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Hybrid organic materials for modeling the biocompatibility of metal implants

Гибридные органические материалы для моделирования биосовместимости металлических имплантатов

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ABSTRACT

High biocompatibility of metal implants can be achieved by creating the organic layers that should have a high affinity to the metal surface, be non-toxic, prevent the adsorption of non-specific proteins, and also contain various ligands that improve adhesion, proliferation, and differentiation of cells, exhibit anti-inflammatory and antimicrobial properties. Modern trends in the development of such coatings imply the design of relatively complex multifunctional structures. The report represents the results on the synthesis of bisphosphonic acid derivatives, including natural polysaccharides and RGD-oligopeptide. The compounds were applied for the Ti surface modified by plasma electrolytic oxidation (PEO). In vitro studies of cell adhesion and proliferation showed that this approach is promising for effective modeling of the biological properties of the surface of metal implants.

KEYWORDS

Bisphosphonates; oligopeptides; hyaluronic acid; titanium implants; antimicrobial coating.

АННОТАЦИЯ

Высокая биосовместимость металлических имплантатов может быть достигнута путем создания органических покрытий, которые должны иметь высокое сродство к поверхности металла, быть нетоксичными, предотвращать адсорбцию неспецифических белков, а также содержать различные лиганды, улучшающие адгезию, пролиферацию и дифференцировку клеток, проявлять противовоспалительные и антимикробные свойства. Современные тенденции создания таких покрытий предполагают дизайн и синтез относительно сложных многофункциональных молекул. Представлены результаты по синтезу производных бисфосфоновой кислоты на основе природных полисахаридов и RGD-олигопептида. Полученные соединения были нанесены на поверхность Ti, модифицированной методом плазменно-электролитического окисления (ПЭО). Исследования адгезии и пролиферации клеток *in vitro* показали, что разработанный подход перспективен для эффективного моделирования биологических свойств поверхности металлических имплантатов.

КЛЮЧЕВЫЕ СЛОВА

Бисфосфонаты; олигопептиды; гиалуроновая кислота; титановые имплантаты; антимикробное покрытие.

Introduction

Currently, the development of metal implant coatings with high biocompatibility remains one of the urgent problems of implantology. In modern medicine, titanium and its alloys are most in demand, because these materials are nontoxic, possess high corrosion resistance and excellent mechanical strength [1]. Modeling the surface properties of titanium implants by the changes in the topography, chemical composition, and, consequently, biological properties of the surface can be achieved through the creation of both inorganic and organic coatings. These approaches are intended to ensure both biomechanical and biochemical compatibility of implants, their corrosion resistance, and, therefore, to launch the processes of osseointegration of metal devices into bone tissue. The bioactivity of inorganic coatings can be significantly improved and adjusted with an organic matrix that can mask the implant or contain functional fragments that actively interact with cells [2]. First, the surface can be treated in such a way to prevent the formation of a nonspecific protein layer to make the device "invisible" to the cells of a body (or antifouling coatings). Polysaccharides, self-organizing monolayers, polyethylene glycols, polyacrylates, etc. are used to form such surfaces. Second, the compounds that would provide appropriate signaling between the implant surface and the receptors on the cell surface (proteins, protein fragments, growth factors, etc.) can be applied. An approach implying the combination of antifouling and biologically active layers seems to be the most promising because the coating will improve the quality of implants due to a decrease or completely blocking the nonspecific adsorption of proteins and the formation of specific binding sites for certain types of cells. The degree of bonding of the organic matrix to the surface can be increased with anchors. Catechols, thiols, siloxanes, phosphonates, and any other functional fragments and groups are used for these purposes. Among them, phosphonate groups have a high affinity to metal ions and their oxides, and they do not undergo a hydrolytic cleavage in contrast to siloxanes [3]. The incorporation of a gem-bisphosphonic group into the molecules increases their affinity for oxidized surfaces in comparison with monophosphonates and increases their solubility in water through the change in polarity of a molecule [4].

