Acta Biomaterialia 46 (2016) 323–335

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Direct silanization of zirconia for increased biointegration

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ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 22 September 2016
Accepted 24 September 2016
Available online 26 September 2016

Keywords:
Zirconia
Functionalization
Biomedical applications
Self-assembled monolayers
Surface modification

ABSTRACT

High-performance bioinert ceramics such as zirconia have been used for biomedical devices since the early seventies. In order to promote osseointegration, the historical solution has been to increase the specific surface of the implant through roughness. Nevertheless these treatments on ceramics may create defects at the surface, exposing the material to higher chances of early failure. In zirconia, such treatments may also affect the stability of the surface. More recently, the interest of improving osseointegration of implants has moved the research focus towards the actual chemistry of the surface. Inspired by this, we have adapted the current knowledge and techniques of silica functionalization and applied it to successfully introduce 3-aminopropyldimethylethoxy silane (APDMES) directly on the surface of zirconia (3Y-TZP). We used plasma of oxygen to clean the surface and promote hydroxylation of the surface to increase silane density. The samples were extensively characterized by means of X-ray photoelectron spectroscopy (XPS) and contact angle, mechanically tested and its cytotoxicity was evaluated through cell adhesion and proliferation tests. Additionally, aging was studied to discard negative effects of the treatment on the stability of the tetragonal phase. No adverse effect was found on the mechanical response of treated samples. In addition, plasma-treated samples exhibited an unexpectedly higher resistance to aging. Finally, silane density was 35% lower than the one reported in literature for silica. However cells displayed a qualitatively higher spreading in opposition to the rounder appearance of cells on untreated zirconia. These results lay the foundations for the next generation of zirconia implants with biologically friendlier surfaces.

Statement of Significance

The use of zirconia-based ceramics in biomedical devices is broad and well accepted, especially in dental implants. However, they do not bond naturally to bone, therefore to ensure fixation surgeons typically rely on roughness at different scales, or on cements. Alternatively in this work we present a new perspective of surface modification through chemistry to enhance the interaction between surface and biological environment, without the downsides of roughness. This surface treatment is proposed for zirconia, which allowed a direct silanization of its surface and a higher cell attachment. The results of this research may open the possibility for the next generation of bioinert ceramic implants with more advanced tailored surfaces for increased osseointegration.

1. Introduction

Among the medical materials, structural ceramics such as zirconia, are projected to be the largest and fastest-growing type of medical ceramics between 2015 and 2020, as the demand in end-use applications is growing rapidly [1]. Typical applications of these materials are dental and orthopedic implants. In these cases, materials are required to withstand high loads and fatigue, as well as resisting any degradative or corrosive attack by physiological fluids [2].

Zirconia materials are typically designed to exploit a specific reinforcing mechanism known as "transformation toughening". This process hinders crack propagation through the transformation of tetragonal grains to monoclinic, which is accompanied by a volume increase. Still, this process can also be triggered by the

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http://dx.doi.org/10.1016/j.actbio.2016.09.034

presence of water, which may degrade the material and compromise the integrity of the implant [3]. This phenomenon is usually referred to as ''hydrothermal aging'', ''Low Temperature Degradation (LTD)'' or simply ''aging''.

Furthermore zirconia materials are considered as 'biologically inert'. This means that when implanted, they (or their degradation products) do not initiate any adverse reaction to surrounding tissue. However, this feature also hampers osseointegration: since bone cannot naturally grow on their surface [4], other means of fixation are necessary, such as cements or metallic shells. One typical strategy to improve a direct fixation of ceramic to bone is to add roughness to the surface at different scales [5–7]. The main risks associated to this strategy are twofold: on the one hand it may decrease the mechanical properties of the implant [8–10] especially as far as ceramics are concerned, and on the other it may provide the perfect host substrate for bacterial adhesion and biofilm formation [11].

In order to increase osseointegration and favor a more natural way of fixation to bone, the implant surface could alternatively (or additionally) present a friendlier chemistry to better interact with cells and proteins. This approach has been explored in multiple works before. For example, it is known that the presence of OH on surface is effective for apatite nucleation *in vivo* [12]. Since the rate of hydroxylation on the surface of zirconia is generally low, some authors have studied surface hydroxylation of zirconia by immersion on different corrosive solvents [12–14]. In most of the cases, the OH rate at the surface was effectively increased, but the treatment etched the material at the same time, creating roughness and hence defects. Other authors have explored the use of hydroxyapatite coatings as a way of mimicking the chemistry of the bone [4,12,15], similarly to the hydroxyapatite coatings that have been used for years on metals. Unfortunately, results on humans have been often controversial [16–18].

