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Full Length Article

Hydrogenated amorphous silicon coatings may modulate gingival cell response

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ABSTRACT

Silicon-based materials present a high potential for dental implant applications, since silicon has been proven necessary for the correct bone formation in animals and humans. Notably, the addition of silicon is effective to enhance the bioactivity of hydroxyapatite and other biomaterials. The present work aims to expand the knowledge of the role exerted by hydrogen in the biological interaction of silicon-based materials, comparing two hydrogenated amorphous silicon coatings, with different hydrogen content, as means to enhance soft tissue cell adhesion. To accomplish this task, the films were produced by plasma enhanced chemical vapor deposition (PECVD) on titanium substrates and their surface composition and hydrogen content were analyzed by means of X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectrophotometry (FTIR) respectively. The surface energy and roughness were measured through optical contact angle analysis (OCA) and high-resolution mechanical profilometry respectively. Coated surfaces showed a slightly lower roughness, compared to bare titanium samples, regardless of the hydrogen content. The early cell responses of human keratinocytes and fibroblasts were tested on the above mentioned surface modifications, in terms of cell adhesion, viability and morphometrical assessment. Films with lower hydrogen content were endowed with a surface energy comparable to the titanium surfaces. Films with higher hydrogen incorporation displayed a lower surface oxidation and a considerably lower surface energy, compared to the less hydrogenated samples. As regards mean cell area and focal adhesion density, both a-Si coatings influenced fibroblasts, but had no significant effects on keratinocytes. On the contrary, hydrogen-rich films increased manifolds the adhesion and viability of keratinocytes, but not of fibroblasts, suggesting a selective biological effect on these cells.

1. Introduction

Dental implants represent a fundamental option to treat edentulism, whether it be partial or complete. Since Brånemark's first studies, research focused on the interaction between bone and titanium fixtures [1]. However, to be clinically used, implants must be connected to an intra-oral prosthesis, which implies the presence of a transmucosal component interfacing also with fibroblasts and epithelial cells [2]. Unwanted, yet frequent, clinical responses such as gingival recession and the so-called peri-implantitis have recently highlighted the importance of the soft tissue around the implant as a possible barrier to bacterial penetration along the fixture [3,4].

Although the extra-osseous part of an implant may be designed to reduce plaque accumulation, several limitations remain as for the soft tissue surrounding the implant. Indeed, the connective tissue and epithelium attach to the cemental root surface of a natural tooth [5], but act differently around implants [6,7]. To this end, numerous surface modifications have been recently proposed [8–11]. Interestingly, controversial data are available as regards the use of roughened surfaces [12–16] in enhancing soft tissue healing. Additive surface modifications are thus increasingly studied as possible means to drive selective adhesion of the two main cell types within the gums: epithelial cells and fibroblasts. Among the most

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promising options, silicon-based coatings, such as sol-gel derived SiO_2/ZrO_2 coatings [17], amorphous silicon (a-Si), as well as silicon-oxygen, silicon-nitrogen and silicon carbon amorphous thin film alloys (a-SiO_x, a-SiN_x, a-SiC_x), play an important role [17–20]. These materials are similar to bioglass pertaining to hardness, mechanical resistance and optical properties, i.e. transparency, and may display antibacterial properties and peculiar features as cell interface [18–21]. These thin-film coatings have attracted a strong interest for medical applications, especially owing to their silicon content, the role of which has been evidenced in the interaction between inorganic surfaces and living tissues since the work of Carlisle in 1970 [22]. The presence of silicon traces in the diet is necessary to achieve a normal growth in chickens [23] and to stimulate cartilage synthesis in ovariectomized rats [24]. Moreover, the dietary intake of Si is proposed to be correlated with bone mineral density in humans [25]. Unsurprisingly, titanium oxide nanostructures have also shown an improvement of their biological interaction after their surface was implanted by Si ions [26], while the use of nitrogen-incorporated a-SiO_x coatings on metal implants accelerates the bone healing process, thanks to their ability to release Si^{4+} ions [27].

While the role of silicon in the enhancement of the interaction with the biological environment has been already demonstrated in the literature, a synergistic role of silicon and hydrogen has been suggested, possibly owing to the ability of hydrogen to tune the hydrophilic/hydrophobic properties of silicon-rich surfaces [28]. Indeed, the hydrogenation of crystalline Si samples improves their interaction with osteoblasts [29]. Hydrogenated amorphous silicon (a-Si:H) layers enhance osseointegration, evidencing their ability to promote apatite formation in simulated body fluid [30]. Despite these interesting results, the effective role of hydrogen in the biological interaction of silicon-based surfaces is still not completely clear, due to the limited number of works on the subject. In addition, most studies concentrate on the osseointegration process, while results regarding the interaction of silicon-based surfaces with soft tissues, such as the gums, are lacking. The present work aims to expand the knowledge of the role exerted by hydrogen in the biological interaction of silicon-based materials, comparing two different hydrogenated amorphous silicon coatings, with different hydrogen content, as means to enhance soft tissue cell adhesion. To accomplish this task, the early cell response of human keratinocytes and fibroblasts was tested on the above mentioned surface modifications.

