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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

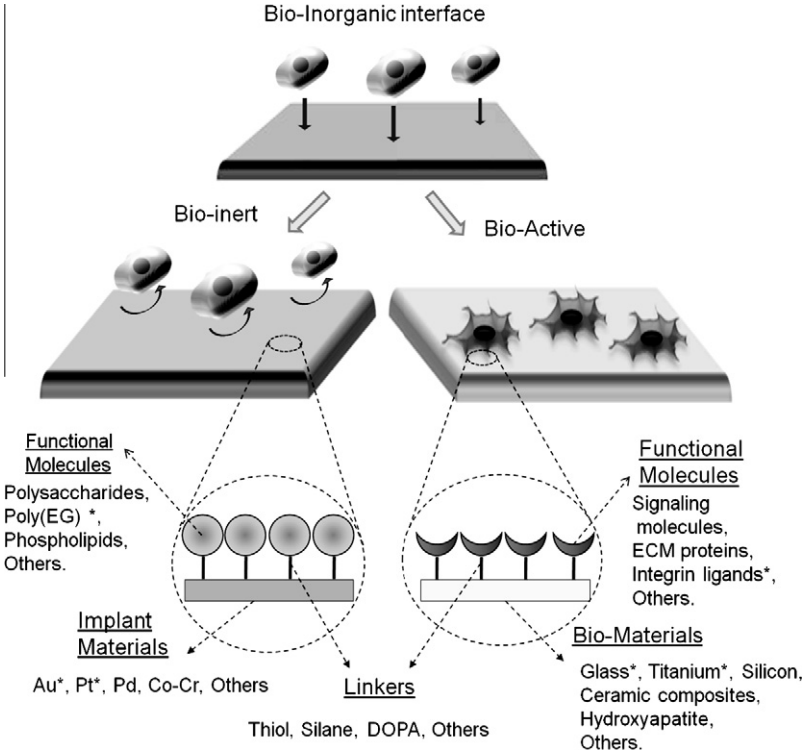

Fig. 1. Schematic representation of common approaches for modification of biomaterial surfaces with functional molecules. The functional molecules and materials used in this study are marked with an asterisk.

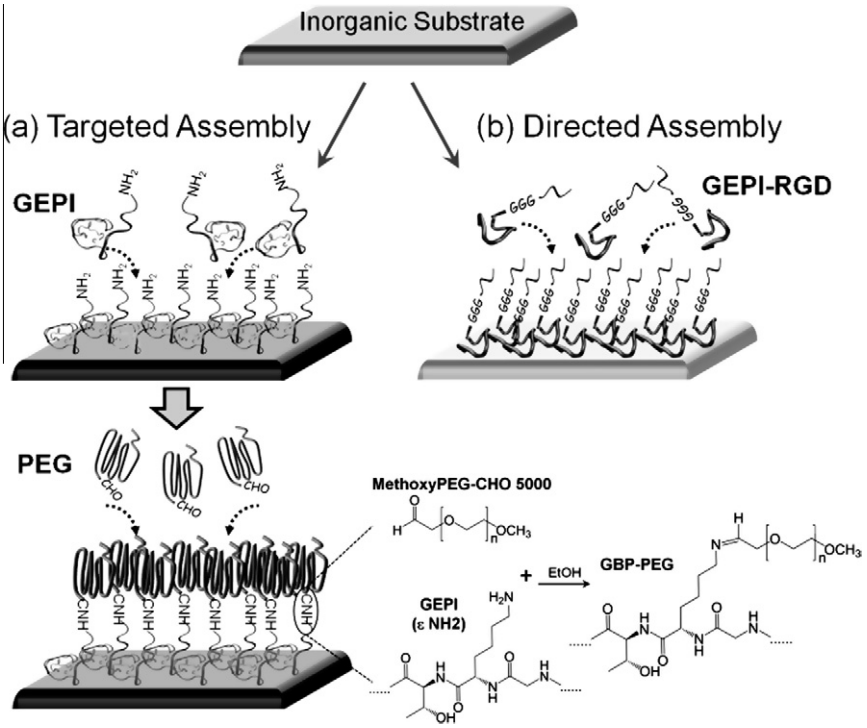

Fig. 2. Schematic representation of surface modification using GEPIs via targeted and directed assembly processes. Targeted assembly (a) is a two-step procedure involving functionalization of the surface with a GEPI and subsequently reacting the PEG with the surface bound peptide. The close-up sketch depicts the reaction between the ε amino group of the GEPI and the aldehyde group of PEG. Directed assembly (b) is a single-step procedure, where the surface is functionalized directly with the bifunctional GEPI–RGD peptide.