The aim of the work is the development of new multifunctional organic coatings based on bisphosphonic acid derivatives, including natural polysaccharides and oligopeptides. The resulting coatings exhibit high adhesion to the metal surface, and depending on the presence of functional pharmacophore fragments, they can increase cell adhesion to the surface or impart antifouling and antimicrobial properties in vitro. The data on the composition, morphology, physicochemical properties, and biological activity of the coating consisting of hybrid organic molecules and an inorganic sublayer is obtained. The results provide a further strategy for the design of hybrid molecules that are in demand in practice for the development of a new type of promising implants with improved biocompatibility and bioactivity.

1. Results and discussion

1.1. Synthesis of oligopeptide-functionalized derivatives.

Short peptide sequences could be used to obtain biologically active coatings. Indeed, it was shown that many extracellular matrix (ECM) proteins contain an amino acid sequence: arginine-glycine-aspartic acid (RGD), to which cells attach with specific receptors on their surface [5]. This discovery led to the identification of a large family of surface cell adhesion receptors, the so-called integrins that specifically recognize the RGD sequences in some ECM proteins. Interacting with ligands, the integrins activate the intracellular signaling pathways and serve as an intermediate in cell migration and adhesion.

We synthesized several derivatives based on bisphosphonates of β-alanine, γ-aminobutyric and ε-aminocaproic acids containing various linkers (BMPS, EMSC, SMCC) modified with an integrin-active linear RGD tripeptide, which were used as organic coatings on titanium modified with plasma electrolytic oxidation (PEO) (Fig. 1) [6–8].

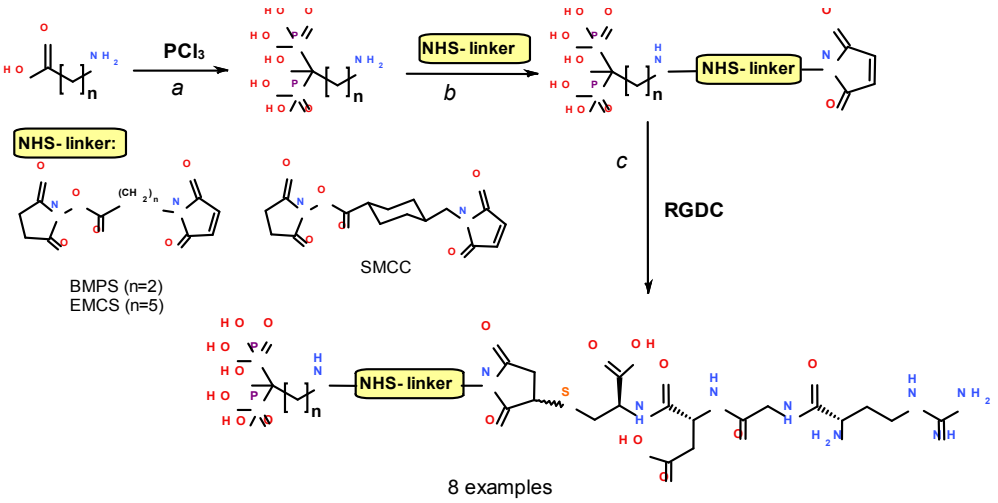

Fig. 1. Reactions and conditions:
a – $MeSO_3H$, 4–5 h, 85–90 °C; b – H_2O: acetone = 1:1, pH = 8–9, 1 h, 38–40 °C;
c – H_2O, pH = 7, 1–1.5 h, 38–40 °C

Рис. 1. Реакции и условия:
a – $MeSO_3H$, 4–5 ч, 85–90 °C; b – H_2O: ацетон = 1:1, pH = 8–9, 1 ч, 38–40 °C;
c – H_2O, pH = 7, 1–1,5 ч, 38–40 °C

RGD-substituted bisphosphonates were introduced into the Ti-PEO surface via physicochemical adsorption from the solutions. The presence of organic molecules in the coating was confirmed by XPS spectroscopy.

