DENTAL MATERIALS 23 (2007) 469–478

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Photocoupling of fibronectin to titanium surfaces influences keratinocyte adhesion, pellicle formation and thrombogenicity

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ARTICLE INFO

Article history:
Received 3 June 2005
Received in revised form
13 February 2006
Accepted 9 March 2006

Keywords:
Dental implants
Titanium
Biofunctionalization
Fibronectin
Anthraquinone photocoupling
Epithelial seal
Keratinocyte adhesion
Platelet activation
Pellicle formation
QCM-D

ABSTRACT

Objectives. Coating of implant surfaces with biomolecules can influence basic host responses and enhance subsequent tissue integration. The biological factors have to be immobilized on the implant material. Human fibronectin (Fn) was used as a model protein and covalently coupled to titanium (Ti) surfaces via silanization and an anthraquinone linker. The impact on several aspects of initial host/biomaterial interactions (keratinocyte adhesion, platelet interactions and pellicle formation) was studied.

Methods. Coupling efficiency was characterized by immunological techniques. The effects of coupled Fn on initial host/biomaterial interactions were assessed. Cell adhesion and spreading were investigated by fluorescent staining, pellicle formation by an acoustic sensor system (quartz crystal microbalance with dissipation, QCM-D), and platelet adhesion as one parameter mediating the inflammatory response by scanning electron microscopy (SEM) and immunological assays.

Results. Coupling efficiency was related to irradiation time used for photochemical coupling of the UV-activated anthraquinone to the silanized Ti surface. With an optimized protocol, the amount of Fn coupled to the surface could be almost doubled compared to standard dip-coating methods. On the anthraquinone-coupled Fn coatings, cell adhesion and spreading of human keratinocytes was significantly enhanced. Online detection of pellicle formation revealed strong reversibility of saliva protein adhesion on Fn coated surfaces compared to the pure Ti surface. Furthermore, the Fn coated Ti showed a low thrombogenicity.

Significance. This study suggests that anthraquinone-coupled biological coatings may be useful for biofunctionalization of Ti dental implants by enhancement of soft tissue re-integration (restoration of the epithelial seal) combined with diminished pellicle formation.

1. Introduction

Host reactions to a biomaterial are determined mainly by the material's surface properties. These properties include morphological characteristics, as well as physical and chemical properties of the surface like wettability, surface free energy, or charge, composition and density of chemical groups or molecules at the surface [1]. While particularly cells will

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doi:10.1016/j.dental.2006.03.005

readily respond to structural properties [2–4], the physicochemical properties will have also a large influence on composition and adhesion strength of the "conditioning film" forming rapidly on each implant surface after insertion. Formation of this film is due to a very rapid adhesion of proteins and other macromolecules from ubiquitous body liquids like blood and saliva [5,6]. The adhering proteins, in turn, will indirectly determine subsequent cellular and bacterial interactions with the surface [7,8], as well as inflammatory and immune responses of the host. Surface modification offers promising approaches to influence certain aspects of these initial host/biomaterial interactions and to improve subsequent tissue integration of established implant materials.

Titanium (Ti) has become the material of choice in many orthopedic and dental applications. Surface modifications of Ti like sand blasting and acid etching have improved the osseointegrative properties of the original "as machined" Ti implant surfaces substantially [9]. The aim of current dental implant research is therefore not only a further increase of bony anchorage but rather an acceleration of the whole healing process [10]. In this context, the rapid and stable establishment of a tight "epithelial seal" which prevents bacterial adhesion and subsequent plaque accumulation is of certain interest.

The adhesion of cells to biomaterials is determined by a complex interaction between material surface, anchor proteins and extracellular matrix (ECM). Besides the surface properties of the biomaterial, type, concentrations and conformation or structure of participating proteins like vinculin, integrins, vitronectin or fibronectin (Fn) influence this process [11,12]. Especially the ECM-molecule Fn determines a couple of critical cellular reactions like adhesion, spreading, but also migration and differentiation [13]. By coating with Fn, adhesion of several cell types can be enhanced, e.g. adhesion of fibroblasts to Ti has been enhanced two- to three-fold [14].