Table 1
Amino acid sequences and the physico-chemical properties of the peptides used in the study.

Peptide	Amino acid sequence	pI	Gravy	M.W.	Net charge
GBP1	**M**H**G**KTQ**A**TSGT**I**Q	8.25	−0.743	1446.6	+1
3GBP1	**M**H**G**KTQ**A**TSGT**I**QS × 3	10.30	−0.743	4304.7	+3
PtBP1	CSQS**V**TSTKSC (cyclic)	8.47	−0.867	923.9	+1
AuBP1	**WAGA**KR**L**V**L**RRE	11.71	−0.567	1454.7	+3
AuBP2	**WAL**RRS**I**RRQSY	12.00	−1.267	1591.8	+4
3AuBP1	**WAGA**KR**L**V**L**RRE × 3	12.10	−0.567	4328.1	+9
3AuBP2	**WAL**RRS**I**RRQSY × 3	12.30	−1.267	4739.4	+12
QBP1	**PPPWLP**Y**MPPW**S	5.95	−0.650	1467.7	0
QBP1-RGD	**PPPWLPYMPPWSGGGRGDS**	6.26	−0.958	2054.3	0
TiBP1	R**P**RE**N**R**G**RER**GL**	11.82	−2.633	1495.6	+3
TiBP1-RGD	R**P**RENR**G**RER**GLGGG**R**G**DS	11.70	−2.211	2082.2	+3
RGD	**GRG**DS	5.84	−1.920	490.4	0

The non-polar residues are displayed in bold. pI indicates the theoretical isoelectric points and GRAVY indicates the calculated grand average hydropathicities of the peptides.

resin via Fmoc chemistry and HBTU activation. The crude peptides were purified by reverse phase high performance liquid chromatography to >98% purity (Gemini 10 μm C18 110A column). The purified peptides were verified by mass spectroscopy (MS) using a MALDI-TOF mass spectrometer (Bruker Daltonics Inc., USA) The amino acid sequences and the physico-chemical properties of the peptides used in the study are shown in Table 1.

2.2. PEG functionalization

Aldehyde-terminated methoxypolyethylene glycol 5000 (PEG-CHO) [42] was prepared through the condensation reaction of methoxypolyethylene glycol 5000 and 4-carboxylbenzaldehyde. The reaction was carried out in a 1:1 mixture of N,N-dimethylformamide (DMF) and methylene chloride, in the presence of 4-(dimethylamino)pyridium 4-toluenesulfonate (DPTS) and 1,3-dicyclohexylcarbodiimide (DCC). The reaction product was purified using a silica gel column with an ethyl acetate to methanol elution gradient.

For selective on-resin functionalization of the AuBP1, PEG-CHO was reacted with the peptide still on the resin and with the ε-NH_2 groups protected by a tert-butoxycarbonyl (Boc) group. In this way, conjugation occurred only through the α-NH_2 at the N-terminus. The conjugation reaction was carried out for 4 h in ethanol under a nitrogen atmosphere while stirring. The resin was then filter washed with 10 ml of ethanol. The C=N double bond at the peptide–PEG junction was reduced with 2.5 mM sodium borohydride in sodium acetate buffer (pH 5.5) at room temperature under nitrogen overnight. The Boc group on the ε NH_2 was later removed from the peptide during its cleavage from the resin.

2.3. Substrate preparation

Standard 22 × 22 mm microscope coverslips (Fischer, USA) were used as the glass surface. The glass slips were cleaned by ultrasonication in 1:1 isopropanol:acetone mixture and were kept in ethanol prior to use. Gold, platinum and titanium surfaces were prepared by sputter coating the coverslips using a Gatan Precision Etching Coating System (Gatan Inc., USA). Both sides of the substrates were coated with 2 nm of chromium followed by 23 nm of gold or platinum, as measured using the built-in quartz crystal microbalance. Titanium substrates were prepared by directly coating a 25 nm titanium layer on glass. The substrates were used immediately after sputtering.

