Nanoporous Stainless Steel Materials for Body Implants

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Contributor: Metka Benčina , Ita Junkar , Alenka Vesel , Miran Mozetič , Aleš Iglič

Despite the inadequate biocompatibility, medical-grade stainless steel materials have been used as body implants for decades. The desired biological response of surfaces to specific applications in the body is a highly challenging task, and usually not all the requirements of a biomaterial can be achieved. Nanostructured surfaces have shown intriguing results as cell selectivity can be achieved by specific surface nanofeatures. Nanoporous structures can be fabricated by anodic oxidation, which has been widely studied for titanium and its alloys, while no systematic studies are available for stainless steel (SS) materials.

stainless steel anodisation nanoporous morphology

1. Introduction

Many body implants, such as orthopaedic, cardiovascular and dental, are made from stainless steel. The most common types of stainless steel used in biomedical applications are 316, 316L and 304 [1][2][3][4]. The main difference between the 316 and 304 grades is that 316 grade contains about 2–3 percent molybdenum [4]. The 316L grade of stainless steel has a lower amount of carbon than 316 grade. Stainless steel of medical grade is characterised by excellent mechanical properties and reasonable chemical inertness. The biocompatibility, however, is often inadequate because of the structure and composition. The rather poor biocompatibility in the case of vascular stents is reflected in thrombogenic response, uncontrolled proliferation of smooth muscle cells and a poor proliferation of endothelial cells, while in the case of orthopaedic devices, poor adhesion of osteoblast cells and a high risk of bacterial infections. To overcome these issues, various coating procedures were developed, which showed more or less success. Deposition of a thin film of highly biocompatible material, such as some types of ceramics, biocompatible polymers, composites and matrixes that incorporate drug releasing agents, has been proposed [5][6][7][8][9], but issues regarding long-term stability, mainly due to the discrepancies between mechanical properties as well as poor adhesion and gradual changes of the interface, still persist. Furthermore, stainless steel contains traces of nickel, which is regarded as an allergenic and cytotoxic material [10][11][12]. The release of nickel from the stainless-steel implants is minimal but, in some cases, sufficiently large to cause serious health problems. It is known that the biocompatibility of metal oxide coatings is often better than the parent metals used as substrates because the oxides usually suppress the release of toxic ions [13][14]. On the other hand, it is also known that the oxidation of stainless steels or similar materials leads to the formation of various Fe and Cr oxides, and not all of them are considered biocompatible. Furthermore, the oxide film is often not compact enough to prevent diffusion of any ions from the bulk stainless steel onto the oxide surface and thus the release of ions. Many

researchers suggested chromium oxide as the most biocompatible oxide, which might be formed on the stainless steel surface.

2. Nanoporous Stainless Steel Materials

Electrochemical oxidation of metals and alloys is an established technique nowadays used in mass production. The basic concept is to dip the workpiece into an ionic solution and apply a positive charge. The workpiece acts as the anode, while the container is usually grounded and therefore acts as the cathode. Alternatively, the container is made from a dielectric, and a counter electrode is immersed in the bath. The technique is usually referred to as anodic oxidation or anodisation. Probably the best-known technique is anodisation of titanium, used for the formation of nanotubular or nanoporous films [15][16] and anodisation of aluminium, which is used for the formation of a thin oxide film [17][18][19], which prevents any further degradation of the material.

The nanoporous structure of metals and their alloys for biomedical applications can also be prepared by other techniques such as non-thermal plasma [20][21]. Although metals, such as titanium, aluminium and stainless steel, spontaneously form a thin oxide film in a harsh environment, anodisation is commonly applied in order to enhance corrosion resistance or biocompatibility of the metallic materials. The reason for using anodisation for oxidation of the stainless steel is twofold: (i) ability to control the composition of the oxide film and (ii) various morphologies of the oxide film on the nano-scale, which, for instance, influence the response of biological material in contact with the anodised surfaces.

Tsuchiya et al. [22] also studied the effect of water in an organic solvent in the anodisation vessel and found that small quantities caused much faster pore formation. When using an electrolyte containing water and ethylene glycol, the diameter of nanopores was increased (about 300 nm) compared to the diameter of nanopores obtained in a water-free ethylene glycol-based electrolyte (about 150 nm). The results were explained by a much larger electrical current in cases where water was present in the electrolyte.

