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Review

Functionalization of titanium dioxide nanotubes with biomolecules for biomedical applications

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ABSTRACT

Titanium (Ti) and its alloys are extensively used in the manufacture of implants because they have biocompatibility. The production of a nanostructured surface can be achieved by means of titanium dioxide nanotubes (TNTs) which can have dimensions equivalent to the nanometric components of human bone, in addition to increasing the efficiency of such implants. The search is ongoing for ways to improve the performance of these TNTs in terms of their functionalization through coating these nanotubular matrices with biomolecules. The biocompatibility of the functionalized TNTs can be improved by promoting rapid osseointegration, by preventing the adhesion of bacteria on such surfaces and/or by promoting a more sustained local release of drugs that are loaded into such TNTs. In addition to the implants, these nanotubular matrices have been used in the manufacture of high-performance biosensors capable of immobilizing principally enzymes on their surfaces, which has possible use in disease diagnosis. The objective of this review is to show the main techniques of immobilization of biomolecules in TNTs, evidencing the most recent applications of bioactive molecules that have been functionalized in the nanotubular matrices for use in implants and biosensors. This surveillance also proposes a new class of biomolecules that can be used to functionalize these nanostructured surfaces, lectins.

1. Introduction

Titanium (Ti) and its alloys have been a raw material for the manufacture of biomaterials because of their biocompatibility and resistance to corrosion [1]. The passivation phenomenon contributes to these characteristics and makes Ti suitable for use in dental and orthopedic implants, without promoting adverse reactions locally or systemically. Importantly, this metal upon exposure to air or aqueous electrolytes forms a passive and stable layer of titanium dioxide (TiO_2) which can reach a thickness of 2–10 nm on its surface in 1 s, providing resistance to the release of metal ions [2,3]. TiO_2 can also be used for the construction of biosensors, because as a semiconductor it allows for the rapid transport of electrons from reactions on its surface to the Ti substrate, improving the performance of these important tools for the diagnosis of diseases [4].

According to the dimensions of the surface characteristics, the roughness of the surface of the implants can be of macro- (varies from millimeters to microns), micro- (1–10 μm) or nano- (1–100 nm) scales [5]. Nanoscale surface topography is preferred for implant making. The fact that bone tissue presents nanoscale structures, such as collagen, allows this nanotopography, with surface energy higher than the other texture scales, to improve the adhesion of matrix proteins, such as fibronectin and vitronectin, and to stimulate cellular migration and proliferation, important steps in the process of osseointegration, *i.e.* the formation of bone around the implant [6,7]. Functionalized and modified nanostructured devices for biomedical applications have become increasingly investigated in the hybrid science field named nanobiotechnology [8]. The metallic, ceramic, polymeric and composite nanomaterial properties may be integrated with biomolecules to promote combined and synergistic effects from the hybrid systems, such as biomolecule-nanoparticles [8,9]. The hybrid nanomaterials combining inorganic and organic or even bioactive components into a single material are promising for using in biomedicine, for example, the organic-inorganic hybrid hydrogel, polymers and the magnetic Janus particles that have physical properties on their two or more dissimilar faces for drug delivery [10–12]. Inorganic/organic hybrid materials can be obtained by atomic layer deposition to promote the modification of polymer surfaces [13].

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Drugs may be delivered with release in a target site of the organism, even at the cellular level for delivery of genes into cells using nanocarriers, such as inorganic nanoparticles and quantum dots, for example, carbon dots developed with glucose and polyethyleneimine [14–16]. Specific nanomaterials to use as vehicles depending of the characteristics of the therapeutic biomolecule; for that the delivery and expression of optogene used in the optogenetics may to stimulate or inhibit the neural activity should be taken into account the optical, electrical, thermal properties and proper bio-functionalization of nanomaterials [17]. Upconversion nanoparticles, luminescent materials that can absorb near-infrared light and emit UV–visible light, covalently conjugated with a photosensitizer has been a highly specific and targeted treatment option in the photodynamic therapy studies [18]. Gold nanostructures can be synthesized with size controlled using reduction of copper; the properties of nanostructures depend on their morphology and are widely used in biomedicine, such as drug delivery and biosensing systems [19,20]. Proteins and peptides from the eggshells participate of the nucleation of calcium carbonate crystals and play an important role in the eggshell biomineralization; such biomolecules can be used in the future with TiO_2 nanostructured for manufacturing of implants [21,22].

TiO_2 nanotubes (TNTs) are tubular and self-organized nanostructures that have attracted considerable attention in implant manufacturing because of their mechanical stability, low cost of preparation and better biocompatibility compared to TiO_2 film [23–25]. TNTs are able to form well-defined nanostructured platforms with favorable transport pathways, good adhesion to the substrate and high surface area, *i.e.*, having a large number of atoms on their surface, available to interact with many biomolecules and allowing their use as an electrode in the manufacture of biosensors [26]. TNTs can be obtained in large quantities by various synthesis techniques, such as the sol-gel method, hydrothermal treatment and electrochemically by anodization [27]. Anodic oxidation (or anodization) is a simple and versatile technique that synthesizes TNTs with controlled structure and morphology, being aligned perpendicularly and easily to the Ti substrate [28,29].