2.4. Surface modification

The GEPIs were attached to the surfaces by submerging the substrates in their respective peptide solutions. The gold substrates

were submerged in 2 ml of 20 μM solutions of the original or triple tandem repeats of GBP1, AuBP1 and AuBP2 for 1 h at room temperature. The platinum substrates were submerged in 2 ml of a 150 μM solution of PtBP1 for 1 h at room temperature. The glass and titanium substrates were submerged in 2 ml of 20 μM QBP1–RGD and 20 μM TiBP1–RGD solutions, respectively, for 1 h at room temperature. Similarly, substrates coated with 20 μM QBP1, 20 μM TiBP1 and 20 μM fibronectin were prepared as controls. All surfaces were washed three times by dilution with water and dried with nitrogen after functionalization.

The gold and platinum substrates were then further modified with PEG by submerging in 2 ml of 100 μM PEG-CHO solution in ethanol overnight. The samples were then washed by dilution using ethanol and deionized water, followed by rinsing with deionized water and drying with nitrogen. The thiol control surfaces were prepared using a thiol oligo(ethylene glycol) (thiol-OEG) conjugate ($HSC_{11}(EG)_3OH$) (Sigma–Aldrich, USA). Gold substrates were submerged in 2 ml of a 1 mM solution of thiol-OEG in ethanol overnight and washed by dilution with ethanol and deionized water, followed by rinsing with deionized water and drying with nitrogen.

The surfaces were characterized by atomic force microscopy (AFM) to check the uniformity of the coverage and polymer agglomeration using a multimode Nanoscope IIIa in tapping mode (Veeco, USA). The presence of the peptide and the polymer on the surface was verified via X-ray photo-electron spectroscopy (XPS), using S-Probe (Surface Science Instruments, USA), at the Surface Analysis Recharge Center (University of Washington, USA).

2.5. Contact angle measurements

Advancing water contact angles were measured on each of the gold and platinum samples to verify the assembly of PEG on the peptides and the consistency of assembly between different experiments. The modified surfaces were allowed to equilibrate in air for 30 min after preparation prior to measurements.

All measurements were made with a Rame-Hart Optical Goniometer 100-00 (Rame-Hart Instrument Co., USA) using >16 MΩ deionized water.

2.6. Cell adhesion and spreading analysis

NIH 3T3 mouse embryonic fibroblasts were used in the cell adhesion assays. The cells were maintained in Dulbecco's modified Eagle's medium (D-MEM) with 10% fetal bovine serum (FBS) and 2 mM glutamine, 100 U/ml penicillin and 100 mg ml^{-1} streptomycin (Invitrogen, USA). Experiments with 3GBP1–PEG, QBP–RGD and TiBP–RGD were carried out in both the absence of serum proteins and the presence of 1% FBS.

The gold and the platinum surfaces were inoculated with 3×10^5 cells and the glass and the titanium surfaces with 5×10^3 cells in 500 μl of medium for 2 h at 37 °C and 5% CO_2. The gold surfaces were also kept for 24 h after cell inoculation to further test the stability of the PEG layer. The samples were then rinsed three times with serum-free medium and the cells were either prepared for phalloidin staining or scanning electron microscopy (SEM), as described in the following sections. The number of cells on the surfaces was determined by counting the cells within four random areas using SEM and, in particular, fluorescence microscopy images using ImageJ image processing and analysis software (NIH, USA). The extent of cell spreading was quantified by measuring the cell contact areas from the detailed SEM images using ImageJ (NIH, USA).

2.7. Phalloidin staining

Following the 2 h incubation, the cells were fixed in 500 μl of 2% glutaraldehyde (Ted Pella, USA) solution in phosphate-buffered saline (PBS) for 10 min at room temperature and dehydrated with a series of ethanol solutions of increasing concentration (10%, 30%, 60%, 90% and 100% ethanol, 10 min in each). The slides were then rinsed twice with PBS and permeabilized in 500 μl of 0.1% Triton X-100 in PBS for 5 min and rinsed twice with PBS. The permabilized samples were blocked with 1% bovine serum albumin (Sigma–Aldrich, USA) in PBS for 30 min at room temperature to minimize non-specific staining. Previously prepared methanolic stock solution of Alexa Fluor488–Phalloidin (Invitrogen Co., USA) was diluted 40 times in PBS to obtain an approximately 165 nM working solution. 200 μl of the final working solution was added on top of each sample and kept at room temperature protected from light for 20 min. The samples where then rinsed twice with PBS and observed using a TE 300L microscope (Nikon, Japan).

