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Self-assembling peptide hydrogels immobilized on silicon surfaces

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ABSTRACT

The hydrogels of self-assembling ionic complementary peptides have collected in the scientific community increasing consensus as mimetics of the extracellular matrix that can offer 3D supports for cell growth or be vehicles for the delivery of stem cells or drugs. Such scaffolds have also been proposed as bone substitutes for small defects as they promote beneficial effects on human osteoblasts. In this context, our research deals with the introduction of a layer of self-assembling peptides on a silicon surface by covalent anchoring and subsequent physisorption.

In this work, we present a spectroscopic investigation of the proposed bioactive scaffolds, carried out by surface-sensitive spectroscopic techniques such as XPS (X-ray photoelectron spectroscopy) and RAIRS (Reflection Absorption Infrared Spectroscopy) and by state-of-the-art synchrotron radiation methodologies such as angle dependent NEXAFS (Near Edge X-ray Absorption Fine Structure). XPS studies confirmed the change in the surface composition in agreement with the proposed enrichments, and led to assess the self-assembling peptide chemical stability. NEXAFS spectra, collected in angular dependent mode at the N K-edge, allowed to investigate the self-assembling behavior of the macromolecules, as well as to determine their molecular orientation on the substrate. Furthermore, Infrared Spectroscopy measurements demonstrated that the peptide maintains its secondary structure (β-sheet anti-parallel) after deposition on the silicon surface. The complementary information acquired by means of XPS, NEXAFS and RAIRS lead to hypothesize a "layer-by-layer" arrangement of the immobilized peptides, giving rise to an ordered 3D nanostructure.

1. Introduction

In the field of nanobiotechnology and biomaterials based on peptides and proteins, self-assembling properties have been recently a focus of considerable research activity [1]. The possibility of manufacturing innovative nanostructured scaffolds with customizable mechanical properties and biological functions makes this class of materials an ideal candidate for countless biomedical applications, including tissue regeneration, drug delivery, protein crystallization, and cellular internalization [2].

The main aim of regenerative medicine is to design a biomimetic and well-defined matrix capable of promoting specific interactions with the cells in order to control and guide their behavior by mimicking their native environment. The ideal matrix must have a 3D geometry similar to the extracellular matrix and must be able to promote cell adhesion, proliferation, infiltration and differentiation aimed at new tissue formation. Nowadays the "top-down" approaches, that are limited by properties of the bulk starting material, are being replaced by "bottom-up" scaffolds nanofabrication [1,3]. For this new approach, a very promising path is the exploitation of self-assembling properties: in fact, self-organization provides molecular nanotechnology with a powerful alternative to both top-down miniaturization and bottom-up nanofabrication methods. In this context, ionic-complementary self-assembling peptides (SAPs) [4–6] appear as ideally suited molecules for the preparation of 3D nanostructured bioactive scaffolds. These simple peptide sequences are able to aggregate in the presence of saline solutions creating hydrogels that can be used as drug carrying vehicles [7], but can also be decorated with adhesive peptides or proteins [8–11], even suitably conjugated with the self-assembling sequence, allowing the scaffold to functionalize, for example, with adhesive signals in a 3D manner by simple co-aggregation. These peptides assemble themselves into well-ordered nanofibers and scaffolds often about 10 nm in diameter with pores between 5 and 200 nm [12].

Abbreviations: SA, Self-assembling; SAP, Self-assembling peptide; APTES, 3-Aminopropyl triethoxysilane; Si, Silicon surface; Sil, Silicon surface silanized with APTES; SAP_{CB}, Covalently bonded SAP on Sil surface; SAP_{CB} + SAP, SAP_{CB} decorated with physisorbed SAP; Sil + SAP, Sil decorated with physisorbed SAP.

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To characterize details of the process occurring during the immobilization of peptides on a surface, it is desirable to obtain an element-specific and bond-specific picture of the reactions that are taking place: X-ray Photoelectron Spectroscopy (XPS), Near Edge X-ray Absorption Fine Structure (NEXAFS) and Reflection-Absorption Infrared Spectroscopy (RAIRS), when opportunely combined, are able to provide such information. XPS is the best suitable technique to investigate the molecular and electronic structure of molecules anchored on a surface, providing information about the atomic composition of the adlayer as well as on the electronic state of the atoms involved in the chemical bonds [13]. By selecting the proper transition energy, NEXAFS allows focusing on a specific valence orbital, projected onto a specific atom in a well-defined oxidation state [14]; what is more, the orientation of the orbital can be determined by the polarization dependence of the specific resonance intensity using optical-dipole selection rules. XPS data analysis allows determining the coverage, i.e. the anchored peptide thickness on the surface. RAIRS can be successfully used to investigate the oligopeptide secondary structure, and to test its stability upon SAP immobilization on the substrate.

