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Biofunctionalization of materials for implants using engineered peptides

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ABSTRACT

Uncontrolled interactions between synthetic materials and human tissues are a major concern for implants and tissue engineering. The most successful approaches to circumvent this issue involve the modification of the implant or scaffold surfaces with various functional molecules, such as anti-fouling polymers or cell growth factors. To date, such techniques have relied on surface immobilization methods that are often applicable only to a limited range of materials and require the presence of specific functional groups, synthetic pathways or biologically hostile environments. In this study we have used peptide motifs that have been selected to bind to gold, platinum, glass and titanium to modify surfaces with poly(ethylene glycol) anti-fouling polymer and the integrin-binding RGD sequence. The peptides have several advantages over conventional molecular immobilization techniques; they require no biologically hostile environments to bind, are specific to their substrates and could be adapted to carry various active entities. We successfully imparted cell-resistant properties to gold and platinum surfaces using gold- and platinum-binding peptides, respectively, in conjunction with PEG. We also induced a several-fold increase in the number and spreading of fibroblast cells on glass and titanium surfaces using quartz and titanium-binding peptides in conjunction with the integrin ligand RGD. The results presented here indicate that control over the extent of cell–material interactions can be achieved by relatively simple and biocompatible surface modification procedures using inorganic binding peptides as linker molecules.
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1. Introduction

Developments in the area of biomaterials have given rise to many compounds, materials systems and devices with a variety of attributes to be used for implantable medical purposes [1–3]. Many of the available materials have already been optimized to have satisfactory physical and mechanical properties. Insufficient or improper interactions between the synthetic materials and living systems, however, remain a major concern, and often result in failure of the implant [4–9]. The most common strategy to overcome this problem, and enhance the biocompatibility of implants, has been to modify their surfaces with functional molecules. Such molecules are usually selected to perform one of two functions: to generate cytocompatible surfaces by carrying specific cell signals or non-fouling surfaces by preventing adhesion of undesired protein and cells. A number of molecular immobilization systems have been successfully employed to modify the implant surfaces. For example, Rezania et al. have utilized N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane and 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) to covalently immobilize cyclic arginine–glycine–aspartic acid (RGD) peptides on oxide surfaces [10]. The exposure of these modified surfaces to cell cultures has resulted in a marked increase in cell adhesion. Likewise, Harder et al. have used self-assembled alkanethiolate monolayers to link oligo(ethylene glycol) (OEG) chains to gold and silver and produce protein-resistant surfaces [11]. Messersmith et al. have utilized 3,4-dihydroxy-L-phenylalanine (DOPA) to non-covalently attach poly(ethylene glycol) (PEG) to gold and titanium. The DOPA–PEG modified substrates exhibited a marked decrease in cell adhesion [12]. Other strategies for surface modification with functional molecules include non-specific adsorption [13,14], photochemical grafting [15], functional self-assembled monolayers (SAM) [16–18], covalent attachment [19,20] and plasma deposition [21], among others.

The conventional immobilization methods listed are often applicable only to a limited range of materials and require the presence of specific functional groups, synthetic pathways or biologically hostile environments [22]. For this reason, the development of biocompatible and versatile linkers for surface engineering of implants has been a major objective in biomaterials science [23–25]. Short peptide motifs that bind to inorganic surfaces, known as genetically engineered peptides for inorganics (GEPI), offer an attractive alternative to achieve this objective and have already been demonstrated to be useful in various molecular immobilization applications [26–29]. The binding affinity of GEPIs is derived from a sum of weak

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electrostatic and van der Waals interactions between the peptide and the solid surfaces, requiring no complex or aggressive chemistries. In addition, the relatively simple cell surface or phage display selection protocols and recent developments in bioinformatics-based techniques have made it significantly easier to identify peptides that can specifically bind to a material of choice [30]. Peptide motifs, 7–20 amino acids long, have been identified that bind to a variety of materials, including polymers [31], metals [32] and oxides [30,33], by exposing a random pool of amino acid sequences, displayed on the surface of a host microorganism, to the target surface and selecting the organisms displaying specific binding sequences [34,35].

