BIOACTIVE SURFACE GRADIENTS TO CONTROL CELL ADHESION

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Introduction

NIST has developed numerous platforms for characterizing the physical properties of sample libraries with orthogonal gradients using combinatorial methodologies. Self-assembled monolayers (SAMs) possessing surface energy gradients have proven to be a versatile substrate useful for examining numerous physical and biological phenomena. Further extension of these methods includes the characterization of surfaces possessing well-defined gradients of bio-active peptides designed to control cell adhesion.

Cell adhesion to extracellular matrix (ECM) components, such as fibronectin (FN) and laminin, involves a collection of complicated dynamic processes that is primarily mediated by the integrin family of heterodimeric receptors. Integrin-mediated adhesion is a highly regulated process involving mechanical coupling to extracellular ligands, and subsequent clustering of bound receptors and rapid association with the actin cytoskeleton to form focal adhesions. Incorporating discrete cell adhesion motifs onto substrates in a defined orientation with variable spacing and increasing concentration offers a robust strategy for measuring cell-material interactions and encouraging biospecific cell adhesion. For instance, the Arg-Gly-Asp (RGD) tripeptide sequence is found in a variety of ECM proteins and is recognized by a number of integrins. The goal of this work is to design bioactive surfaces that provide specific signals to cells through well-characterized integrin-ligand interactions. This approach may lead to methods for measuring cellular responses and improved tissue formation in tissue engineering applications. The syntheses, characterization and preliminary biological assessment of bioactive films possessing gradients in RGD peptides will be described.

Results and Discussion

Our approach to the fabrication of bioactive surface gradients has been to develop and characterize a “universal substrate” to which various species can be attached. The first step is the generation of a surface energy gradient on a dimethylsilyl octyl SAM. This process has proven to be a very reproducible method for gradient fabrication and yields an increasing amount of terminal acid groups whose spatial concentrations were quantified reproducibly using x-ray photoelectron spectroscopy (XPS), into an alkoxyamine using synthetic methods similar to those published previously by Benoit et al. The GRGDS peptide was synthesized by solid phase synthesis using FMOC chemistry and further derivatized with a commercially available azo linker. Following cleavage, reverse-phase HPLC purification and characterization by LC-MS, the peptide-derivatized linker was attached to the surface using conditions found in the literature. Further optimization of these conditions and characterization of peptide concentration are ongoing.

The spatial concentration and distribution of initiating sites on the gradient remains difficult to characterize. Of critical importance is concentration information at the low end of the substrate. Many peptides, carbohydrates or other species of interest lack the spectroscopic handles necessary for precise and accurate measurements. Quantitative characterization of the bioactive species has encouraged the design of labeling strategies that incorporate $^{19}$F stable isotope labels, which are readily identified using x-ray photoelectron spectroscopy (XPS), into an alkoxyamine initiating species. $^{19}$F atoms are incorporated easily into nitroxides and alkoxyamine using synthetic methods similar to those published previously by Benoit et al.

A systematic study looking at focal adhesion complex number, size, anisotropy and distribution as a function of surface peptide density is ongoing. More detailed analyses of extracellular matrix production are also planned.

Conclusions

The fabrication and characterization of a “universal substrate” to which any number of bioactive species can be attached will afford the ability to probe biological hypotheses where ligand concentration and orientation are important.

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References


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