Titanium Base Substrates

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Titanium base alloys are state-of-the-art implant materials in various surgery applications, including dentistry, bone and craniofacial reconstruction/fixation, and temporary anchorage devices (TAD), etc. Titanium is a lightweight and bioinert metal that possesses a high specific strength (strength to density ratio) and an elastic modulus close to that of human bone. Titanium additionally features excellent corrosion resistance and relatively high X-ray translucency, thus facilitating post-treatment diagnosis.

Keywords: zwitterionic polymer coating ; photopolymerization ; antibiofouling ; cytotoxicity ; titanium substrate

1. Overview

Biofouling and biofilm formation on implant surfaces are serious issues that more than often lead to inflammatory reactions and the necessity of lengthy post-operation treatments or the removal of the implant, thus entailing a protracted healing process. This issue may be tackled with a biocompatible polymeric coating that at the same time prevents biofouling. In this work, oxygen plasma-activated silanized titanium substrates are coated with poly(sulfobetaine methacrylate), a zwitterionic antibiofouling polymer, using photopolymerization. The characterization of polymer films includes FT-IR, AFM, and adhesion strength measurements, where adhesion strength is analyzed using a cylindrical flat punch indenter and water contact angle (WCA) measurements. Both cytotoxicity analysis with primary human fibroblasts and fluorescence microscopy with fibroblasts and plaque bacteria are also performed is this work, with each procedure including seeding on coated and control surfaces. The film morphology obtained by the AFM shows a fine structure akin to nanoropes. The coatings can resist ultrasonic and sterilization treatments. The adhesion strength properties substantially increase when the films are soaked in 0.51 M of NaCl prior to testing when compared to deionized water. The coatings are superhydrophilic with a WCA of 10° that increases to 15° after dry aging. The viability of fibroblasts in the presence of coated substrates is comparable to that of bare titanium. When in direct contact with fibroblasts or bacteria, marginal adhesion for both species occurs on coating imperfections. Because photopolymerization can easily be adapted to surface patterning, smart devices that promote both osseointegration (in non-coated areas) and prevent cell overgrowth and biofilm formation (in coated areas) demonstrate practical potential.

2. Titanium Base Alloys

Antibiofouling coatings based on polymer brushes are of paramount importance in various biomedical and biotechnological applications. They afford an environmentally benign and sustainable way to preventing the adhesion of proteins and different cell types [1][2][3]. Among the plethora of materials available, zwitterionic brushes are the most promising as most of them combine biocompatibility with protein and cell repellent properties. Furthermore, they can be used with various substrates, including metals, ceramics, and polymers, using well-known grafting techniques [3][4][5][6][7][8]. The mechanisms governing the antibiofouling properties of these polymers have been amply discussed in the relevant literature [3][5][9]. It appears that their high hydrophilicity leads to a watery surface (a hydrated layer that has been proven to be non-structured, i.e., the water hydrogen bonds are not perturbed) that prevents adsorption as no free energy is gained by the adsorption of the protein on the watery surface. Furthermore, steric effects and surface neutrality that precludes ion exchange also seem to relate to the main factors inhibiting protein adsorption [3].

