Peptide microarrays for the combinatorial discovery of bioactive surfaces that guide cellular processes

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Statement of Purpose: Cellular behavior is controlled by a complex environment containing many different molecules including extracellular matrix (ECM) proteins, growth factors, and proteoglycans. The ability to control the interaction between these various components will be invaluable in developing new materials and assays for biomedical applications. We report a method using copper-catalyzed azide-alkyne cycloaddition to immobilize combinations of peptides in a self-assembled monolayer (SAM)—in a single step—to study the combinatorial effects of these different components. Peptides were selected from motifs present in adhesion proteins, cytokines, growth factors and proteoglycan binding proteins and arrayed together in multiple combinations.

Methods: Surfaces were prepared by evaporating 5 nm of Ti followed by 20 nm of Au using E-beam deposition. Stock solutions of peptide ligand (1mM in H2O), TBTA and azide-terminated alkanethiolate solution. Copper solution (10mM Cu, 10mM sodium ascorbate in DMSO) was prepared fresh prior to click modification. Reactions containing peptide, click solution, and azide-terminated alkanethiolate solution were robotically prepared in a 384-well plate and printed in subarray format on the gold-coated surfaces using a Gene Machines OmniGrid Microarrayer. Human Adipose derived stem cells (ADSCs), mouse embryonic fibroblasts (MEFs) and mouse melanoma cells (B16F0 and B16F10) were seeded onto our microarray and fixed with 4% PFA, permeabilized with 0.1% Triton X-100 and blocked with 1% BSA. Primary antibody labeling occurred overnight at 4ºC and secondary labelling for 20 min at 37ºC. Immunofluorescence imaging was performed on a high-content imaging system, the IN Cell Analyzer 2000 (GE). A minimum of 16 fields of view were taken for each sample condition.

Results: We synthesized ECM adhesion peptides (YIGSR, GRGDS), a BMP-7 and BMP-2 derived peptide (KPSSAPTQLN, DWIVA), heparin binding peptides (KRSR, FHRIKKA), and arrayed them, alone and in combination, onto gold coated coverslips (**Figure 1**). Self-assembled monolayers were characterized by X-ray photoelectron spectroscopy (XPS) and contact angle goniometry, and arrayed peptide combinations were seen to differentially bind to ADSCs, MEFs, B16F0 and B16F10 cells. We further investigated the long-term culture of ADSCs on SAMs containing adhesion peptides and BMP-7 peptide in both standard culture and osteogenic differentiation media. We demonstrate that the BMP-7 peptide can upregulate the expression of osteogenic markers in adherent ADSCs when used alone or in combination with adhesion peptides, and without the use of osteogenic supplements.

Conclusions: Our results demonstrate the utility of a peptide microarray of self-assembled monolayers on gold for the study of specific ligand interactions with multiple cell types. By using azide-alkyne "click" chemistry in a single spotting step, we can easily tune ligand combinations to screen for combinatorial effects. Cells seeded onto the spotted regions remain localized for over a week, allowing for use of this method for long-term culture experiments. The adhesion and proliferation characteristics of different cell types on the combinations of peptides suggests that cells adhere via combinations of different surface receptor. We more thoroughly investigated a combination of an adhesion peptide and BMP-7 derived peptide combination and found that the presence of the BMP-7 peptide promoted osteogenic differentiation by upregulating Runx2 and OPN expression. We anticipate this technique will be useful in the future for screening many different growth factor and adhesion peptides in combination for a variety of cell types.

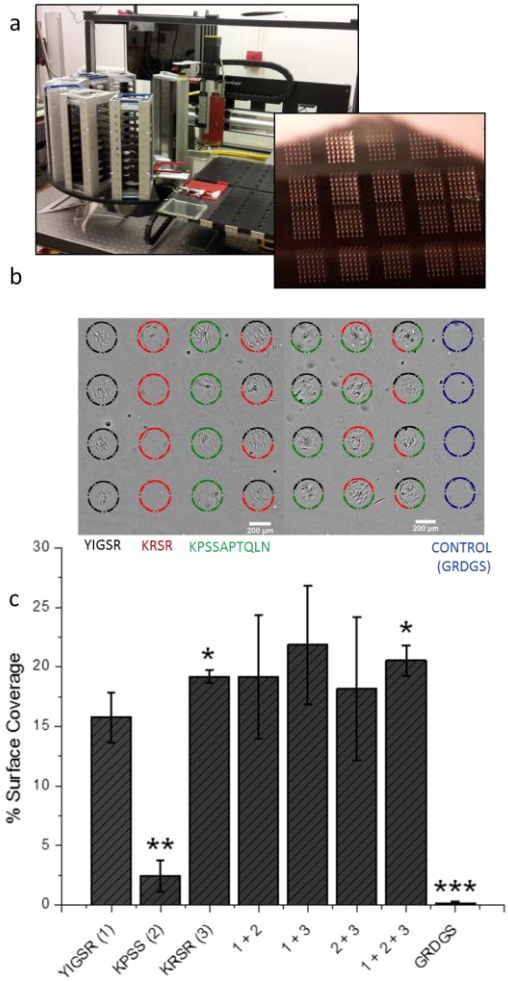

Figure 1. a) Photograph of the microarrayer and spotted slide; b) Adipose derived stem cells (ADSCs) adherent to the array of adhesion peptide (YIGSR – black), proteoglycan binding peptide (KRSR – red) and morphogen derived (KPSSAPTQLN – green) and combinations. A scrambled adhesion peptide sequence was used as control (GRDGS).