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Chapter 1. DIAZONIUM CHEMISTRY AS A ROBUST APPROACH FOR THE BIOFUNCTIONALIZATION OF TITANIUM SURFACE

1.1. Introduction

Metallic biomaterials are most commonly used as implants for bone replacement or support. These metals include stainless steel, cobalt alloys, and titanium and its alloys which have become widespread since the early 1970s [1, 2]. Titanium is characterized by an excellent corrosion resistance and high strength-to-density ratio, overcoming that of steel. Additionally, it is regarded as an inert material in physiological conditions [2]. This set of conducive, mechanical, physical, and biological properties has made Ti the most attractive metal for biomedical applications, such as dental and orthopedic implants [3]. Another advantage of Ti implants is their non-ferrous character, which allows the patients to be safely examined with magnetic resonance imaging [1]. The resistance to corrosion is associated with a spontaneous passivation of Ti surface [4]. Grade V Ti alloy (Ti-6Al-4V) is more prone to corrosion, resulting in the possible release of cytotoxic vanadium and aluminium in long-term implantations, whereas commercially pure Ti (Grade I-IV) remains more resistant [2, 5].

Surface properties of Ti implants, including surface chemistry, topography and wetting angle, have a decisive effect on the process of osseointegration, affecting cellular response and bacterial colonization [6]. The surface of the implant is especially susceptible to bacterial infection after the surgery, when the tissue gets disrupted and has reduced number of blood vessels [7]. Therefore, the immunological response at the implant/tissue interface is diminished. Additionally, defects in the

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passive layer and insufficient wear resistance of Ti implants can compromise their corrosion resistance and durability [8]. All these issues can be addressed by appropriate surface functionalization. The efforts to modify Ti surface include chemical and physical methods, e.g. surface texturing, plasma treatment, thermal oxidation, chemical etching, or physical vapour deposition, especially to obtain coatings like TiN or CrN [6, 9].

For decades, scientists have paid close attention to the functionalization of different surfaces in a wide range of applications [10]. Diazonium salts are one of the most versatile compounds in organic science, also they are frequently utilized in a variety of reactions [11]. Because of their high reactivity, diazonium salts are now widely used in surface modification. For instance, Chehimi et al. [12] modified the surface of TiO_2 with diazonium chemistry to initiate radical polymerization of methacrylate. Also Ti6Al4V alloy was modified with diazonium ions to create poly(hydroxyethyl) methacrylate and polyetheretherketone layers [13]. A number of articles has addressed electrochemical deposition of various metals on TiN surfaces as a simple and cost-effective approach [14, 15]. Although there are several coating techniques described in the literature, a diazonium-based surface modification is preferred over the rest, due to its ability to form covalently bonded aryl-layers with desired functionalities, such as alkyl, nitro, cyanide, carboxylic, ester, alcohol, thiol, and halogenated groups [16]. As-formed coatings exhibit good adherence and homogeneity [17]. One of the main benefits of the diazonium-based surface modification is that it may be used on any type of surface, regardless of size, shape, or geometry.

In this chapter, we present the process of surface modification of Ti with a 4-nitrobenzenediazonium salt, resulting in the formation of a layer of nitrobenzene molecules covalently attached to the surface at para position (denoted as Ti-NO_2). To extend the number of possible biofunctionalization pathways, Ti-NO_2 surface was subjected to an additional electrochemical reduction reaction, resulting in the reduction of nitrobenzene moiety to aniline (Ti-NH_2). To verify whether the functionalization procedure was successful, unmodified and modified Ti discs were investigated by means of electrochemical impedance spectroscopy. As-formed Ti-NH_2 surfaces can be further biofunctionalized by a variety of methods to immobilize particular biomolecules (DNA, enzymes, proteins, peptides, etc.), metal complexes, polymers, various nanoobjects and molecular species (carbon nanotubes, fullerenes, metal nanoparticles, etc.), in order to equip Ti surface with unique biological characteristics.