Titanium base alloys are state-of-the-art implant materials in various surgery applications, including dentistry, bone and craniofacial reconstruction/fixation [10][11], and temporary anchorage devices (TAD), etc. Titanium is a lightweight and bioinert metal that possesses a high specific strength (strength to density ratio) and an elastic modulus close to that of human bone. Titanium additionally features excellent corrosion resistance and relatively high X-ray translucency, thus facilitating post-treatment diagnosis [10][12]. Titanium is known to promote osseointegration, which is desirable in most cases of dental and orthopedic reconstructive surgeries. Nevertheless, implant removal is indicated in case of complications, including “infections, non-union, failure of fixation, pain after fracture consolidation, etc.” [13]. The literature also contains reports on complications related to TADs, such as periimplantitis, inflammations, and cell overgrowth [14]. A surface treatment that could prevent bacteria and cell adhesion while maintaining biocompatibility is consequently highly desirable in many cases. Various strategies have been devised to endow biomaterial surfaces with anti-biofouling properties [3][4][5][6][7][15][16][17][18]. Zwitterionic polymer films appear to be among the most promising strategies as they fulfill the required functionality with simultaneous proven biocompatibility [3]. In recent works [19][20], it has been shown that polySBMA (poly(sulfobetaine methacrylate)) can be successfully grafted from/onto the pore walls of porous aluminum and titanium oxide films (obtained via anodization of the metal) using a versatile and environmentally friendly photografting method. The polySBMA films were shown to wet the pore walls, leading to the creation of a 3D nanocomposite that was mechanically resistant and particularly effective at repelling proteins and bacteria. Furthermore, the same authors demonstrated that it is possible to structure the surface of the substrate in neighboring areas in order to achieve high protein adhesion (non-coated with polySBMA) and high protein repellant (coated with polySBMA) effects. As such, the authors demonstrated that it is possible to endow a particular implant with areas of high cell adhesion where cell adhesion is desirable, e.g., for osseointegration, and areas with poor or even no cell adhesion where such functionality is required.

3. Conclusions

Biofilms are known to form on numerous surfaces, with sometimes deleterious and sometimes beneficial effects, depending on the microbial composition [21][22]. The microbiome of the oral cavity is a good example for this issue, where a balanced environment favors symbiosis and oral health, whereas an imbalance creates a dysbiotic state with destructive/corrosive outcomes in regard to biological tissues [23][24]. Far-reaching consequences may be encountered in the context of dental surgery, where a rapidly growing dysbiotic biofilm covering an oral implant surface impairs the surrounding tissue, often resulting in peri-implant diseases [11][25]. Microbial growth on implants is thus of great concern, since most of the widely used implant materials, such as titanium and its alloys, meet the physical, chemical, and tissue biocompatibility properties well but do not prevent microbial fouling [26][27][28][29][30]. To address these issues, various strategies (often inspired by nature) have been suggested [31][32][33][34][35]. Essentially, the main approaches rely on (1) destroying the “intruder” or (2) preventing adhesion. The first approach mainly relies on leachable microbicides, such as silver nanoparticles [36][37][38], antibiotics, anti-microbial peptides [39], nitric oxides, and others [40][41], by way of temporarily impregnating the implant surface. Surface coatings with stable polymer coatings, in some cases together with nano- or micro-patterning, constitute the second approach [19][42][43][44][45]. Moreover, the topographical effects on biofilm formation have been also investigated, and limited favorable effects of roughened and chemically non-modified surfaces have been reported for biofilm formation in comparison to smooth surfaces [29][46].

Surface modification with functional coatings affords a great variety of choices. The resulting coatings can be classified with regard to their chemical nature and physicochemical properties, e.g., hydrophobic, surface hydration, or amphiphilic properties, as well as in terms of their mechanism of action. A great deal of work has been carried out with surface-hydrating coatings, aiming to create surfaces with low interfacial energy with water, which are sometimes called “inert” surfaces [47]. Among others, oligo-, poly(ethylene glycol)-, and zwitterionic acrylates come into focus. Their hydrophilicity, expressed in very low water contact angles, has often been cited and linked to measurable antifouling effects; however, hydrophilicity is not equivalent to antifouling as many hydrophilic surfaces exhibit no antifouling properties, as is the case for glass. What seems to drive the development of non-adhesion properties in the case of hydrophilic surfaces (including superhydrophilic surfaces) is the interaction mode of water molecules at the interface. The greater the H-bonding structure of the interfacial water film resembles that of bulk water, the greater the energetic state of this film favors antifouling, thus preventing the replacement of interfacial water molecules with fouling species [3][7]. This latter point seems to constitute the difference between zwitterionic- and ethylene glycol-based coatings [3][7]. Recent computational studies have confirmed the stronger interaction of zwitterionic polymers with water and consequently better antifouling properties [48]. A secondary outcome with a different interfacial water structure between coating classes includes higher resistance of zwitterionic surfaces towards increasing salt concentrations [3][7][49][50].

