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Multifunctional polymer coatings for titanium implants

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ABSTRACT

The aim of this work was to modify the surface of the titanium implants by application of multifunctional polymer coatings based on polyurethane and its composites with graphene and β-TCP. Graphene was used as an antibacterial agent, TCP as a bioactive component, and polymer coating as a corrosion protection of metal. As a result, materials with different surface characteristic, from hydrophilic to hydrophobic, varying in bioactivity and biocompatibility, were obtained. Wettability of the materials was tested by the sessile drop method; surface roughness was assessed on the basis of R_a parameter, measured by contact profilometry. The surface characteristic was complemented by microhardness testing. Also, in vitro immersion tests in fluids and cell tests were performed. Obtained results suggest that it is possible to fabricate, on the surface of titanium implants, multifunctional composite coatings based on polyurethane, with optimal composition for bone surgery and dentistry applications. The study further showed that the chemical structure (composition) of the polymer and the graphene content are crucial in terms of biocompatibility of the final material, while addition of tricalcium phosphate affects its bioactivity.

1. Introduction

Surface of the implant plays a key role in a biological response of the organism to the implant. It is the surface that initially interacts with physiological fluids, cells and surrounding tissues. One of the first phenomena occurring after implantation is so called 'race for the surface' [1]. Host cells and bacteria compete to colonize as large implant surface as possible. The result of this competition decides upon success of the implantation. In case of the bone implants, bacterial infections are especially dangerous due to the difficulties in maintaining, within bone tissue, effective concentration of the drugs administrated traditionally. Infections often lead to local bone resorption, that further cause implant loosening and the need for removal surgery [2,3]. One of the solutions that allows minimizing the risk of infection-related implant loss is the application of antibacterial coatings. Polymer brushes consisting of layer of oriented polymer chains tethered to the surface are a good example [4]. In most cases, effectiveness of the polymer brushes depends on the bacteria strain and the polymer type. Sometimes, bacteria adhesion might be hindered, but the colonization itself is not fully prevented. Polymer brushes can deteriorate adhesion of bone cells. Moreover, application of pure polymer brushes does not affect remote bacterial cells, which can migrate and colonize beyond the reach of the coating.

Application of antibacterial coatings that not only kill bacteria adhering to the surface, but also release agents preventing the surface from recolonization (reinfection) is a much more effective solution. Silver is one of the most well-known antibacterial agents. It is used both in its ionic form, and as nanoparticles, however it is extremely difficult to find the silver's so-called therapeutic window, i.e. the amount that expresses antibacterial activity without being cytotoxic [5,6]. Hence, the presence of silver within the implant often negatively affects both bacteria, and host cells. Among biopolymers, chitosan is considered an alternative for silver. Owing to its antibacterial properties, it has been successfully applied for polymer and composite coatings. However, adhesion and durability of the chitosan coating might raise some concerns [7].

Also nanoparticles based on carbon allotropes, including graphene and its family, are tested in terms of antibacterial activity. Graphene, being two-dimensional, single layer carbon hexagonal lattice with sp^2 orbital hybridization, can have different forms [8]. Graphene family materials (GFM) consist of two-dimensional carbon nanoforms based on graphene sheet. This includes pristine graphene (pG), few-layer graphene (FLG), graphene nanosheets (GNS), graphene oxide (GO), and reduced graphene oxide (rGO). Chemical structure and morphology of the GFM nanomaterials substantially affect their properties and interaction with biomolecules, therefore their activity against

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microorganisms. What's more, GFM materials can vary in number of layers, dimensions, and purity – all those parameters are essential in biological applications [9]. Despite good antibacterial properties, biological applications of GFM materials to be successful require further enhancement of the GFMs biocompatibility. That is why, composite systems based on biocompatible polymers modified with antibacterial forms of graphene are often used. It was proven that the GFM toxicity strongly depends on tested material – number of layers, surface characteristic, presence of functional groups, wettability, but also presence of polymer, time of incubation, and the type of cells used. Literature study [10–12] shows that in general, GO is less toxic than pG and rGO, and that fabrication of composites with biopolymers increases biocompatibility of the carbon nanoforms.

Antibacterial activity of the GFM materials can result from few different mechanisms [8]:

- destruction of delicate cell membranes as result of the direct contact with the sharp edges of the nanoparticles;
- oxidative stress in contact with GO and rGO;
- destructive extraction of phospholipids from bacterial membrane.

Another very important feature of the bone implant surface is bioactivity, i.e. ability to form strong, chemical bond with bone tissue. Bioactive components used in implants are mostly calcium phosphates (e.g. hydroxyapatite – HAp, tricalcium phosphate – TCP) and bioglasses; but also among polymers it is possible to find ones that can improve biological activity of the implant [13,14].

Previously mentioned chitosan or polyurethanes can be an example. Polyurethanes are used in medicine also due to favourable mechanical properties, high biocompatibility and low trombogenecity [15].