2.8. SEM sample preparation

The cells were fixed in 500 μl of 2% glutaraldehyde (Ted Pella, USA) solution in PBS for 10 min at room temperature and dehydrated with a series of increasing concentration ethanol solutions (10%, 30%, 60%, 90% and 100% ethanol, 10 min in each). After ethanol dehydration the samples were coated with platinum using an SPI sputter coater (SPI Supplies, USA) and observed using a JSM 7000F scanning electron microscope (JEOL, Japan).

3. Results and discussion

Here we demonstrate the versatility of inorganic binding peptides (GEPIs) as multi-purpose molecular tool for the surface modification of implant materials. Due to the diverse applications of implants, we have utilized GEPIs for bio-inert and bio-active surface modification. Targeted assembly is a two-steps procedure where the material surfaces are, first, functionalized with GEPIs and, then, PEG conjugation is carried out on the peptides bound to the surface for bio-inert surface preparation (Fig. 2a). Directed assembly, on the other hand is a single-step procedure, where the bifunctional peptides are directly applied to the surface (Fig. 2b). The sequence information and the physico-chemical properties of all the inorganic binding peptides and their bifunctional combinations used in the current study are provided in Table 1.

3.1. Bio-inert surface modification

The surface adsorption and uniformity of PEG on gold were monitored by AFM and contact angle measurements over a range of assembly conditions. The AFM characterization showed a uniform distribution without agglomeration of the polymer on the surface under the experimental conditions described above (Fig. 3a and b). The contact angle measurements confirmed that the gold and the platinum surfaces were successfully functionalized with PEG through GEPIs. No significant changes in contact angle were observed on the surfaces incubated with PEG-CHO alone with respect to the unmodified negative control: about 60° for gold and 48° for platinum (Fig. 3c). The adsorption of the GEPIs alone on the surfaces resulted in about a 25% decrease in the contact angles. This decrease is consistent with the abundance of hydrophilic and polar residues in the peptides (Table 1). The conjugation of PEG with immobilized 3GBP1 and PtBP1 further decreased the contact angles to about 32° for both surfaces. This value is consistent with those reported in the literature and reproduced in this study for OEG immobilized on gold through thiols (Fig. 3c) [11,16]. Additionally, high resolution XPS analysis of the carbon photo-electron emission spectrum of GEPI–PEG modified substrates showed the presence of both amide –CO–NH– groups indicative of the peptide and –C–O–C– groups indicative of PEG (Fig. 3d).

The gold and platinum surfaces modified with GEPI–PEG conjugates exhibited a significant decrease in the non-specific adhesion of NIH 3T3 cells as compared with the unmodified controls. After 2 h incubation with cells in serum-free conditions, and subsequent washing, less than one cell mm^{-2} was observed on the GEPI–PEG modified surfaces, while 100-fold higher non-specific adhesion was observed on the control surfaces (Fig. 4a–e). Similar results were obtained with OCCM-30 cells (see Fig. S1). As shown in Fig. 4e, the difference between the number of cells per unit area on both the bare and peptide-only surfaces was negligible.

The stability of the 3GBP1–PEG system was also tested in the presence of 1% FBS and 24 h cell incubation. The rich variety of proteins in the FBS provides an environment similar to in vivo conditions by introducing possible peptide–protein, PEG–protein or surface–protein interactions. As expected, the presence of FBS increased the number of cells attached 1.5- to 3-fold in all of the samples compared with the serum-free conditions. This increase was due to the interaction of serum proteins with the material surfaces, which in turn created a more compatible surface for the attachment of cells. The specific classes of serum proteins adsorbed on the substrate surfaces would create specific adhesion points for the cells. [43] The increase in cell attachment on the GBP–PEG modified substrates, however, was still negligible compared with the negative controls. The average number of the cells attached to the 3GBP1–PEG surfaces was 3.1 ± 0.75 cells mm^{-2}, while about 50-fold higher cell attachment was observed on the negative control surfaces (Fig. 5a). From this observation it can be deduced that the 3GBP1–PEG layer not only resists the adhesion of cells, but also the adsorption of serum proteins. SEM analysis showed that the 3GBP1–PEG layer was still stable after 24 h cell incubation in 1% FBS. After 24 h the cells on the bare gold surface displayed the typical fibroblast morphology, with some of the cells displaying clustering rather than a homogeneous distribution (Fig. 5b). On the 3GBP1–PEG modified surfaces no additional cell adhesion was observed at 24 h. Energy-dispersive X-ray spectroscopy (EDXS) analysis confirmed that the PEG layer was still present on the surface with a prominent carbon peak (Fig. 5b, inset).