Therapeutic failure in the use of implants may occur due to insufficient bone formation in the tissue surrounding the biomaterial, as ineffective bone fixation may lead to bacterial infection [30]. Research has been carried out with the aim of improving the functionality of these implants made of TNTs, for example, by the immobilization of biomolecules on the surface of these nanotubular matrices. The administration of growth factors such as bone morphogenetic protein 2 (BMP2) in TNT implants may improve their osteoinductive capacity [31,32]. TNTs can be used as matrices for the immobilization of proteins and enzymes for use in biosensors, such as the enzyme glucose oxidase (GOx) on the surface of TNTs for the preparation of an enzymatic biosensor capable of detecting glucose [33].

The objective of this review is to address some techniques used for the immobilization of biomolecules in TNTs, since its synthesis, to make the applicability of these functionalized TNTs in biomedicine more efficiently, either as implants or as biosensors.

2. Immobilization of biomolecules in TNTs

The growth of TNTs by electrochemical anodization occurs in aqueous electrolytes with fluoride ions and organic electrolytes such as glycerol or ethylene glycol. This method is based on an oxidation-reduction reaction that occurs in an electrochemical cell in which titanium is used as the anode and at the cathode is an inert material such as platinum [29,34]. An electrical potential from a power source is applied in the process to promote an electrical field and thus the diffusion of oxygen ions present in the electrolyte to form an oxide layer on the surface of the anode [35].

Fig. 1 shows the scanning electron microscope (SEM) image of TNTs at a magnification of 100,000 × (Fig. 1a), showing an ordered layer of the nanotubes obtained by the anodization process. Fig. 1b is at 7000 × and bacterial *Staphylococcus aureus* cells can be seen forming clusters on the surface of the TNTs.

Fig. 2 presents a schematic showing the formation of TNTs and their functionalization with biomolecules. The reaction that occurs on the anode describes the growth of oxide on the surface of Ti (Fig. 2a), in which the oxidized species of the metal react with the O^{2-} ions, provided by the water molecules, to form the TiO_2 layer (Fig. 2b). The fluoride ions, present in the electrolyte, have the ability to form $[TiF_6]^{2-}$ complexes, which are soluble in water and promote a chemical attack, that is, the dissolution of the TiO_2 formed. As soon as the oxide layer is obtained, there is decay of the current applied in the anodization and then the nanopores begin to grow on the surface of the metal (Fig. 2c). The equilibrium state is reached when the growth rate of the nanopores at the oxide-metal interface is the same as the rate of dissolution of the oxide, allowing continuous growth of the nanotubes (Fig. 2d) on the Ti surface [36]. Depending on the application, the TNTs can undergo a heat treatment to convert their amorphous structure into nanocrystalline structures such as anatase and rutile [34].

The reaction that leads to the synthesis of TNTs in Ti (a) begins with the formation of the TiO_2 layer on the metal (b); then this oxide undergoes dissolution by fluoride ions that leads to the appearance of nanopores (c); these nanopores become deeper and deeper until they form an orderly and compact layer of TNTs (d). Nanotubes can still have their functionality enhanced by the immobilization of biomolecules by loading and/or coating them (e).

The biomolecules can be immobilized in the nanotubular matrices by coating the surface of the TNTs and/or by loading them (Fig. 2e). The adsorption of biomolecules in TNTs can occur by physical methods such as hydrophobic interactions, hydrogen bonds and electrostatic interactions, or by chemical methods such as the covalent attachment with formation of ether, amide and thioether linkages, for example.

The sol-gel coating methods have been classified into two types: dip coating and spin coating [37]. These techniques are being applied to promote the coating of TNTs with biomolecules, such as the coating of these nanotubular matrices with chitosan biopolymer [38,39]. Spin coating is based on the application of a solution on a rotating substrate with subsequent ejection and evaporation of the solvent. Dip coating consists of immersing a substrate in a solution followed by gravitational drainage and evaporation of the solvent. Both methods allow the formation of a homogeneous film on the surface of the substrate [40].

Spin coating and dip coating can also be used for Layer-by-Layer (LbL) assembly, which is a technique capable of forming Polyelectrolyte Multilayers (PEMs) by adsorption of oppositely charged polyelectrolytes attracting by electrostatic interaction, on the surface of a given substrate [41]. TNTs could be coated by LbL using the polysaccharides chitosan and sodium hyaluronate, with positive and negative charges, respectively [42].

Spin coating is a fast method to obtain films homogenous with thickness easily changed mainly by changing spin speed, or the viscosity of the solution to be deposited [40,43]. A limiting factor to the spin coating technique is the substrate size because large substrates cannot be spun at a sufficiently high rate in order for formation thin film [43]. Besides, dip coating does not need sophisticated apparatus characterizing a low cost solution deposition; a thin film of solution onto a plate, cylinder, or irregular shaped substrate, that is, without defined geometry, is a distinguishing feature of this technique [44–46]. There are those who consider one drawback the fact that in the dip coating process occurs the film formation in both sides of the substrate [45]. However, this factor may be favorable for the immobilization of biomolecules on the TNTs, since in certain anodization conditions the TNTs formation occurs in both sides of the Ti foil, so, unlike spin coating that promotes the immobilization on a single side, the dip coating may to allow the functionalization in the two sides of the anodized Ti foil, that is, more functionalized biomolecules on the TNTs may be obtained.

Biomolecules are also covalently immobilized on TNTs. BMP2 can