1.2. Materials and methods

1.2.1. Reagents

Ti discs (diameter: 1 in., thickness: 0.02 in., in line with ASTM-B-265/ASME-SB-265 GR 2 specifications), 4-nitrobenzenediazonium tetrafluoroborate, NBF (97%), potassium chloride, KCl (>99.0%), and tetrabutylammonium hexafluorophosphate, nBu_4NPF_6 (>99.0%), were obtained from Sigma Aldrich (Saint Louis, MO, USA). nBu_4NPF_6 was vacuum dried before use. Deionized water (Millipore quality), ethanol (99.8%, Avantor) and acetonitrile (ACN, HPLC grade, Sigma Aldrich) were used as solvents.

1.2.2. Surface modification

The electrochemical functionalization of Ti surface was performed by means of a PARSTAT 2273 potentiostat (Ametek, Berwyn, PA, USA) by a cyclic voltammetry (CV) scanning. A standard three-electrode setup was used, comprising a Ag/AgCl (3 M KCl) reference electrode (ET073, EDAQ, Denistone East, Australia), a platinum plate counter-electrode (1 cm^2) (Mennica Polska, Warsaw, Poland), and a Ti disc working electrode (exposed area of 0.283 cm^2) (Sigma Aldrich, Saint Louis, MO, USA). Ti surface grafting with NBF (5 mM) was carried out in 0.1 M nBu_4NPF_6 ACN solution, within a potential range from -1.0 V to 0.5 V (vs. Ag/AgCl), for 5 CV cycles at a scan rate of 100 mV/s. Further electrochemical functionalization of Ti was performed in the 0.1 M KCl ethanol:water (1:9 v/v) solution, within a potential range from -1.0 V to 0 V (vs. Ag/AgCl), for 5 CV cycles at a scan rate of 100 mV/s.

1.2.3. Surface characterization

Electrochemical characterization of unmodified and modified Ti surfaces was performed by means of an electrochemical impedance spectroscopy (EIS) by collecting EIS spectra in 0.1 M KCl solution with frequencies ranging from 100 kHz to 100 mHz, an AC amplitude of 40 mV (vs. Ag/AgCl) and a DC potential equal to 0 V (vs. Ag/AgCl).

1.3. Results and discussion

Since diazonium chemistry has been known as an efficient approach for surface functionalization [18, 19], grafting and subsequent reduction of 4-nitrobenzenediazonium (NBF) salt was used for the covalent modification of a surface of model Ti disc electrodes (Fig. 1). The first step of functionalization involved an electrochemical reduction of NBF at the surface of Ti electrode. A distinct reduction peak at the potential of -0.4 V (vs. Ag/AgCl) was observed in a cyclic voltammetric (CV) curve associated with this process. NBF reduction peak, however, was observed particularly in the first CV cycle, and disappeared in the subsequent CV cycles, suggesting that Ti surface was successfully modified at the beginning of the reduction process and subsequent CV cycles did not further affect surface chemistry. As a result of the first step of functionalization, the surface of Ti disc was coated with a layer of nitrobenzene molecules covalently attached to the surface at para position (denoted as Ti-NO_2). To extend the number of possible biofunctionalization procedures, Ti-NO_2 surface was subjected to an additional electrochemical reduction reaction in a protic medium (water:ethanol solution), resulting in the reduction of nitrobenzene moiety to aniline (Ti-NH_2).

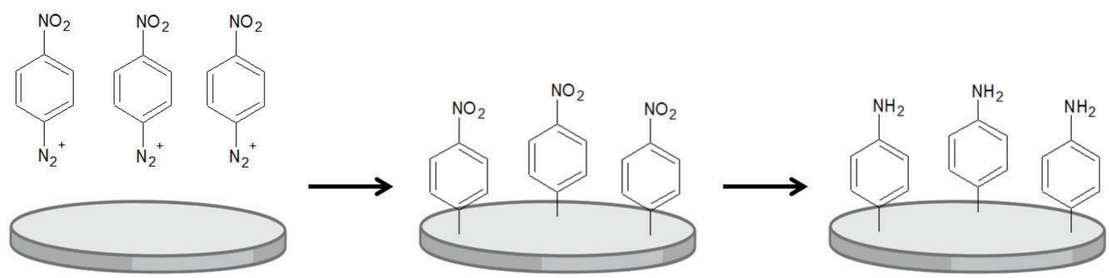

Fig. 1. Schematic representation of the process of Ti surface functionalization with diazonium chemistry (on the example of 4-nitrobenzenediazonium surface grafting)