Additionally, we have attempted to achieve PEG conjugation on gold with two other gold-binding peptides (AuBP1 and AuBP2) selected via bacterial cell surface display [44]. Although all of these peptides have demonstrated selective affinity for gold, conjugation of PEG with AuBP1 and AuBP2 did not result in a sufficient decrease in the water contact angles (Fig. 6). This may be due to the fact that AuBP1 and AuBP2 are much smaller in size (12 a.a.) compared with 3GBP1 (42 a.a.), facilitating conjugation with PEG. To test this possibility, we prepared a single repeat of GBP1 and triple tandem repeats of AuBP1 and AuBP2 (3AuBP1 and 3AuBP2) and attempted PEG functionalization as described above. As expected, the triple repeat 3AuBP2 resulted in better coverage compared with the original peptide, but not 3AuBP1. The failure to achieve complete coverage with both the original and the triple repeat of AuBP1 implies that the conjugation of PEG with lysine residues impairs the stability of the peptide on the surface. The lysine residues

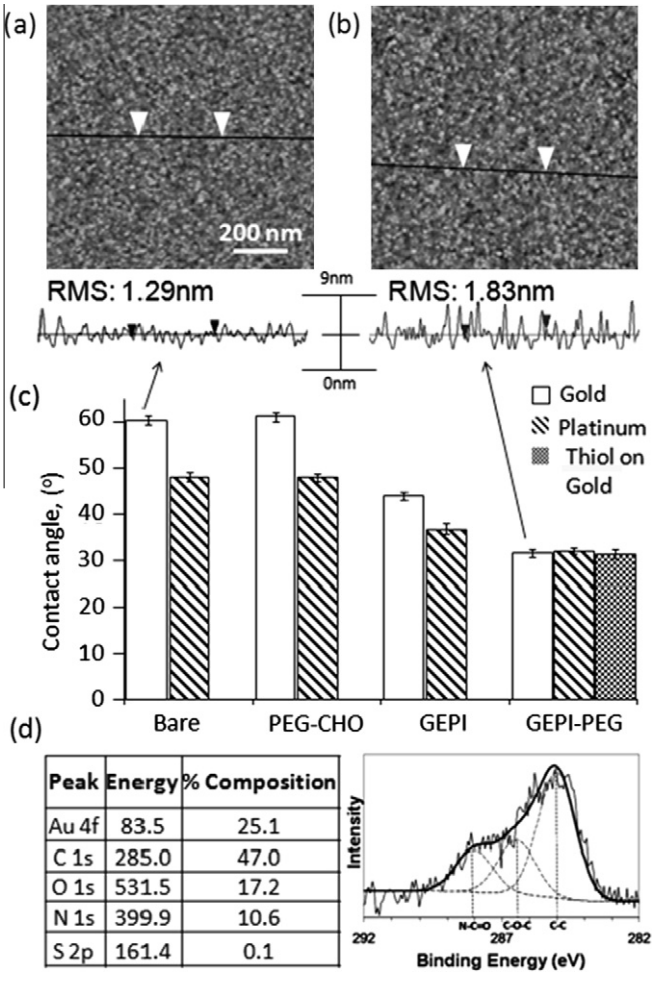

Fig. 3. AFM micrographs and section analysis of a bare (a) and a GEPI–PEG modified (b) gold surface. The size bars correspond to 100 nm. (c) Water contact angles on gold and platinum surfaces after treatment with PEG-CHO alone, GEPIs alone and PEG-CHO conjugated via GEPIs. Thiol-OEG on gold is shown as a positive control. (d) High resolution XPS analysis of the carbon photo-electron emission spectrum of GEPI–PEG modified substrates.

