Colloids and Surfaces B: Biointerfaces 136 (2015) 74–83

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Surface modification of zirconia with polydopamine to enhance fibroblast response and decrease bacterial activity *in vitro*: A potential technique for soft tissue engineering applications

Mingyue Liu[a], Jianfeng Zhou[b], Yang Yang[b], Miao Zheng[b], Jianjun Yang[c], Jianguo Tan[b,*]

[a] Outpatient Department, School and Hospital of Stomatology, Peking University, Beijing 100034, China
[b] Department of Prosthodontics, School and Hospital of Stomatology, Peking University, Beijing 100081, China
[c] Department of Oral and Maxillofacial Surgery, The Affiliated Hospital of Medical College, Qingdao University, Qingdao, Shandong, 266001, China

ARTICLE INFO

Article history:
Received 28 October 2014
Received in revised form 22 April 2015
Accepted 24 June 2015
Available online 30 June 2015

Key words:
Zirconia
Surface modification
Polydopamine coating
Implant interface
Cellular response
Bacterial adhesion

ABSTRACT

The quality of soft-tissue integration plays an important role in the short- and long-term success of dental implants. The aim of the present study was to provide a surface modification approach for zirconia implant abutment materials and to evaluate its influence on fibroblast behavior and oral bacteria adhesion, which are the two main factors influencing the quality of peri-implant soft-tissue seal. In this study, polydopamine (PDA)-coated zirconia was prepared and the surface characteristics were evaluated using scanning electron microscopy, atomic force microscopy, a contact-angle-measuring device, X-ray photoelectron spectroscopy, and Raman spectroscopy. The responses of human gingival fibroblasts (HGFs) to PDA-coated zirconia; i.e., adhesion, proliferation, morphology, protein synthesis, and gene expression, were analyzed. Additionally, the adhesion of *Streptococcus gordonii* and *Streptococcus mutans* to zirconia after PDA coating was assessed by scanning electron microscopy and live/dead staining. The material surface analyses suggested the successful coating of PDA onto the zirconia surface. The PDA coating significantly increased cell adhesion and proliferation compared with pristine zirconia. HGFs exhibited a high degree of spreading and secreted a high level of collagen type I on PDA-modified disks. Upregulation of integrin α_5, β_1, β_3 and fibronectin was noted in HGFs cultured on PDA-coated zirconia. The number of adherent bacteria decreased significantly on zirconia after PDA coating. In summary, our result suggest that PDA is able to modify the surface of zirconia, influence HGFs' behavior and reduce bacterial adhesion. Therefore, this surface modification approach holds great potential for improving soft-tissue integration around zirconia abutments in clinical application.

1. Introduction

The use of dental implants as an alternative to certain traditional rehabilitation methods for completely and partially edentulous patients has been widely applied and has achieved a remarkable success. The long-term success of dental implants in terms of form and function depends not only on the integration of the implant into the surrounding bone but also on the quality of soft tissue seal. Previously, most of the advances in dental implant research are about the optimization of osseointegration, but the research on the improvement of peri-implant soft-tissue reactions is relatively less. The soft-tissue seal structure, consisting of epithelium and connective tissue, has been histologically evaluated *in vivo* and is defined as the 'biological width' [1]. It protects the underlying bone and implant from bacterial penetration, prevents the loss of crestal bone, and maintains the normal shape of the gingiva [2]. In extensive investigations of soft tissue responses to oral implant surfaces, it has been shown that physical chemical properties of implant materials significantly influence the quality of soft tissue seal [3]. Therefore, the modification of implant abutment surfaces to promote early formation of enduring and effective soft-tissue barriers has been a focus of investigation.

The main cell type in peri-implant soft tissues is human gingival fibroblasts (HGFs) [4]. Fibroblasts synthesize and maintain the components of the extracellular matrix, are involved in the maintenance of connective tissue homeostasis, and are responsible for tissue repair and regeneration in the wound-healing process [5]. HGFs play an important role in establishing and maintaining the

* Corresponding author at: Department of Prosthodontics, School and Hospital of Stomatology, Peking University, 22# Zhongguancun South Street, Haidian District, Beijing 100081, China. Fax: +86 1082 195531.
E-mail address: tanwume@vip.sina.com (J. Tan).

http://dx.doi.org/10.1016/j.colsurfb.2015.06.047
0927-7765/

mucosal seal of implants [6]. Investigations have suggested that surface modification of implants significantly influences the behavior of fibroblasts – such as adhesion, proliferation, morphology, and differentiation – thereby influencing the reaction of the soft tissue on the implant surface [7–9].

Microbial infection is another main factor influencing peri-implant soft-tissue seal. During and immediately after surgery, bacterial colonization and subsequent biofilm formation occurs on implant surfaces [10]. Such infections are not easy to treat because bacteria are protected by the biofilms, leading to the destruction of adjacent tissue or even implant failure [11]. *Streptococcus* spp. are the predominant initial-colonizing bacteria; colonization by these microorganisms provides a surface suitable for later bacterial colonization [10]. The surface properties of the implant materials – such as roughness, surface free energy, and chemical characteristics – have a marked impact on bacterial accumulation [12]. Therefore, numerous clinical and *in vitro* studies have focused on reducing biofilm formation by modifying implant surfaces. Various surface treatment methods – such as dry ion implantation of F+ [13], deposition of silver ion and titanium (zirconium) nitride [14,15], coating of antibacterial polymers (e.g., silica, chitosan-lauric acid) [16,17] and peptides (e.g., silk, and a multilayered film containing an antimicrobial peptide) [18,19], and immobilization of antibiotics [20] – have been developed to improve the antibacterial properties of implant materials.