Coating of biomaterial surfaces with Fn or other biological adhesion or proliferation factors is therefore a promising approach to optimize healing processes and tissue integration of implants in vivo. In previous work we have demonstrated that initial key events during re-establishment of the epithelial seal, like cell attachment and spreading of human keratinocytes, can be modified by coating of materials with proteins of the extracellular matrix like Fn or laminin, at least in vitro [15–18]. In a clinical situation, however, the benefit of a cell adhesion-promoting coating may be compensated by potentially harmful side effects like an enhanced bacterial adhesion or increased inflammatory response. The impact of any surface modification on these processes has therefore to be considered.

Bacterial adhesion in the oral cavity is mediated through the acquired pellicle [19], a macromolecular film which covers within seconds every available surface. Composition and adhesion strength of the pellicle depend on physicochemical surface properties, and in turn determine strongly the subsequent adhesion of oral microorganisms [5,20,21]. In initial stages of biofilm formation, bacteria may be readily detached from a biomaterial by desorption of the underlying pellicle film [22]. Adsorption and especially desorption characteristics of saliva macromolecules are therefore key processes for initial plaque formation on biomaterials. For investigation of adsorption/desorption processes on biomaterial surfaces, the applied analytical methods should work online without interfering with the system. Besides, for example, optical methods [23], acoustic sensors such as quartz crystal microbalances (QCM) are now widespread since their first description as mass sensitive sensors in gas phases [24]. During the last years, QCM has been increasingly used in the fields of bioadhesion and of biosensors [25–30]. However, a problem with respect to QCM sensors used in liquid phases is that the quartz frequency is not only a function of the adsorbed mass but is influenced by the viscoelastic properties of the interface. The QCM system, used in our studies, allows the online detection not only of the frequency but also simultaneously of a dissipation signal and thus allows to distinguish between adsorbed mass and viscoelastic effects [31].

As mentioned above, another point to be addressed in preclinical testing of newly developed implant surfaces or coatings are interactions of the implant surface with the blood system, since one of the first steps during surgical implantation of a dental implant is the contact with blood, leading to an adsorption of plasma proteins as well as blood cells. Several authors have hypothesized that this step is of decisive importance for the consecutive in-growth and healing process of the implants [32–34]. Implant-adsorbed fibrinogen quickly changes to polymer fibrin fibers entangled by activated platelets, which release growth factors (PDGF, etc.) probably leading to an improved osseointegration. However, high thrombogenic surfaces additionally show a strong inflammatory response, which may lead in severe cases to the loss of the implant.

This study shows effects of blood contact to surface modified Ti to develop an in-depth understanding of the mechanisms which are involved in blood–biomaterial interactions.

Main aim of this work is the performance optimization of Ti dental implants. Biological coatings consisting of ECM-molecules and/or growth factors provide one powerful approach to influence cell/surface, as well as initial saliva/surface and blood/surface interactions. The molecules should be tightly bound to the surface to avoid disruption during implant insertion. The first objective of the current study was therefore the investigation and evaluation of a specific method for covalent chemical coupling of biological factors to Ti implant surfaces. Fn was used as a model protein. The coupling efficiency was determined by immunological methods.

After preparation, the Fn coatings were further investigated with regard to (a) enhancement of cell adhesion and spreading, (b) platelet interactions and (c) pellicle adsorption and desorption characteristics.

2. Materials and methods

2.1. *Preparation and characterization of coatings*

2.1.1. *Sample preparation*

Ti grade two discs of 10 mm Ø and 2 mm height were polished with a graded series of SiC-wet-grinding papers (320, 600, 1200 and 4000 grid) cleaned by ultrasonication in distilled water and dried under N_2.