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Original Article

Relevant Properties for Immobilizing Short Peptides on Biosurfaces

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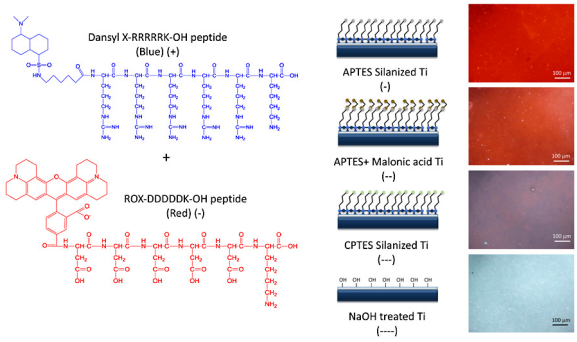
Received 23 January 2017; received in revised form 24 May 2017; accepted 12 June 2017

Graphical abstract



Abstract

Understanding protein and oligopeptide adsorption on biomaterial surfaces is important to develop new biomaterials with improved properties. The phenomenon of peptide adsorption is determined by many parameters such environmental pH, surface topographical features, surface polarity, peptide structure, and/or surface and peptide electric charges. We assessed the effect of surface and peptide net charges on oligopeptide adsorption on synthetic surfaces under different conditions. We have also assessed the ability of immobilizing peptides on the surface generating covalent bonds or electrostatic attraction. Direct relation between the amount of peptide adsorbed on the surface and the difference in net charge between surface and peptides was determined. No relation between the difference in net charge and the ability to promote covalent bonds between peptide and surface was found. Competitive adsorption experiments confirmed these findings. Understanding the specific interactions during adsorption of peptides onto synthetic surfaces improves our ability to develop strategies for the efficient immobilization of oligopeptides on biomaterial surfaces.

Keywords: Biofunctionalization; Silanization; Surface charge; Titanium; Peptide; Adsorption

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http://dx.doi.org/10.1016/j.irbm.2017.06.003

1. Introduction

Although the processes involved in protein adsorption on a synthetic substrate are not yet fully understood, protein adsorption on biomaterial surfaces is a relevant topic that relates to many novel applications in implantology, regenerative medicine and biotechnology. A number of surface and protein parameters have be proposed to be key in determining the amount of proteins adsorbed on the surface as well as the adsorbed protein stability and conformation. Namely,

- Environmental pH [1,2]
- Basicity and acidity of the amino acid side chains that compose the protein [3–5]
- Surface topography [6,7]
- Primary, secondary, tertiary and quaternary structure of proteins [2,8,9]
- Protein–protein interactions [10–12]
- Surface hydrophilicity/hydrophobicity [13–16]
- Surface charge [1,3,17]

When a synthetic device comes in contact with body fluids, hundreds of different proteins interact and compete for interacting with the device surface [18]. As there is a wide range of different materials with many different surface treatments that are used for biomedical devices and implants, the assessment of the specific protein adsorption on those surfaces is unpredictable in the majority of cases. That is in spite of tremendous progress made in this research field [19]. Oligopeptide adsorption on biomaterial surfaces may be less complex than protein adsorption due to the fact that short peptides do not adopt tertiary and quaternary structures [4,5,9,16].

Immobilization of oligopeptides on biomaterial surfaces has been frequently achieved by chemical reaction with formation of covalent bond between the peptide and the surface. The most common approach used for this purpose is the generation of amide bonds between primary amines and carboxylates using a coupling reagent to activate the carboxylate and thus, catalyze the reaction [20–22]. Other methods generate disulfide bonds using glutaraldehyde as a cross-linker [23,24] or induce nucleophilic–electrophilic substitution reactions involving a halogen atom [25,26].

The aim of this work was to elucidate the effect of surface and peptide charge on the physical and chemical immobilization of oligopeptides on metallic biosurfaces. To achieve this objective two different fluorescent labeled peptides with opposite polarities were fabricated. Additionally, four titanium surfaces with strongly different ζ-potentials and capability to generate covalent reactions with the peptides were developed. Both peptides were led to interact with the different surfaces and measurements of peptide adhesion were carried out.

Understanding the specific interactions during adsorption of peptides onto synthetic surfaces will improve our ability to develop strategies for the efficient immobilization of oligopeptides on biomaterial surfaces.

2. Design of the elements of the study

A negatively charged peptide (Dansyl X–DDDDDK–OH) was composed by a chain of five aspartic acids and a lysine and a positively charged peptide (Dansyl X–RRRRRK–OH) was composed by a chain a five arginines and a lysine. The lysine residue would provide the capability to generate covalent reactions on both peptides. In order to detect the peptides by fluorescence analysis techniques both peptides were labeled with a neutral dansyl probe on its N-terminus. The Dansyl probe provided an intense blue fluorescence to the peptides. Additionally, a minor part of the negatively charged peptide (ROX–DDDDDK–OH) was labeled with ROX™ fluorescent dye which provides an intense red fluorescence in order to differentiate both peptides when deposited on the different titanium surfaces.

Four different titanium surfaces were treated and silanized in order to obtain different surface properties. The first surface (Ti NaOH) was treated with sodium hydroxide in order to obtain a highly electronegatively charged surface due to the presence of a high amount of electronegative species on the surface [27,28]. The second surface was silanized with 3-aminopropyltriethoxysilane in order to obtain a surface with a high amount primary amines which would provide a decrease on the electronegative character of the Ti surface. A third surface was silanized with 3-chloropropyltriethoxysilane which would increase the electronegative character of the surface due to the presence of Cl^- species on the surface. Additionally, this silanized surface has the potential to develop a covalent bond with the designed peptides through a nucleophilic substitution between the silane and the lysine residue of the peptide [25,29–31]. On the fourth surface, esterified carboxylic acids were covalently anchored to the titanium surface. Similarly to the third surface, this one is able to interact with the lysine residue of the peptides but, in this case, to generate an amide bond between surface and peptide [32].

3. Experimental section

3.1. Materials

The base synthetic substrates for the experiments were disks of commercially pure Grade 2 titanium (Ti) cut from bars (Daido Steel Co, Japan). Surface activation of titanium surfaces was performed with sodium hydroxide pellets (Sigma-Aldrich, USA). 96% ethanol (Panreac, Spain), acetone PAI-ACS (Panreac, Spain), Milli-Q Water (Millipore, USA), 99% 2-propanol (Sigma-Aldrich, USA), and 99.5% cyclohexane anhydrous (Sigma-Aldrich, USA) were used to clean Ti surfaces.

Silanization was performed using 3-aminopropyltriethoxysilane (APTES) [33] or 3-chloropropyltriethoxysilane (CPTES) [29] in combination with anhydrous toluene, all from Sigma-Aldrich (USA). Crosslinking between the silanes and the oligopeptides was carried out using malonic acid, N,N-Diisopropylethylamine (DIEA) (Sigma-Aldrich, USA) and N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (NovaBioChem- EMD group, USA).

Please cite this article in press as: Sevilla P, et al. Relevant Properties for Immobilizing Short Peptides on Biosurfaces. IRBM (2017), http://dx.doi.org/10.1016/j.irbm.2017.06.003