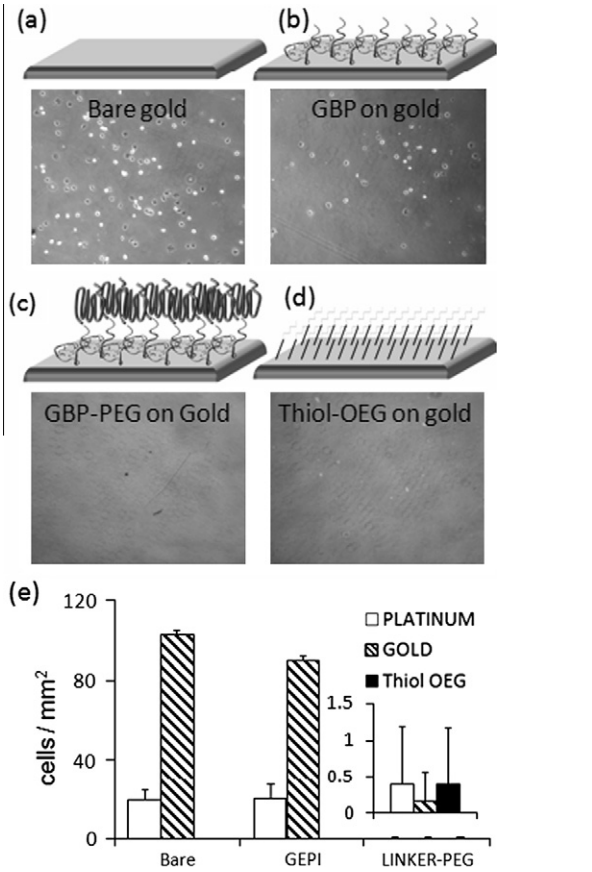

Fig. 4. (a–d) Optical microscopy images of NIH 3T3 cells remaining after washing on modified and unmodified gold substrates. (e) The number of NIH 3T3 cells per area remaining after washing on modified and unmodified gold and platinum surfaces.

may be essential for binding, either by directly interacting with the surface or by contributing to the structural stability of the peptide on the surface. To investigate this hypothesis, we selectively conjugated PEG-CHO with AuBP1 while the primary amine on the lysine residue was protected and only the N-terminal primary amine was available. In this way, conjugation can only occur through the primary amine at the N-terminus. As expected, selectively conjugated AuBP1 resulted in a contact angle comparable with the positive controls (Fig. 6).

The differences in the degree of functionalization with different peptides indicate that both the relative size of the peptide and the number of available groups suitable for conjugation are important factors governing the extent of surface functionalization. Since combinatorial selection against a surface usually produces not only one but a set of multiple peptides for the target surface, one can select the most suitable peptide for the desired application among the set of peptides, as demonstrated here.

It is interesting that PtBP1 does not obey this generalization. Despite the relatively small size of the peptide compared with the PEG molecule, sufficient conjugation and coverage was achieved without the need for a tandem repeat of the peptide. This may be due to the constrained nature of the peptide. The disulfide bridge between the cysteine residues introduces a structural constraint and, therefore, increases the stability of the peptide. Constraint peptide architecture, in turn may render the peptide less susceptible to steric effects.

3.2. Bio-active surface modification

Cellular adhesion and spreading was evaluated on titanium and glass surfaces using NIH 3T3 mouse fibroblasts in the absence and presence of serum proteins to assess the cyto-compatibility of the surfaces. Adhesion of the NIH 3T3 cells to glass and titanium surfaces modified with RGD chimeric peptides were increased by 3.5- to 5-fold in serum-free and 2-fold in 1% FBS conditions, compared with negative controls (Fig. 7a and 7b). A decrease in adhesion was observed especially on glass surfaces incubated with RGD alone in serum-free conditions. Such a decrease may originate from the dissociation of non-specifically adsorbed RGD peptides from the surface into the medium. The unbound RGD in the medium may then interact with the RGD-binding sites on the integrin receptors and act as an integrin antagonist to prevent further cellular interactions with the surface [45,46]. Conversely, a slight increase in adhesion was observed on the surfaces modified with QBP1 and TiBP1 alone. Although within the margin of error, this effect may be due to the inherent chemical properties of the peptides. The abundance of non-polar residues in the peptides (Table 1) is likely to result in an increase in the hydrophobicity of the glass surface. Contact angle measurements showed that QBP1 indeed resulted in a 19% increase to a value of 46° compared with bare glass. Consequently, when amphiphilic molecules on the cell membrane, such as proteins, interact with the non-polar residues an increase in non-specific adsorption on the surface may be observed.