Rys. 1. Schematyczne przedstawienie procesu funkcjonalizacji powierzchni Ti za pomocą soli diazoniowych (na przykładzie szczepienia powierzchniowego soli 4-nitrobenzenodiazoniowej)

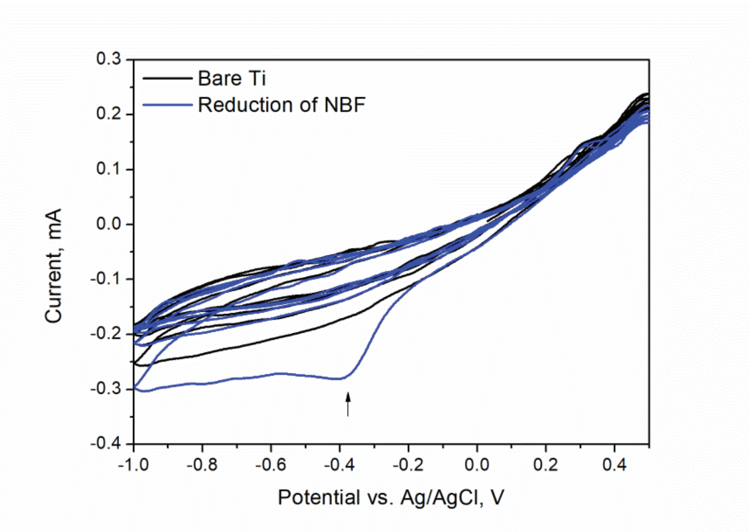

Fig. 2. Cyclic voltammetric curve collected during the reduction of NBF on Ti surface compared with the cyclic voltammetric curve collected during the reduction of Ti in the absence of NBF
Rys. 2. Krzywa woltamperometryczna zarejestrowana podczas redukcji NBF na powierzchni Ti, porównana z krzywą woltamperometryczną zarejestrowaną podczas redukcji Ti w roztworze bez NBF

To verify whether the functionalization procedure was successful, unmodified and modified Ti discs were investigated by means of electrochemical impedance spectroscopy (EIS). Due to the fact that EIS allows to detect minor changes in the mechanism of charge transfer, by comparing the shape of EIS spectra (Fig. 3), it is possible to assess the efficiency of surface modification. EIS data can be analyzed in three different ways. Impedance vs. frequency plots (Fig. 3A) can be used to compare the impedance behaviour of investigated samples in a wide frequency range. By observing the increase/decrease in the impedance values, it could be assessed whether the modification strategy leads to the decrease/increase in the conductivity of the surface, respectively. Phase angle vs. frequency plots (Fig. 3B), on the other hand, can be used to assess the capacitive behaviour of the samples. The number of peaks is related to the number of capacitive processes taking place in the investigated system, and the position of the peak is related to the time constant of the system. Finally, negative imaginary impedance vs. real impedance plots (Fig. 3C), also known as Nyquist plots, combine both the impedance and phase angle into the one plot in the complex plane. The semicircles observed in the Nyquist plots are associated with electrochemical processes such as charge transfer. In general, the larger the diameter of the semicircle, the less conducting is the investigated system.

EIS data clearly indicated the variation in Ti surface impedance at different stages of the functionalization procedure. Bare Ti discs were found to be highly electroactive, exhibiting a low impedance profile and a single phase angle peak at 100 Hz. The impedance profile was found to be typical for metal electrodes, with a low impedance at high frequencies and its increase at low frequencies [20]. The formation of Ti-NO_2 at the first stage of functionalization was found to have a deteriorating effect on surface conductivity by elevating the impedance profile and shifting a phase angle peak to higher frequencies (decreasing time constant). Interestingly, if the reduction of Ti was carried out in the absence of NBF, the resulting material (Ti-red) exhibited the worst electrochemical characteristics among all investigated materials, as evidenced from the largest semicircle observed in the Nyquist plot. Further reduction of Ti-NO_2 to form Ti-NH_2 resulted in the consecutive changes in impedance (decrease in the impedance profile) and capacitive (increase in time constant) behaviour of Ti surface. Basing on the EIS data, it could be concluded that since the electrochemical behaviour of Ti-NO_2 and Ti-NH_2 was different than that noted for bare Ti, both surface functionalization steps were successful in changing the surface characteristics of Ti. The presence of NBF-derived organic moiety on the surface of Ti electrodes was indirectly confirmed by observing the differences in electrochemical behaviour among Ti-NO_2, Ti-NH_2, and Ti-red samples, since the latter one was formed in the absence of NBF.