In the present study we have chosen glass, titanium, gold and platinum as model materials to showcase the versatility of the potential use of peptide-based surface modification platforms. Noble metals have been the material of choice in many biomaterial applications because of their excellent corrosion resistance, mechanical properties and relatively good biocompatibility. They have been widely used in dental implants, stents and tube plating, wiring for electronic implants and pacemaker electrodes [36,37]. Glass- and titanium-based materials have found utility mostly in orthopedic implants. They have been used as bone grafts and coatings for metal implants to improve osteointegration and as bone replacements due to their mechanical properties and excellent stability [38]. Depending on the material, implantation site and intended function, certain devices may call for increased tissue integration rather than a bio-inert behavior, or vice versa (Fig. 1). For example, applications involving noble metals, such as stents and pacemaker electrodes, often require minimum interaction with the biological environment. In contrast, orthopedic applications of oxides, glasses and glass-based composites favor increased cyto-compatibility to promote hard tissue integration [3,39].

With this in mind, we used an engineered gold-binding peptide (3GBP1) [40] and a platinum-binding peptide (PtBP1) [41], developed in our laboratories, to generate bio-inert gold and platinum surfaces, respectively. By exploiting the primary amine groups present on the peptides, we covalently bound aldehyde-terminated poly(ethylene glycol) (PEG-CHO) to the peptides on the surface through targeted assembly (Fig. 2a). Such PEG chains, if properly functionalized, can be further modified with specific targeting molecules to render the surface inert to all but particular interactions. Water contact angle and cell adhesion assays showed that the PEG density and functionality achieved were comparable with surfaces prepared via conventional immobilization methods, such as covalent thiol binding [11,16]. Similarly, we used a quartz-binding peptide (QBP1) and a titanium-binding peptide (TiBP1) to generate glass and titanium surfaces with enhanced cyto-compatibility. Although the regular glass used in the study was not a material suitable for implant applications, it was still useful as a model surface since many of the commercially available glass implants are based on silicate glasses [38]. We synthesized bifunctional QBP1–RGD and TiBP1–RGD peptides via solid phase peptide synthesis and immobilized these peptide conjugates on the surface through directed assembly in a single step (Fig. 2b). Cell adhesion and spreading assays have shown that QBP1 and the TiBP1 facilitate the immobilization of RGD on both surfaces while preserving its functionality as a recognition site for cells.

The results presented here indicate that control over the extent of cell–material interactions can be achieved by relatively simple and biocompatible surface modification procedures using GEPIs as linker molecules.

2. Materials and methods

2.1. Peptide synthesis

The peptides were produced by solid-state synthesis using a CSBio 336s automated peptide synthesizer (CSBio, USA) on Wang

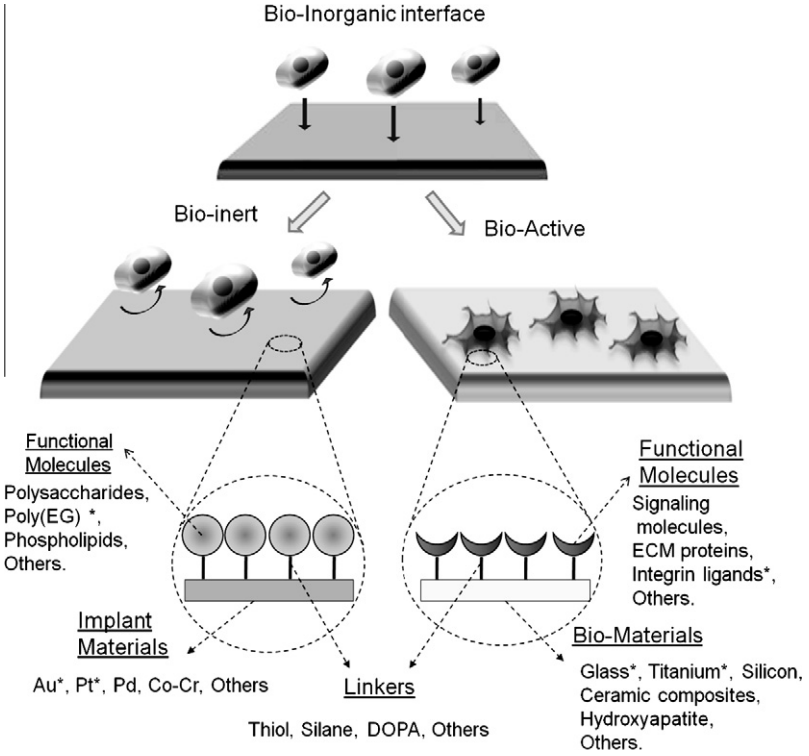

Fig. 1. Schematic representation of common approaches for modification of biomaterial surfaces with functional molecules. The functional molecules and materials used in this study are marked with an asterisk.

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