Currently, titanium is the most widely used material and has become the gold standard for both implant and abutment due to its excellent mechanical properties and biocompatibility. However, as an abutment, its dark grayish color limits its application in aesthetic zone [21]. Zirconia, introduced as an alternative to titanium, provides a better aesthetic outcome. In addition, it has demonstrated lower bacterial colonization and similar soft-tissue attachment to titanium, possibly providing favorable soft-tissue integration [22,23]. However, the surface of zirconia is bio-inert and difficult to modify. Numerous surface treatment methods to improve the bioactivity of titanium are available; however, such research for zirconia remains in its infancy. Several physical and chemical surface modification techniques have been developed to enhance the bioactivity of zirconia in terms of tissue reactions, such as optimizing the surface texture (sandblasting, acid-etching) [24], ultraviolet irradiation [25], laser application (e.g., Er:YAG, CO_2 or diode laser) [26,27], and coating with micro-arc oxidized zirconia films [28], calcium phosphate [29], or fluor-hydroxyapatite [30]. Among those approaches, the physical methods require specialized equipment and conditions, while bioactive coatings frequently demonstrate poor adhesion to the material and require complex procedures. In addition, most of the above surface modification methods are used to promote osseointegration; few studies have focused on soft-tissue integration. Therefore, there is an urgent need for a simple and effective surface modification method to enhance the bioactivity of zirconia in terms of soft-tissue integration.

3,4-dihydroxy-L-phenylalanine (L-DOPA) was found to be an important component in the adhesive structure of mussels [31]. Dopamine, precursor of L-DOPA, can self-polymerize and adhere strongly to a wide range of organic and inorganic materials. Coating can be achieved by simply dipping objects in an alkaline dopamine solution (e.g., pH 8.5) [32]. Furthermore, polydopamine (PDA) film, which is enriched in catechol and amino groups, provides a surface for secondary reactions via Michael addition or Schiff base chemistry to create multifunctional coatings. Although the molecular mechanism of the polymerization process has not been well documented, the PDA coating technique has been applied in various fields such as energy science, water treatment, sensing, and biomedical science [31]. Because coating does not require a complex procedure, and is solvent-free and non-toxic, it is particularly suitable for biomaterial application. PDA coatings on bio-inert surfaces facilitate protein adsorption and cell adhesion [33–35]. Moreover, PDA has also been employed to fabricate antimicrobial surfaces. Iqbal et al. [36] found that PDA itself has an antimicrobial effect on *Escherichia coli*. Recently, Liu et al. [37] first applied dopamine derivatives (L-DOPA) onto the zirconia surface to promote the osteogenesis of implants. Nevertheless, the influence of PDA coating on the soft-tissue integration of zirconia implants and oral bacterial colonization remains unexplored. In addition, dopamine performs better than L-DOPA in terms of increasing surface hydrophilicity, the index of which is critical for the regulation of cellular and bacterial behavior [38]. Therefore, dopamine coating holds greater potential in terms of improving tissue reactions around zirconia compared with L-DOPA.

The objective of the present study was to enhance the bioactivity of zirconia abutment materials using PDA modification technique. The effects of PDA coating on the behavior of HGFs and bacterial adhesion were investigated *in vitro*. In addition, specific cell adhesion- and differentiation-related genes expressions were detected at molecular level. We hypothesized that PDA can deposit onto zirconia surface and the coating would facilitate the adhesion, proliferation, and differentiation of HGFs and reduce bacterial adhesion.

2. Material and methods

2.1. Specimen preparation

Zirconia disks (Zenostar, Wieland Dental, Germany; 20-mm diameter, 2-mm-thick) were first obtained using a cutting machine. The crystallographic structure of zirconia was analyzed previously and the results suggested that it fitted the properties of zirconium yttrium oxide [25]. Zirconia disks were wet-grinded and polished to an 800-grit SiC abrasive paper. The specimens were successively ultrasonically cleaned using absolute ethanol and distilled water each for 20 min and then dried in the oven at 50 °C before surface treatment.

After polishing, PDA coating was performed as described previously [32]. In brief, the disks were immersed in a dopamine solution (2 mg/mL in 10 mM Tris-HCl, pH 8.5) and gently shaken for 24 h at room temperature. The PDA-coated disks were then rinsed extensively with distilled water to remove unattached dopamine molecules and then dried under a N_2 stream. Zirconia disks were immediately used after coating and denoted as PDA-zirconia. Untreated disks were used as controls. After surface preparation, all disks were sterilized with 75% ethanol for 40 min, washed three times in phosphate-buffered saline (PBS), and then placed into 24-well culture plates for cell and bacterial assays.

2.2. Surface characterization

2.2.1. Surface topography and surface roughness

Atomic force microscopy (AFM) (P9 XPM; NT-MDT, Russia) was used to examine the surface topography of the samples. Peak-to-valley surface roughness (Ra) measurements were taken from the roughness profile. The surface morphologies of the zirconia disks were also observed by scanning electron microscopy (SEM) (S-4800; Hitachi, Tokyo, Japan). Three samples from each group were visualized at five randomly chosen locations per sample.

2.2.2. Surface wettability

The surface wettability of the substrates was determined by the contact angle of a 1 μl double-distilled water droplet measured using a contact-angle-measuring device (SL200; USA Kino Indus-