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Engineering Interfaces for Controlled Cellular Activities

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INTRODUCTION: Cell adhesion to adsorbed extracellular matrix (ECM) proteins and adhesive sequences engineered on synthetic surfaces plays critical roles in biomaterial, tissue engineering, and biotechnological applications [1]. Cell adhesion to these adhesive motifs is primarily mediated by integrin receptors [2]. In addition to anchoring cells, integrin binding activates signaling pathways regulating cell survival, proliferation, and differentiation. While tethering short adhesive peptides derived from ECM ligands (e.g., RGD for fibronectin) promotes cell adhesion and function in several cell systems, these biomimetic strategies are limited by reduced biological activity compared to the native ligand, lack of specificity among integrins, and inability to bind non-RGD integrins. We have engineered biointerfaces that mimic the secondary and tertiary protein structure of fibronectin and type I collagen. These surfaces convey integrin binding specificity, focal adhesion assembly and signaling as well as bone and muscle cell adhesion, proliferation, and differentiation.

METHODS: Self-assembled monolayers of ω-functionalized alkanethiols on gold and microcontact printing were used to engineer surfaces with well-defined chemical properties. Bioadhesive ligands that mimic the primary and secondary protein structure for fibronectin and type I collagen were tethered onto protein-adsorption-resistant supports (19:1 tri(ethylene glycol)-terminated/COOH-hexa(ethylene glycol)-terminated alkanethiols) to target specific integrin adhesion receptors. Cell adhesion was analyzed in terms of integrin binding, focal adhesion assembly and signaling, and adhesion strength using biochemical and functional assays. Cell differentiation (gene and protein expression, mineralization) was assessed via real-time RT-PCR, immunostaining, and histochemical staining.

RESULTS: A recombinant fragment of fibronectin spanning the 7th-10th type III repeats of fibronectin and encompassing the PHSRN and RGD motifs was tethered to non-fouling supports to specifically bind alpha5beta1 integrin and trigger focal adhesion assembly and signaling. Binding of this receptor is critical to osteoblast proliferation, differentiation, and matrix mineralization. To target alpha2beta1 integrin, a triple-helical collagen-mimetic peptide incorporating the GFOGER motif was tethered to model non-adhesive supports. These biomimetic surfaces supported alpha2beta1 integrin-mediated adhesion and focal adhesion assembly and directed osteoblast specific-gene expression and matrix mineralization to higher levels than conventional culture supports. Second-generation interfaces have been developed to display controlled fibronectin-/collagen-mimetic ligand mixed densities to independently target alpha5beta1 and alpha2beta1 integrins. These mixed ligand surfaces synergistically modulate cell adhesive activities. Finally, these approaches have been combined with micropatterning techniques to generate biointerfaces that control cell-substrate adhesive area and integrin binding.

DISCUSSION & CONCLUSIONS: By focusing on bioadhesive ligands that recapitulate the secondary and tertiary structure of ECM proteins, we have engineered surfaces that direct integrin binding and signalling to elicit specific cellular responses. These biomolecular engineering strategies provide a basis for the rational design of robust biointerfaces that tailor adhesive interactions and elicit specific cellular responses for the development of bioactive implant surfaces, scaffolds for enhanced tissue reconstruction, and growth supports for enhanced cellular activities.

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