In vitro studies were carried out to estimate the proliferation and viability of fibroblasts, mesenchymal stem cells, and osteoblast-like cells on the metal surface [6–8]. It was shown that various types of cells exhibited different behavior on a surface. Moreover, the molecule bioactivity depends on the structure of the bisphosphonate anchor and linker. Thus, RGD derivatives with relatively short bisphosphonate anchors and BMPS or SMCC linkers increase cell proliferation on the surface of PEO-modified titanium.

1.2. Synthesis of hyaluronic acid bisphosphonates.

Recently, great emphasis has been attracted on surface modification with anti-inflammatory and immunomodulating agents [9]. The coatings based on polysaccharides, in particular glycosaminoglycans (GAGs), have great potential for development, because they combine properties such as low toxicity, high anti-inflammatory properties, etc. [10]. Among GAGs, commercially available hyaluronic acid (HA) shows unique biological properties [11]. Valuable properties of HA are biocompatibility, water-holding, and reparative-regenerative properties together with the ability to form high-viscosity hydrogels. HA participates in most biological processes: cell motility, proliferation, tissue organization, wound healing, angiogenesis, and morphogenesis, and in the development, growth, and reconstruction of a skeleton [12]. Moreover, HA is a key regulator of inflammation due to the interaction with CD44 and TSG-6 (tumor necrosis factor-stimulated gene-6) receptors [13].

The synthesis of HA bisphosphonates was carried out via the reaction of hyaluronic acid with aminobisphosphonates using the Michael addition (Fig. 2). For this purpose, the SH-derivative of the polysaccharide obtained according to the known method [14], was used. The addition of maleimidobisphosphonates to the SH-substituted HA occurred through the maleimide fragment. The target products were further isolated by the dialysis.

