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Peptide-functionalized zirconia and new zirconia/titanium biocermets for dental applications

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ABSTRACT

Objective: Titanium materials have been functionalized with biomolecules as a modern strategy to incorporate bioactive motifs that will expand and improve their biomedical applications. Here, we have biofunctionalized biomaterials based on zirconia of much interest for dentistry: the widely used bioceramic 3Y-TZP and a newly developed 3Y-TZP/Ti biocermet.
Methods: The biosurfaces were activated, silanized, and functionalized with coatings made of oligopeptides. Surface activation by plasma or alkaline-etching was optimized. The surfaces were coated by tethering a purposely-designed RGD-containing peptide. We selected this oligopeptide as a model peptide to validate the effectiveness of the biofunctionalization process. Successful treatments after each step of the process were assessed by surface physical and chemical characterization with water contact angles and XPS, respectively. Coatings' stability was evaluated after 2 h sonication in water. Pre-osteoblasts adhesion on the functionalized surfaces was also studied.
Results: 10-min air-plasma treatment effectively activated all types of materials with no detrimental effects on the material structure and hardness. Nitrogen XPS-peak confirmed that RGD-peptides were chemically-attached on the silanized samples. This was further confirmed by visualizing the functionalized surfaces with flourescence-labelled RGD-peptides before and after ultrasonication. Furthermore, RGD-functionalized surfaces significantly enhanced osteoblast adhesion on all types of substrates, which demonstrated their successful bioactivation.
Conclusions: We successfully developed stable functional biocoatings on zirconia and biocermets made of oligopeptides. Surface bioactivation of zirconia-containing components for dental implant applications will enable their improved clinical performance by incorporating signalling oligopeptides to accelerate osseointegration, improve permucosal sealing, and/or incorporate antimicrobial properties to prevent peri-implant infections.

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1. Introduction

The replacement of hard tissues by synthetic devices is in many cases the last and most costly solution to deal with the depletion of bone quality and/or the inability to preserve the natural hard tissues. Biomaterials used nowadays to replace those structures, such as those used for making dental implants are not fully compatible with the host bone.[1,2] Mechanical incompatibility between the implant and the surrounding bone and lack of bioactive interactions between the inert biomaterial surface and the natural tissues are among the most significant causes for implant failures at mid and long term.[3] Thus, the development of materials with improved biomechanics that have additional osseostimulative surfaces are of need for better addressing the clinical demands.[4,5]

Surface functionalization is currently considered as an effective and modern approach to design new multifunctional materials by changing composition, structure and/or morphology of surfaces without altering the bulk properties.[6,7] Physical (increased roughness[8,9]), chemical (deposition of calcium phosphate phases, chemical etching or incorporation of specific ionic species[10,11]) and/or biological (bioinspired coatings[12,13]) approaches can be pursued to functionalize surfaces. Specifically, covalent bonding of biomolecules at surfaces has become pivotal to induct bioactivity on biomaterials[14–16] and also for other strategies like assay technologies, biosensors, imaging devices, therapies, etc.[17] One of the approaches used in dentistry to covalently immobilize biomolecules on inorganic materials involves methodologies based on silane-chemistry.[18–20] Biomolecules with varied complexities like arginine-glycin-aspartate (RGD) sequence ,[21,22] other oligopeptides,[23,24] proteins,[25–27] aptamers,[28] recombinamers[13,29] or even multiple peptides with cooperative activities[30] have been tethered to metal surfaces using silanes as coupling agents. We have extended this approach to develop mechanically and thermochemically stable biofunctional coatings on titanium which resist surgical shear stresses during implantation as well as the challenging bioenvironments derived from the contact with physiological fluids.[31] Thus, titanium has been functionalized with different bioactivities either to influence on the physiological paths involved in bone regeneration or to prevent bacterial colonization of the surface.[32]

Although the methodologies of biofunctionalization are developed for titanium materials with promising results, the potential of chemically biofunctionalizing other biomaterials of more recent interest in dentistry, particularly those that are based on zirconia, is less known.[17] Recently, the biofunctionalization of fully-dense zirconia-based materials has been achieved, but coatings were made of adsorbed (physically adsorbed) organic-molecules.[33,34] Thus, the strong and stable immobilization of covalently tethered biomolecules on materials containing zirconia is a topic of notable interest still to be investigated. Yttria-stabilized tetragonal zirconia (TZ-3Y-E) presents the best fracture toughness among oxide ceramics, optical properties (colour, translucency) that fit to the aesthetic dental standards and negligible wear debris.[35–37] Moreover, ceramic/metal composites (cermets) have been designed as a new generation of materials that pursue to combine synergistically the dissimilar properties of the monolithical components.[38,39] As novel cermets sintered by spark plasma sintering (SPS), ceramic/titanium composites 40,41 displayed tailored mechanical properties as well as a set of surface features that provided biocompatibility parallel or enhanced to the one of its pure counterparts.[42] In this work, we aimed biofunctionalizing zirconia (TZ-3Y-E) and a biocermet containing zirconia (zirconia/titanium composite with 75% vol. Ti) using silane chemistry. We based the biofunctionalization route in our already developed method for titanium surfaces.[24,30] First, two different methods of activation with different experimental conditions were investigated to optimize this very important step of the process. Then, we used (3-chloropropyl) triethoxysilane (CPTES) as covalent linker between the activated surface and the oligopeptide (KKKGGGGRGDS) containing the RGDS-sequence. This amino acid sequence is the most extensively studied cell adhesion motif to functionalize all types of biomaterials,[43,44] including those used in dentistry, such as dental implants.[45] Indeed, the literature is abundant of examples of substrates coated with RGD peptides, which facilitate the recruitment of cells involved in the formation of bone (osteoblasts), soft tissues (epithelial cells) or pulp regeneration.[46] This short cell-binding sequence is expressed in extracellular matrix proteins, such as fibrin, collagen, fibronectin, vitronectin, osteopontin and bone sialoprotein.[47] The cell adhesive properties of the RGD sequence rely on being recognized by integrins, the most prominent family of cell membrane receptors. Among them, osteoblasts mostly express $\alpha_5\beta_1$ and $\alpha_v\beta_3$.[48,49] In addition, our tailored peptide incorporates four glycines and three lysines in the N-terminus for providing spacing between the active RGDS-motif and the inorganic substrate to enable the correct accessibility of the RGD sequence to the integrins;[46] and extra groups with free amines to facilitate the nucleophilic reaction with the chlorine-group of the silane molecules, respectively. Thus, we used this bioactive coating as a model to validate our route of bio-immobilization by assessing the enhancement of cellular adhesion of murine pre-osteoblasts in vitro. Coated titanium surfaces were also tested as a reference material.

2. Materials and methods

2.1. *Materials preparation*

Tetragonal zirconia stabilized with 3% mol. yttria (TZ-3Y-E), zirconia/titanium (75% vol. Ti) composites and titanium (commercially pure, grade I) samples were obtained following our previous protocols[40] and named as ZrO_2, Z-75Ti and Ti, respectively. Briefly, stable suspensions were prepared by a wet-processing route of powders with an organic surfactant. After homogenization, drying and sieving, the starting powders were spark plasma sintered (SPS; FCT Systeme GMBH, HPD 25, Germany) at 1250 °C, 80 MPa for 5 min within vacuum, heating at 100 °C/min, to produce composites with high density avoiding side products. The specimens were machined to 2-mm thick square samples (5 mm × 5 mm), grinded with SiC discs and finally mirror-polished with 25.5 μm and 1 μm alumina suspensions. Samples were

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