2. Material and methods

2.1. Sample preparation

Ti-Al-V samples were machined to obtain 8 mm × 3 mm cylinders (2r × h) (Titanmed s.r.l., Milan, Italy). Crystalline silicon samples of approximately rectangular shape and dimensions of about 10 mm × 20 mm, cut from p-type (100) wafers of about 50 Ohm cm resistivity, were also prepared. All the samples were cleaned in an acetone ultrasonic bath for 10 min, immersed in isopropanol for 10 min, then rinsed in deionized water and finally dried by blowing them with dry nitrogen gas. Films grown on silicon were used to allow the FT-IR analysis in transmission mode, since the metallic Ti-Al-V alloy absorbs the IR radiation, while the high-resistivity crystalline Si is IR-transparent. In the following, the prefix 'Ti' in the sample nomenclature refers to films grown on Ti-Al-V substrates, while the prefix 'Si' identifies the films grown on silicon substrates. Moreover, the terms "titanium" or "machined titanium" are used in the following, for simplicity, to refer to the uncoated Ti-Al-V alloy samples.

Table 1
Set of the deposition parameters used to grow the a-Si coatings by PECVD.

Sample Name	$T_{Substrate}$ °C	Gas Flow Rate sccm	Gas Pressure mTorr	RF Power Density W/m²
a-Si_90 °C	90	30	450	208
a-Si_350 °C	350	30	450	208

Two different amorphous silicon (a-Si) coatings (named a-Si_90 and a-Si_350), showing different amount of hydrogen incorporation, were grown by *radio frequency - plasma enhanced chemical vapor deposition* (RF-PECVD) technique using silane (SiH_4) as the Si precursor on both titanium and Si substrates. The hydrogen content was tuned by modifying the substrate temperature, exploiting the enhanced diffusion of H atoms in the films at high temperature [31,32]. The growth reactor consisted of a capacitively coupled PECVD system, composed of two parallel electrodes of 12 cm × 12 cm area each, located at a relative distance of 20 mm. The substrates were coated on the whole surface in order to perform the biological tests. The precursor gas was injected inside the reactor through a gas manifold, using a *mass flow controller* (MFC) to control the flow rate. The reaction byproducts and exhausted gases were removed through a pumping system, consisting of a turbomolecular pump connected to a downstream mechanical pump. The plasma discharge was ignited by means of an RF generator (RFPP–RF5S), working at a fixed frequency of 13.56 MHz, and an impedance matching network (Huttinger - FR 1500A) was used to tune the load impedance. All the process parameters, except the substrate temperature, were kept fixed for all the deposition runs and are summarized in Table 1. The heater temperature was measured by a KF thermocouple fixed on the heating unit and the substrate temperature, which was calculated by means of a calibration curve, was varied between 90 °C and 350 °C. The deposition time was fixed at 30 min, resulting in an approximate film thickness of 300 nm for all the films.

A representative number of samples underwent physical and chemical characterization before the biological experiments. Pristine titanium samples were also kept as unmodified controls during further experiments.

2.2. X-ray photoelectron spectroscopy

X-ray Photoelectron Spectroscopy (XPS) was carried out by using a PHI 5000 VersaProbe (Physical Electronics) system. The usual surface pre-cleaning of the samples by means of Ar^+ ion bombardment was not intentionally performed so as not to alter the surface chemical composition that was present at the moment of the biological assays. The X-ray source was a monochromatic Al Kα radiation. The binding energy scale was calibrated by assigning a binding energy value of 284.8 eV to the main C1s contribution.

XPS signals were collected using a SPECS (Phoibos MCD 150) X-ray photoelectron spectrometer, with Mg Kα radiation (1253.6 eV) as X-ray source, having a power of 150 W (12 mA, 12.5 kV). The spot size of the analyzed region was 7 mm × 20 mm. The emission of photoelectrons from the sample was analyzed at a take-off angle of 90° under UHV conditions. No charge compensation was applied during the acquisition. After collection, the binding energies were calibrated using as reference the adventitious carbon C1s peak. The accuracy of the reported binding energies (BEs) can be estimated to be ± 0.1 eV. The XPS peak areas were determined after subtraction of a background. The atomic ratio calculations were performed after normalization using the Scofield factor of each element. All spectrum processing was carried out using the Casa XPS v2.3.13 software (Casa Software Ltd.) package and Origin 7.1 (Origin Laboratory Corp.). The spectral decomposition was performed by using