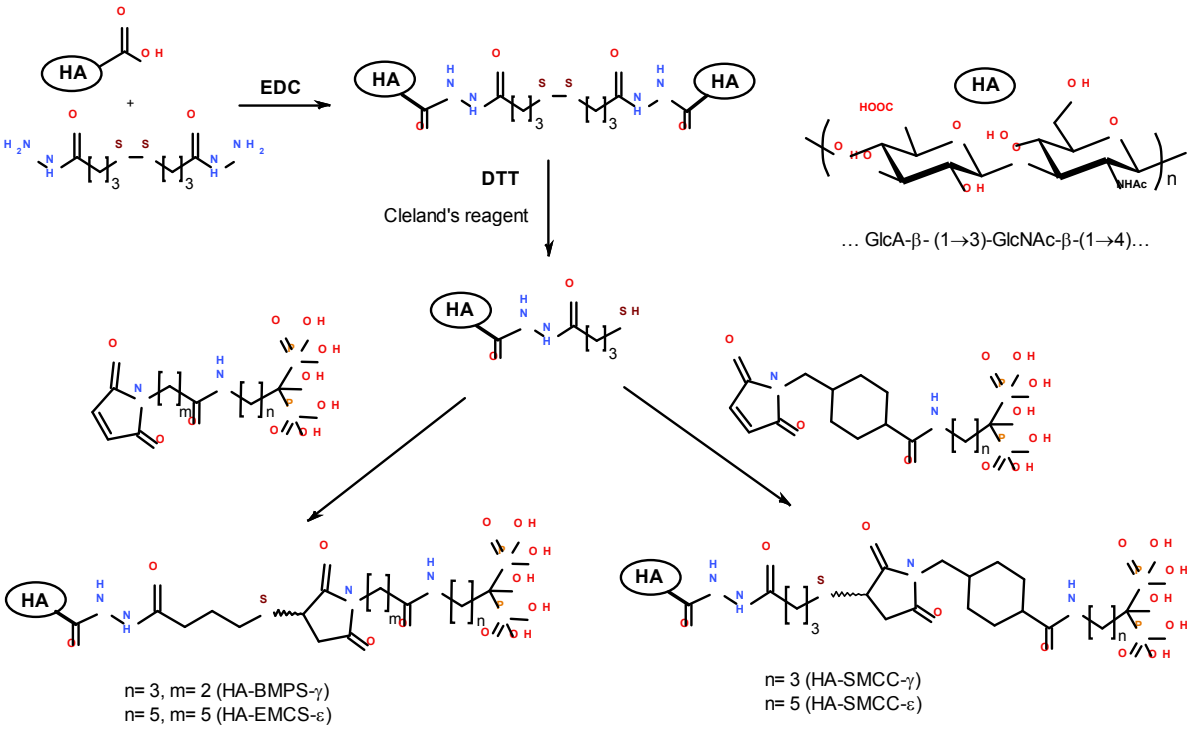

Fig. 2. Sinthesis of hyaluronic acid bisphosphonates

Рис. 2. Синтез бисфосфонатов гиалуроновой кислоты

It was shown that HA bisphosphonates are non-toxic and also promote cell growth. The organic coating of Ti-PEO by hyaluronic acid derivatives significantly decreased the proliferation of fibroblasts, MSC, and MG-63 on a surface. Moreover, a dependence of the degree of both MG-63 and MSC proliferation on the structure of the organic coating was found. The in vitro studies on antibacterial properties showed that the HA bifunctional molecules effectively reduce the adhesion of P. aeruginosa, E. faecium, and S. aureus bacteria cells. In this regard, HA phosphonates can be proposed as a promising base for the development of antifouling and antimicrobial coatings for PEO-modified Ti implants.

2. Experimental

The bisphosphonate derivatives were synthesized according to Refs. [7, 8]. Spectroscopic studies of compounds were carried out using NMR spectroscopy on a Bruker AVANCE-500 spectrometer (500.17 MHz (^{1}H), 125.78 MHz (^{13}C), and 202.46 MHz (^{31}P)). D_2O and $CDCl_3$ were used as the internal standards and solvents. Samples were prepared in a standard ampoule with a diameter of 5 mm. One and two-dimensional NMR spectra (COSY HH, NOESY) were recorded using standard Bruker pulse sequences. IR spectra (thin films) were obtained with the use of a Bruker Vertex 70v spectrometer. Mass spectra were obtained on spectrometer MALDI-TOF/TOF Autoflex III (Bruker) using 2.5-dihydroxybenzoic acid (2.5-DHB) or α-cyano-4-hydroxycinnamic acid (CHCA) as matrixes.

Titanium Grade 4 (ASTM F67) was chosen as the substrate material in this research. Titanium nanostructuring was performed using Conform type of an equal channel angular pressing (ECAP-C) with consequent drawing [15]. Cylindrical samples with a diameter of 8 mm and a thickness of 0.5 mm were assessed. The samples were coated via plasma electrolytic oxidation (PEO) [16]. The surface was characterized by scanning electron microscopy (SEM) and X-Ray Photoelectron Spectroscopy (XPS).

To deposit the organic coating, discs were sterilized and put into 10^{-3} M solution of the bisphosphonate derivatives, which have been preliminarily filtered through the CA 0.22 μm filter. In 3 h the samples were dried on air in a laminar box. Further, all the samples were put into a plastic 48-well tissue culture plate.

Human embryonic lung fibroblasts (FLECH-104), human adipose tissue mesenchymal stem cells (MSC) and human osteosarcoma cells (MG-63) were used to study cell adhesion and proliferation on a metal surface [7, 8]. To assess the cytotoxicity of compounds, the MTT test was used. Antimicrobial studies were carried out with the using of test cultures of S. aureus P 209, P. aeruginosa ATCC 27853, and E. faecium Ef79OSAU.

Conclusions

As a result, hybrid molecules with bisphosphonate anchors were synthesized. Compounds can be introduced into the pores of the PEO coating by the physicochemical adsorption from solutions. The in vitro studies showed the dependence of biological activity on the compound structure and surface morphology. The obtained results could be used further to develop a new generation of implants with improved bioactivity and compatibility.

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