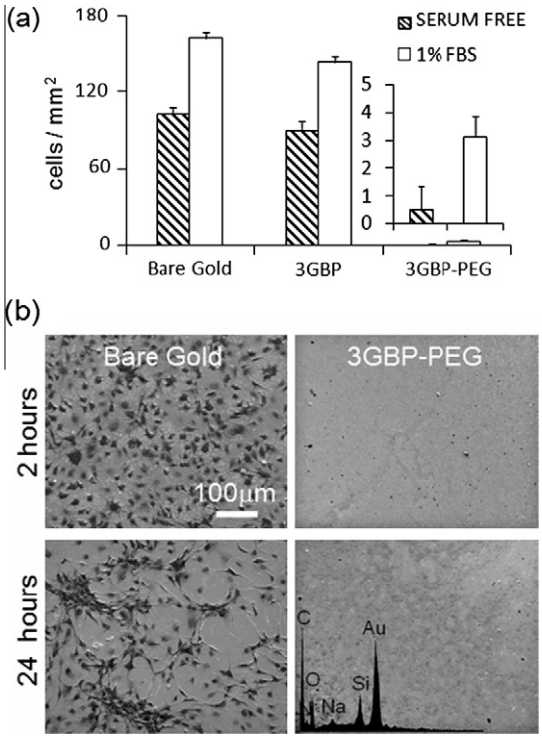

Fig. 5. (a) Cell adhesion in serum-free and 1% FBS conditions on the gold surface after 2 h. (b) SEM micrographs of the bare and 3GBP1–PEG modified gold surfaces after 2 and 24 h. The inset displays the EDXS spectrum of the 3GBP1–PEG modified surface at 24 h showing prominent C K_α (0.277 keV) and O K_α (0.523 keV) peaks, consistent with the coverage of the surface with PEG.

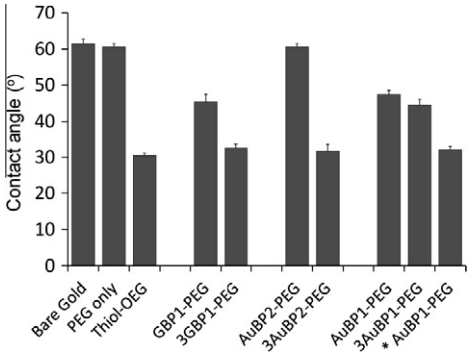

Fig. 6. Water contact angles of the gold surfaces modified with single and triple tandem repeats of the three different gold-binding peptides, GBP1, AuBP1 and AuBP2.

As opposed to serum-free conditions, no significant differences in adhesion were observed between the three negative control surfaces (Bare, GEPI alone and RGD alone) in 1% FBS conditions. Cell coverage on all of the control surfaces was about 300 cells mm^{-2}. Adsorption of serum proteins on the surfaces rendered the control surfaces cytocompatible [43]. On the GEPI–RGD modified surfaces, however, a combined effect of the serum proteins and the bifunctional peptides was observed. The number of cells increased by 30–35% in the presence of serum proteins on GEPI–RGD modified surfaces relative to the serum-free conditions (Fig. 7c and d).

In tissue engineering applications the integrin ligands were not only used to modify surfaces for increased cell adhesion but also for enhanced cell viability and function. To test whether the RGD chimeric peptides resulted in any enhancement in cell viability, cell spreading was evaluated in addition to cell adhesion. ImageJ image processing software (NIH) was used to analyze cell spreading. An increase of about 1.6-fold in cell spreading was observed on both materials modified with chimeric RGD peptides compared with bare surfaces (Fig. 7e). A similar increase was observed on fibronectin modified surfaces. The results indicate that the presence of both material binding and RGD domains in the designed bifunctional molecular construct is necessary to promote cell adhesion and proliferation.

4. Conclusions

We demonstrate the utility and versatility of solid-binding peptides to control cell–surface interactions through the immobilization of functional molecules on various implantable materials, e.g. gold,