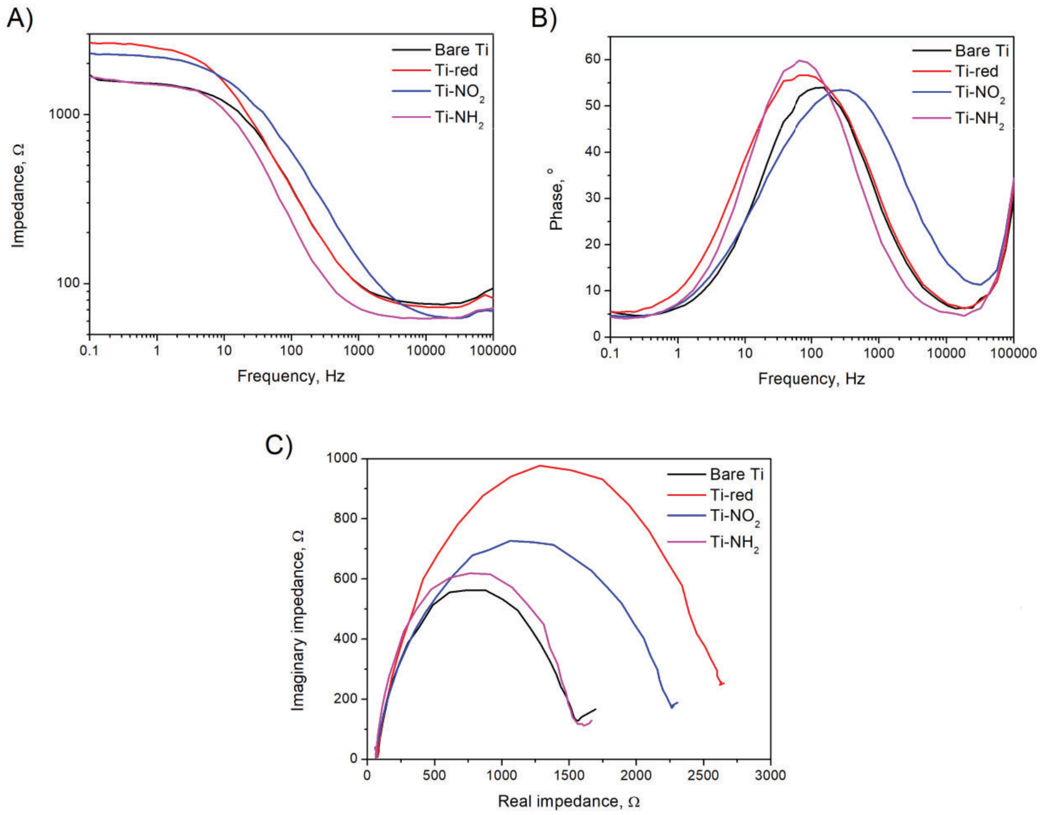

Fig. 3. EIS data in the form of (A-B) Bode plots showing the frequency-dependent behaviour of the (A) impedance modulus and (B) phase angle, and (C) Nyquist plots of bare Ti electrodes, as well as Ti electrodes subjected to reduction, NBF modification and post-modification

Rys. 3. Wyniki EIS w formie (A-B) wykresów Bodego przedstawiających zależność (A) impedancji i (B) kąta fazowego od częstotliwości, oraz (C) wykresy Nyquista dla czystej elektrody Ti, elektrody Ti poddanej redukcji, modyfikacji NBF oraz post-modyfikacji

Diazonium-grafted surfaces can be further biofunctionalized by a variety of methods (Fig. 4), including amide coupling, "click chemistry", and post-diazotization, among others [21]. Therefore, the biological properties of Ti-NH_2 could be easily tailored to specific needs by the immobilization of particular biomolecules, such as DNA, enzymes, proteins, peptides, etc., in order to equip Ti surface with unique biological characteristics. Additionally, the chemistry of Ti-NH_2 allows for the immobilization of metal complexes, various nanoobjects, and molecular species, such as carbon nanotubes, fullerenes, metal nanoparticles, etc. Besides, covalently attached organic moieties can be used as precursors for surface-initiated polymerization reactions, leading to the formation of polymer brush-like structures of defined physicochemical characteristics and a great potential in biomedical engineering, including biosensing, cell culture and regenerative medicine [22].