In this work, we propose to combine favourable properties of polyurethane, TCP and graphene in new composite materials intended for use as multifunctional coatings of metallic implants. The presence of polymer coating increases corrosion resistance of metal, incorporation of graphene gives antibacterial activity, while addition of bioactive ceramic particles can improve osteointegration. Thereby, application of such modified implants can improve durability of bone tissue binding and post-implantation safety. We decided to use two extreme graphene concentrations to check physicochemical properties, biocompatibility and cell proliferation. Even as low graphene content as 0.25% may influence properties of the coating. On the other hand, graphene concentration at the level 4% was the upper limit, at which we could obtain proper graphene dispersion in substrates before polyurethane synthesis. In the next research step, a detailed analysis will be performed on the most promising systems.

2. Materials and methods

2.1. Materials

Commercially available pure titanium Grade 2 plates (10 × 15 mm) and disks (ϕ - 10 mm) (Torresin Titanio SRL, Limena, Italy) were used in this study. The samples were cleaned with 70% ethanol and distilled water. Titanium plates were modified by double acid etching in a mixture of hydrofluoric acid, nitric acid and water 4:7:60 (POCH, Poland) for 5 min. Then, samples were immersed in a 10 M solution of sodium hydroxide (Avantor Performance Materials Poland S.A., Gliwice, Poland) for 24 h at 60 °C to develop a nanometric surface topography; improve adhesion of applied layers and implant osteointegration (Control Group Ti).

2.2. Surface modification

For titanium coatings, polyurethanes (PU) produced by one-step polymerization technique, whose synthesis was described previously [16], were used. The following reagents were used in stoichiometric amounts to obtain PU coatings:

- poly(ethylene glycol) (PEG) with mass-average molar mass 8000 – used as a soft segment, dried under reduced pressure in 110 °C for 2 h (Sigma-Aldrich);
- 4,4′-diisocyanate diphenylmethane (MDI) (Sigma-Aldrich);
- 1,4-butanediol (BDO) – chain extender (Sigma-Aldrich).

The concentration of graphene (Graphene Supermarket, USA) in polyurethane coatings was 0.25 wt% or 4 wt%. A known amount of graphene was introduced to melted PEG at 65 °C and homogenized by sonication. Then, in the case of polyurethanes with chain extender, BDO was introduced. Previously melted MDI was added to PEG + G or PEG + G + BDO system and the whole reaction mixture was mixed thoroughly. Next, the reaction mixtures were cast into pre-heated glass Petri dishes lined with aluminium foil, vacuum degassed, and heated for 2 h in 100 °C for actual polyaddition reaction to take place, and then for another 8 h in 80 °C [17].

In order to fabricate composites with TCP (Sigma-Aldrich), the polyurethane systems described above were dissolved in *N*,*N*-dimethyl formamide (DMF, POCH) in a ratio of 1 g of polymer per 10 mL of DMF. Next, 2 wt% of TCP was added to the PU solutions and homogenized.

The coatings were deposited by dip coating method with the samples being withdrawn from the PU solution at a constant speed of 50 mm/min. The polyurethane solution with graphene and TCP addition were homogenized with a sonicator (Vibra-Cell, Sonics) prior to dip coating to prevent agglomeration and sedimentation of the nanoparticles.

Twelve (12) types of coatings were obtained. Table 1 summarizes samples labelling and description.

2.3. Methods

2.3.1. Microscopic observation (optical microscope, SEM)

Titanium substrates with deposited coatings were observed using a digital microscope (VHX 5000, Keyence) and scanning electron microscope (NOVA NANO SEM 200). The observations were repeated after incubation of the samples in distilled water and simulated body fluid.

2.3.2. Surface roughness and wettability

The surface roughness (R_a) was measured using a surface profilometer (Tester T 4000, Hommelwerke GmbH, VS-Schwenningen, Germany). The surface wettability was measured by a sessile drop method using 0.20 µL liquid droplets (DSA10; KRÜSS GmbH, Germany). The results were expressed as mean values (n = 5 and n = 10, respectively) ± standard deviations (SDs).

2.3.3. Microhardness

The microhardness of the deposited coatings was determined using Vickers microhardness tester (Leco LM700at, USA), with a dwelling time of 10 s (250 mN). To determine the influence of simulated body fluid on the coating hardness, the measurements were performed again

Table 1
Obtained samples and their labelling.

	Primary solution	*Solution with TCP*
PU without BDO	Ti PU	Ti PUT
	Ti PU0.25	Ti PU0.25T
	Ti PU4	Ti PU4T
PU with BDO	Ti PUz	Ti PUzT
	Ti PUz0.25	Ti PUz0.25T
	Ti PUz4	Ti PUz4T

Ti – titanium plate; PU – polyurethane; z – polymer with chain extender; 0.25, 4 – wt% of graphene; T – addition of 2 wt% of tricalcium phosphate.