Zwitterionic polymer films (2D) and coatings (3D) may be used with various material surfaces and nanoparticles using well-established protocols [2][3][6]. Among such processes, photopolymerization has only received limited interest, despite the fact that it is a relatively simple coating method, commonly implying often short processing times and only requiring a few precursors, e.g., a monomer, initiator, and appropriate solvent [19][51]. As outlined above (see the experimental section), a simple photopolymerization process was developed in this work for the coating of titanium substrates with polySBMA, but this process can be extended to other substrates [19][20]. SBMA was polymerized into a bio-, and hemocompatible polysulfobetaine in the presence of PPD, an initiator substance of certified food grade quality, with water as solvent, in order to minimize cytotoxicity and environmental impacts. A short processing time of a few minutes under mild UV radiation (360 nm) makes the scale-up of the coating process easy. On mildly activated as-received TiCP, the polySBMA coating demonstrated stable hydrophilicity over at least 5 days of dry storage. Furthermore, treatment in an ultrasonic bath for over 3 min did not result in excessive damage of the coatings, and nano-indentation studies revealed good adhesion properties, even at high salt concentrations, which is in good agreement with previous reports regarding the salt stability of zwitterionic coatings [7]. These properties are to be traced on the one hand to the strong bonding between the primed substrate and the coating, and, on the other hand, to the particularly tethered morphology of the nanostructured coating.

Emphasis in this work has been placed on the interactions of the processed polySBMA coatings with dental plaque microbes and hgF. The non-cytotoxic nature of the coating was demonstrated using XTT-testing and bright field microscopy with hgF in direct and indirect contact with the coated samples. There were no significant differences between the behaviors of the coated TiCP samples in comparison to bare TiCP samples. These results confirm the expectations with regards to the choice of non-toxic precursors well and attest to the efficiency of the polymerization method to achieve quasi precursor-free coatings.

The antifouling investigations were conducted using dental plaque and hgf. What makes dental plaque interesting is that it contains a great number of adherent species. The use of hgF was based on the fact that these cells are easily cultured and are characterized by adherent growth on various substrates and playing prominent roles in the overgrowth of devices such as TADs. The assessment of the interaction of polySBMA films with dental plaque microbes revealed a considerable reduction in biofilm formation on the coated surfaces. A similar observation was made for hgF, where adherent fibroblast cultures on coated surfaces were solely observed in cases of isolated coating damage, such as scratches and non-coated sharp sample edges, etc. Bearing in mind the demonstrated cytocompatibility, these findings can then be explained in terms of the existence of an “energy barrier” [3] that rules out the displacement of water molecules out of the hydrating layer by adherent species (e.g., proteins, microbes, and fibroblasts). The polySBMA coating on an implantable material thus impeded microbial growth and biofilm formation and can be qualified as non-cytotoxic, and, at the same time, precludes the adhesion of fibroblasts. These properties denote the attributes of a cytocompatible anti-biofouling coating. At first glance, this overall assessment may raise questions as to the use of such coatings in implantable devices. There is no doubt regarding the benefits of the antifouling properties described here, but the anti-adhesion effect on anchorage-dependent cell cultures may be a concern. Still, in cases where a high degree of tissue integration is not intended, as is the case for TAD and traumatology implants, polySBMA coatings may be valuable in terms of biofilm reduction and the tunable cell adhesion and proliferation options. This issue may be illustrated on miniature screws as orthodontic TADs. Such a device may be divided in three main parts, namely, the (1) head coupled to the active orthodontic appliance and facing the oral cavity, (2) gingival collar designed to promote tight gingival contact, and finally (3) the thread that ensures endosteal insertion. In some cases, inflammation, mucosal overgrowth, and infections related to the gingival insertion part may occur and delay a successful treatment [14][52][53]. In this case, a biocompatible antifouling coating of the gingival collar that at the same time excludes cell adhesion/overgrowth might be useful. Furthermore, photopolymerization allows for a patterned coating process which enables one to leave areas of sample parts uncoated, for instance, in areas where tissue integration is needed (as for the thread), and coating in other areas, i.e., where antifouling and tight fitting to tissue are required. Ongoing work is devoted to the demonstration of the usefulness of such an approach and will be published in an upcoming report.

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