Scientists at NIST have developed numerous platforms for characterizing the physical properties of sample libraries with orthogonal gradients using combinatorial methodologies. We extend these methods to the design and characterization of surfaces possessing well-defined gradients of immobilized biomolecules designed to control cell adhesion and direct cell function. Our approach to the fabrication of bioactive surface gradients has been to develop and characterize a functional gradient substrate to which a variety of species can be attached. The first step was the generation of a UV-ozone induced surface energy gradient on a dimethylsilyl octyl self-assembled monolayer (SAM). This process has proven to be a very reproducible method for gradient fabrication and yields a linearly increasing amount of terminal acid groups. A propargyl-derivatized linker was then attached to the surface energy gradient to yield a surface possessing an increasing amount of alkyne groups in one direction. Once characterized, the propargyl gradient surface acts as a universal substrate to which any azo-derivatized species can be attached using “click chemistry”. Using this technique to incorporate discrete cell adhesion motifs onto substrates in a defined orientation with increasing density offers a robust platform for quantitatively measuring cell-material interactions.

A practical application of this work is to design bioactive ligand density gradients that provide specific signals to cells through well-characterized integrin-ligand interactions. Such well characterized substrates can be tools to investigate cell function dependence on ligand density and orientation. This approach will lead to novel methods for measuring cellular responses and reveal factors that improve tissue formation in wound healing and tissue engineering applications. As a prototype example we have fabricated a density gradient of GRGDS peptide within a nonadhesive background and measured cell adhesion and spreading as a function of ligand density. The Arg-Gly-Asp (RGD) tripeptide sequence is found in a variety of extracellular matrix proteins and is recognized by a number of integrins. We will describe the syntheses, characterization and preliminary biological assessment of these versatile bioactive substrates.

**Figure 1.** a: Functional gradient surfaces with defined spatial orientation and complete chemical specificity fabricated by UV-ozone radiation of chlorosilane SAMs, and subsequent coupling of an alkyne-terminated linker. b: “Universal substrates” derivatized with an azo-terminated bioactive species (e.g. GRGDS peptide) via the highly selective 1,3 Huisgen dipolar cycloaddition reaction. This reaction proceeds with a high degree of dependability and specificity using bio-compatible reactants.

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