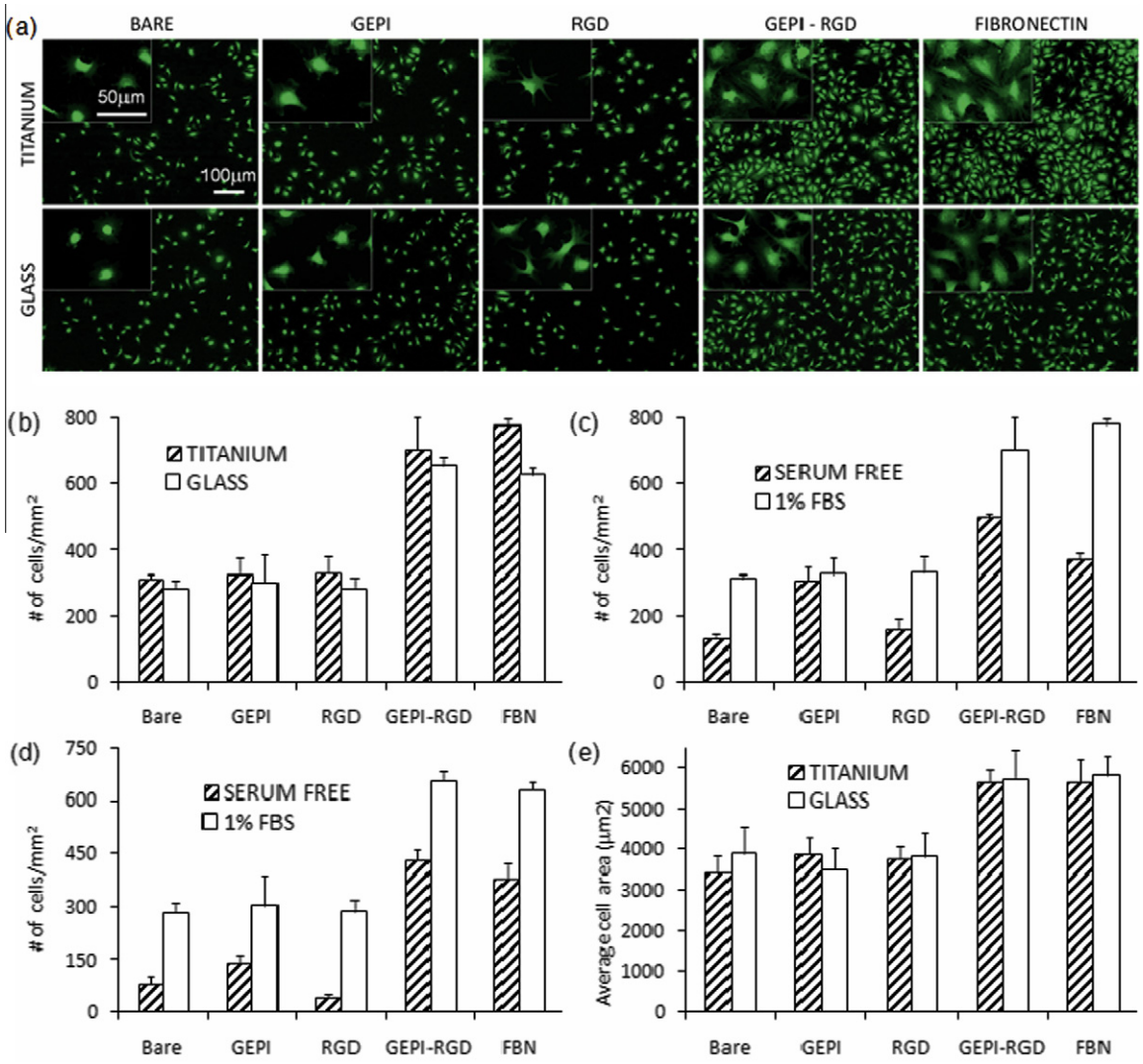

Fig. 7. (a) FM micrographs of phalloidin stained NIH 3T3 cells on glass and titanium surfaces. (b) The number of adhered NIH 3T3 cells per mm^2 in the presence of 1% FBS. The comparison of cell adhesion in serum-free and 1% FBS conditions on the titanium (c) and glass (d) surfaces. (e) Average cell spreading on titanium and glass surfaces in the presence of 1% FBS.

platinum, titanium and glass. We also identified basic design criteria for the choice of the most suitable peptides for specific potential applications. Targeted assembly proved to be an efficient way of immobilizing large molecules (i.e. PEG) through, first, coating the inorganic binding peptides and then performing the conjugation reaction. Directed assembly, on the other hand, is preferred for the immobilization of small molecules by synthesizing a single chimeric molecule with bifunctional domains. Our results have demonstrated that engineered peptide-based surface modification is an adaptable platform to meet the specific requirements that may arise due to the inherent properties of the materials used for a particular application, the demands of necessary functional molecules to be conjugated and the molecular characteristics of the solid-binding peptides themselves. Single step surface modification using solid-binding peptides conjugated to medically relevant molecules may prove, in further studies, to be a robust and biocompatible approach to functionalization of multi-material implants and devices in clinical applications.

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Appendix Figures. with essential colour discrimination

Certain figures in this article, particularly Figure 7, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.06.004.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2010.06.004.

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