Inspired by the exhibited potential of silanes as surface modifiers for biomedical devices to enhance cell adhesion [19–24], and following the works of Schickle et al. [25,26], where different silanes were successfully grafted on the surface of alumina via an intermediate silica coating, in this work we investigated the modification of the surface chemistry of zirconia through the direct grafting of 3-aminopropyldimethylethoxy silane (APDMES, amine-terminated silane), without silica coating. APDMES binds to the surface OH, and its expected binding process to zirconia is schematically represented in Fig. 1. The direct grafting of the silane to zirconia presents some advantages that make it worth to be explored. To begin with, it would reduce the number of steps in the production process and consequently the associated costs. In addition, the elimination of the intermediate silica layer would eliminate the dependence on the affinity between substrate and silica.

In literature, some authors have already explored the direct silanization of zirconia with other kind of applications in mind: as coupling agent for dental resin [27], or for polar sorbents for hydrophilic interaction chromatography [28]. The results obtained by these authors revealed some disadvantages of zirconia that may limit the success of such treatment, but we did not find enough evidence in previous literature to discourage its potential use to enhance cell interaction with implanted zirconia. For example, one of the main disadvantages of zirconia is the low density of surface OH, which would affect silane density on the surface. In this work O_2 plasma was therefore used to eliminate the carbon contamination and to promote hydroxylation of the surface without damaging it [29].

To summarize, we investigated a direct silanization of zirconia to form a monolayer on its surface [25,26,30–32]. The samples were chemically characterized, mechanically tested and their resistance to LTD was analyzed to check for changes related to the modification. Finally, the functionalized zirconia was evaluated for cytotoxicity and compared to untreated zirconia.

2. Materials and methods

Sintered discs of medical grade Yttria-stabilized Tetragonal Zirconia Polycrystal (Y-TZP) Nacera® Pearl were bought from DOCERAM (MOESCHTER Group, Dortmund, Germany) (Table 1) measuring ∅20 mm and 1 mm thickness. The samples were obtained by conventional ceramic technology (cold isostatic pressing, followed by sintering at 1550 °C for 2 h, to achieve >99% of theoretical density).

Four types of samples were prepared, as summarized in Table 2. All samples were first polished up to specular finish, with an average roughness (Sa) of 5.3 nm and a developed interfacial area ratio (Sdr) of 0.04%, measured by optical interferometry (Veeco Wyko 9300NT, Bruker, Billerica, USA). At this stage, a reference group (labeled ''NT'' for No Treatment) was taken out. The remaining samples were first ultrasonically cleaned in isopropanol, subjected to O_2 plasma cleaning and labeled as ''PC'' (Plasma Cleaned). Only the polished face of each sample was plasma-cleaned. We used a NGP 80 Reactive Ion Etching system (Oxford Instruments, Abingdon, UK) to generate the O_2 plasma of 100 W for 5 min, under

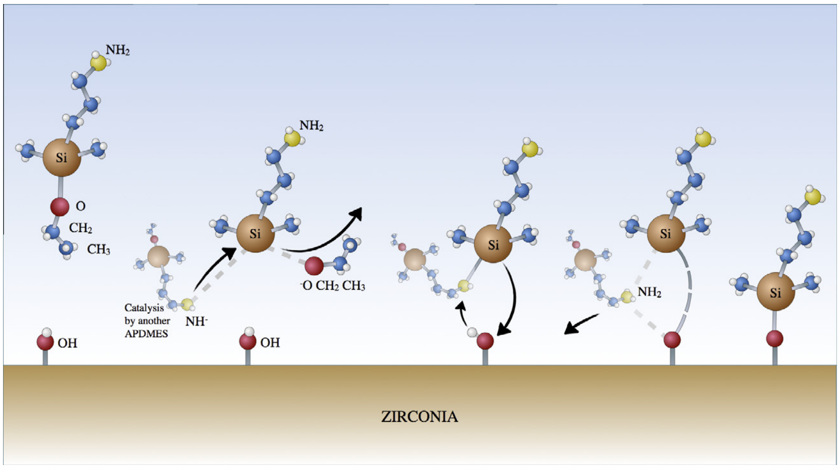

Fig. 1. Schematic representation of silanization.