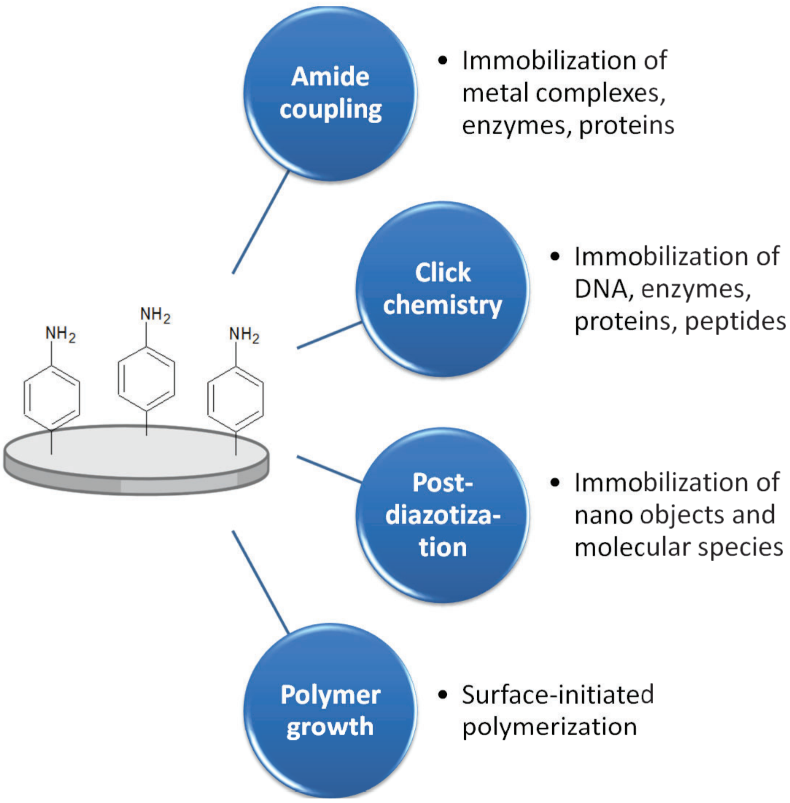

Fig. 4. Further routes of biofunctionalization for Ti-NH_2 surfaces
Rys. 4. Dalsze możliwości biofunkcjonalizacji powierzchni Ti-NH_2

1.4. Conclusions

Diazonium chemistry has been confirmed as an easy and straightforward approach to modify the surface of Ti by means of an electrochemical grafting procedure. As a result, two types of surface chemistries have been formed: Ti-NO_2 and Ti-NH_2, which could be further modified by a variety of methods to immobilize particular biomolecules (DNA, enzymes, proteins, peptides, etc.), metal complexes, polymers, various nanoobjects and molecular species (carbon nanotubes, fullerenes, metal nanoparticles, etc.), in order to equip Ti surface with unique biological characteristics suitable for particular biomedical needs.

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Abstract

Due to an excellent corrosion resistance and high strength-to-density ratio, titanium is most commonly used as an implant material for bone replacement or support. Surface properties of Ti implants, including surface chemistry, topography and wetting angle, have a decisive effect on the osseointegration, and can be easily tailored by surface modification. In this chapter, we present the process of surface modification of Ti with a 4-nitrobenzenediazonium salt, resulting in the formation of a layer of nitrobenzene molecules covalently attached to the surface at para position. To extend the number of possible biofunctionalization procedures, as-formed surface was subjected to an additional electrochemical reduction reaction, resulting in the reduction of nitrobenzene moiety to aniline. To verify whether the functionalization procedure was successful, unmodified and modified Ti discs were investigated by means of electrochemical impedance spectroscopy.

Keywords: diazonium salts, electrografting, titanium, biofunctionalization