gradient by triazole cycloaddition.

**Results/Discussion:** We used x-ray photoelectron spectroscopy (XPS) to measure elemental surface concentrations along the gradient and calculated the immobilized peptide concentration (Figure 2). Automated microscopy was used to assess SMC adhesion on RGD gradients (Figure 3). The number of adherent cells increased (>4 fold) as a function of position along the gradient before reaching saturation (≈100 pmol/cm$^2$).

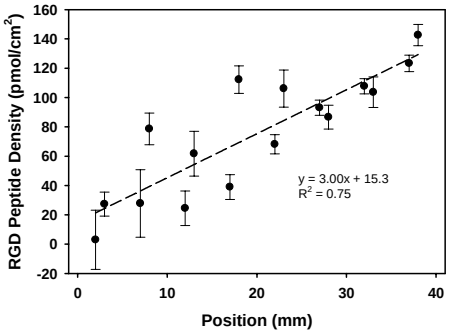


**Figure 2.** Surface conjugated RGD peptide density measured by XPS.

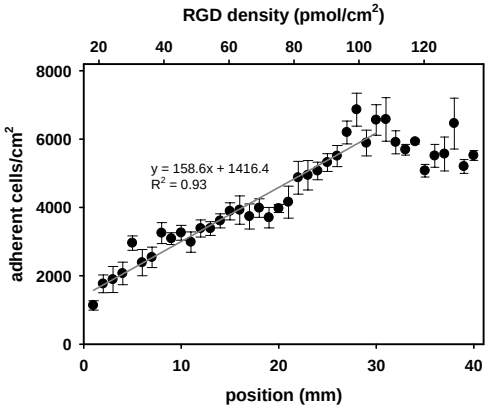


**Figure 3.** Cell adhesion to RGD conjugated gradients versus position. A second axis (top) was derived from the linear regression in Figure 2 and added to indicate adhesion as a function of approximate RGD density.

**Conclusions:** We show that versatile surface concentration gradients can be reproducibly fabricated and that the bioactive species of interest is easily attached via click chemistry. A density gradient of an adhesive RGD peptide was fabricated and we observed that cell attachment was enhanced as the density of immobilized RGD increased. This work demonstrates the ability to modulate a cell response with a bioactive peptide functionalized gradient substrate, and is an example of the broad utility of this technology for biomaterials research.

**References:** [1] Roberson, S. V.; Fahey, A. J.; Sehgal, A.; Karim, A. Appl. Surf. Sci. 2002; 200: 150-164. [2] Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Angew. Chem. Int. Ed. 2001; 40: 2004-2021.

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 Error bars represent standard error of the mean and are the estimate of standard uncertainties.