In this work, the silicon oxidized surface, previously acid-etched (Si), was silanized with APTES (3-aminopropyl triethoxysilane), obtaining the sample indicated as Sil in Fig. 1. Subsequently, the "activated" surface was functionalized covalently and selectively with the carboxyl terminus of the ionic-complementary SA peptide Ac-EAbuK (sequence: CH_3CO-Abu-E-Abu-E-Abu-K-Abu-K-Abu-E-Abu-E-Abu-K-Abu-K-OH where Abu = L-α-aminobutyric acid, E = L-glutamic acid, K = L-lysine) [15] yielding sample SAP_{CB} (CB = covalently bonded) shown in Fig. 1. The hydrogel layer was furtherly decorated with the physisorbed peptide EAbuK (sequence: H-Abu-E-Abu-E-Abu-K-Abu-K-Abu-E-Abu-E-Abu-K-Abu-K-NH_2, sample SAP_{CB} + SAP, covalently bonded SAP decorated with physisorbed SAP), possibly resulting in multilayer formation. For comparison, an activated Sil surface simply decorated with physisorbed EAbuK was also prepared (sample Sil + SAP, physisorbed SAP). The grafting of a SAP layer to silicon allows switching from 2D to 3D bioactive interfaces where the cells can immerse themselves; what is more, the proposed approach enables us to add nanometer roughness to the implant surface. It is well known that the peptide nanofibrous network, like other chemically produced nanophase materials, could improve cellular activities when compared with conventional micro-rough materials [16]. The characterization of the steps leading to the 3D bioactive interfaces reported here was performed by XPS, angular dependent NEXAFS and IR analysis.

In a previous paper [17], we reported the immobilization of SAP by covalent immobilization through APTES on rough Ti surfaces. Surface roughness is necessary to mimic the bone structure, promoting adhesion and proliferation of human osteoblast. However, rough surfaces do not allow determination of molecular order and orientation of the immobilized peptide with respect to the sample surface by incidence angle depending techniques such as NEXAFS spectroscopy.

In this paper, investigations performed on flat Si surfaces aimed at determining molecular order and orientation of the peptide overlayer. Moreover, reflectivity of the Si flat surface allows performing RAIRS measurements and investigating the peptide secondary structure.

2. Experimental

2.1. Peptide synthesis

2.1.1. EAbuK

The synthesis of EAbuK (H-Abu-E-Abu-E-Abu-K-Abu-K-Abu-E-Abu-E-Abu-K-Abu-K-NH_2) was carried out on 0.72 mmol/g Rink Amide MBHA resin using Fmoc chemistry by a Syro I synthesizer [18] The

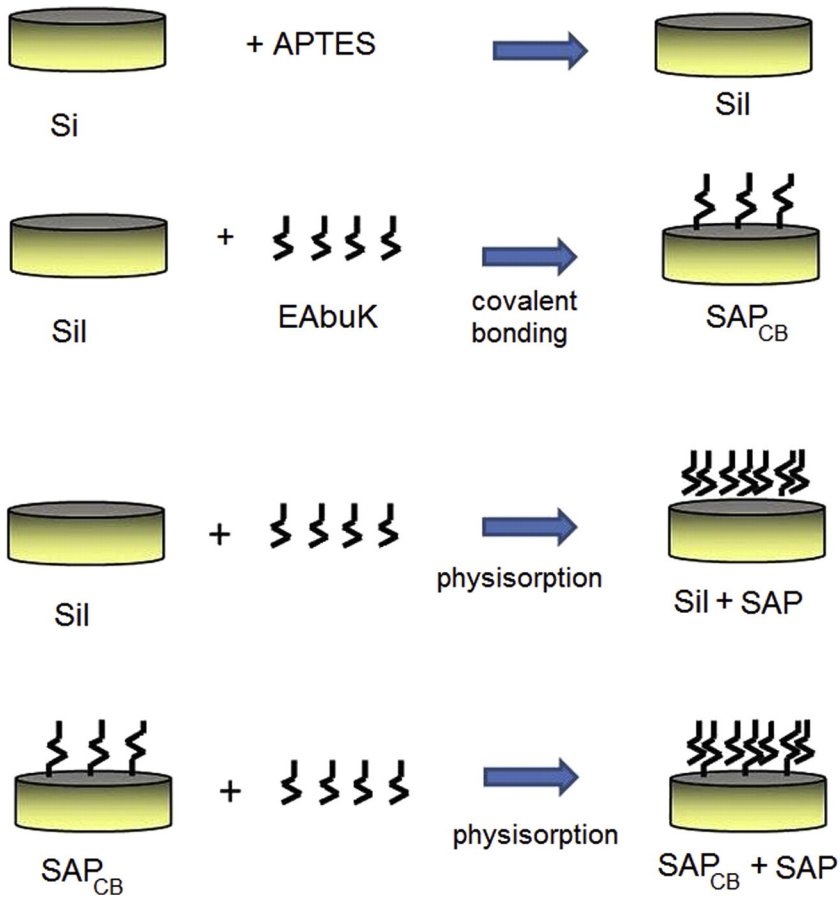

Fig. 1. Reaction scheme. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)