Bio-functionalization of Inorganic Surfaces through Inorganic Binding Peptides

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Statement of Purpose: One of the main goals of the bio-materials field is to engineer surfaces which would interact appropriately with the complex environment of the body. A very promising approach to this problem has been the immobilization of bio-active or bio-inert species on a material chosen for its other desirable properties, such as strength, conductivity, or chemical stability. A number of immobilization techniques have been studied, but many remain limited in their applications due to inherent inflexibility (Love JC. Chem. Rev., 2005;105:1103-1169). Genetically engineered peptides for inorganics (GEPIs) are a class of combinatorially selected and computationally enhanced short amino-acid chains which have been designed to bind strongly to a particular inorganic surface (Tamerler C. *Phil. Trans. R. Soc. A* 2009 **367**, 1705-1726). Due to their immense informational content GEPIs offer material and even phase selectivity not usually available to conventional chemistry, as well as unprecedented flexibility in materials, binding strengths, and other properties. In addition, their natural origins allow these linkers to be easily combined with other functional proteins and peptides, for example, the integrin binding sequence RGD. Perhaps most importantly, GEPIs can bind their respective materials under mild aqueous conditions, at room temperature and neutral pH, making them particularly advantageous to use in medical applications, and those involving sensitive or unstable materials. Here we present several experiments that serve as proof of principle demonstrations for the application of GEPIs in bio-functionalization of surfaces.

Methods: In the present study we chose, gold and platinum as model materials since noble metals have been employed in many biomaterial applications because of their excellent corrosion resistance, physical properties, and low toxicity. We also selected SiO_2 glass because glass- and ceramic-based materials have found utility mostly in orthopedic implants, where integration with the surrounding tissue is important. By exploiting the primary amine groups present on the gold- and platinum-binding peptides (3rGPB1 and PtBP1), we covalently bonded the anti-fouling polymer aldehyde-terminated poly(ethylene glycol) (PEG-CHO) to the peptides assembled on the substrates. The surfaces were characterized by water contact angle goniometry, atomic force microscopy, x-ray photoelectron spectroscopy, and though cell adhesion assays. The integrin binding sequence RGD was synthesized directly with the silica-binding peptide, which was then used to immobilize the construct on the glass surface in a single step. The effects of this surface were assessed through cell adhesion assays.

Results: Bio-inert surfaces we produced via the method above appear to prevent cell adhesion as well as oligo-ethylene glycol – thiol monolayers. See figure 1.

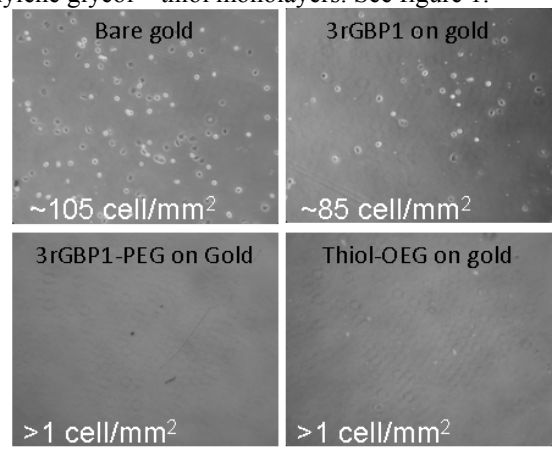

Figure 1. Reduced adhesion of fibroblast cells to GEPI-PEG-functionalized gold (Optical x200 magnification)

Similar results were observed with osteoblast cells, as well as with both cell lines on platinum using PtBP1. Peptide-immobilized RGD sequence on glass has induced a 3.5-fold increase in the number and a 1.6-fold increase in the spreading of osteoblast cells and comparable increases in fibroblast cells. See figure 2.

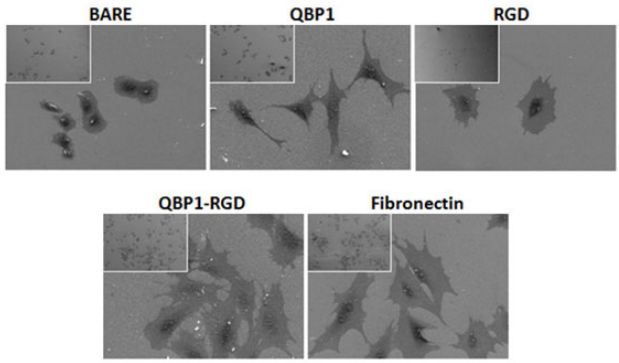

Figure 2: Improved adhesion of osteoblast cells to GEPI-RGD-functionalized silver (Scanning Electron Microscopy)

Conclusions: The data demonstrates the viability of using inorganic binding peptides as linkers to immobilize bio-functional molecules on inorganic materials. In future research we would like to take advantage of GEPIs' unique features, such as material selectivity and self-assembly to simultaneously impart different bio-functionality to regions on a multi-material substrate.

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