The biological response of the anodised nanostructured (nanotubular and nanoporous) titanium surfaces has been immensely studied [15][16][23] The biological response of the nanoporous stainless steel was elaborated by Pan et al. [24]. Different nanopores were fabricated on 316L stainless steel by anodisation. They used perchloric acid and ethylene glycol monobutylether as the electrolyte and studied the evolution of the surface morphology. Unlike Tsuchiya et al. [22], who performed anodisation at room temperature, Pan et al. [24] selected temperatures between 5–10 °C. At the anodisation voltage of 30 V, they found the diameter of nanopores was about 40 nm, roughly three times smaller than Tsuchiya et al. [22]. The next voltage probed was 45 V, and the pore diameter was 75 nm. This is significantly smaller than what was observed by Tsuchiya et al. [22] at a voltage of 40 V. The discrepancy is difficult to explain only from the anodisation parameters, but it should be stressed that Pan et al. [24] did not use pure ethylene glycol but rather ethylene glycol monobutylether. Furthermore, the treatment times adopted by Pan et al. [24] were much longer, at about half an hour. Pan et al. [24] managed to synthesise top-quality nanopores in a broad range of diameters from about 40 nm to 210 nm. They also determined the mean roughness as deduced from AFM measurements. The proliferation of fibroblast cells was studied on samples of various pore diameters. The best

results in terms of enhanced cell proliferation were observed for nanopores of small diameters, between about 40 nm and 80 nm.

The performance and behaviour of human cells in response to the different nanotopographies have already been shown previously. It has been shown that, typically, small nanofeatures (raised above 10 nm but below 20 nm high) increase cell adhesion, size and spreading of, for example, endothelia, fibroblasts and mesenchymal stem cells, but larger nanofeatures (app. 100 nm high) generally inhibit cell spreading, cytoskeletal organization and functional differentiation [25][26][27][28][29]. Initial interaction between cells and nanotopography is through the filopodia, which are fine cell membrane projections that collect the nanotopographical information from the materials surface [29].

Similar experiments as in the pioneering work of Tsuchiya et al. [22] were also performed by Ni et al. [30]. They used exactly the same electrolyte, but the concentration of the perchloric acid was only 5.3 vol. %. Unfortunately, Ni et al. [30] did not report the treatment time nor the electrolyte temperature. They probed four voltages, i.e., 20 V, 30 V, 40 V and 50 V. At a voltage of 20 V, no pores were observed by SEM. Interestingly enough, the voltage of 30 V enabled the formation of a uniform nanoporous honeycomb morphology with an average pore diameter of 25 nm only. At a voltage of 40 V, they observed pores of a 50 nm diameter, and at the maximal voltage used of 50 V, they obtained the pores of a diameter of 60 nm. Ni et al. [30]., therefore, reported much smaller pore diameters than Tsuchiya et al. [22] and Pan et al. [24]. Almost identical results were reported by the same group almost simultaneously in the proceedings [31].

Tsuchiya [32] published another research two years after his report about the formation of nanoporous 316 stainless steel [22]. The experimental conditions were similar to those in his research [22], except that he also used sodium perchlorate, not only perchloric acid-containing electrolyte. He concentrated on rather large voltages of 50 V or 60 V. At a voltage of 50 V, Tsuchiya obtained ordered pore arrays with an average diameter of about 210 nm. He also estimated the depth of the pores from AFM line profiles and found an average depth of about 15 nm. Other experiments were performed at a voltage of 60 V. When only ethylene glycol and sodium perchlorate were used, he found a pore diameter of about 260 nm, which is not far away from the results reported in [22]. When about 5 vol. % water was added, the average pore diameter increased to 330 nm, and when the water concentration was 12 vol. %, the diameter slightly increased to 350 nm. Interestingly enough, when the water content was 20 vol. %, Tsuchiya [32] did not observe any pores. In conclusion, Tsuchiya [32] mentioned that this technique could also be applied to other metallic substrates, in particular, Inconel 600 and Co–Cr–Mo alloys, and supported the statement with two images of porous surface finishes. Despite the large dimension of the pores, Tsuchiya [32] reported increased osteoblast-like cell proliferation. The results are not sound with the work of Pan et al. [24] and Khaw et al. [33], who found such large pores obsolete in terms of cell proliferation.

Lu et al. [34] also performed the synthesis on stainless steel type 904L. Several different voltages were used. At the voltage of 30 V, the authors measured the current density versus treatment time at different temperatures of the electrolyte from 4 °C to 30 °C. The initial current density significantly depended on the electrolyte temperature. The current densities were about 0.15 A/cm^2, 0.3 A/cm^2 and 0.5 A/cm^2, and in terms of temperature, they were 4 °C, 12 °C and 30 °C, respectively. The initial current density decreased significantly (by an order of magnitude) after about

30 s and stabilised at a very low value for the case of 4 °C, whereas the current density at 30 °C remained moderate at a value of about 0.07 A/cm^2. Lu et al. [34] also acquired SEM images of the samples treated for 10 min at those temperatures and found perfectly ordered nanopores at 4 °C, whereas the nanopores at 30 °C were stochastically distributed and somehow larger.

In addition, Lu et al. [34] performed anodisation of stainless steel 904L at different voltages, but at the same treatment time of 600 s and electrolyte temperature of 4 °C. They found an enormous dependence of the pore diameter on voltage. The pore diameter at 20 V was as small as about 40 nm, and the structures were highly ordered. Increasing the voltage to 30 V resulted in the formation of highly ordered nanopores with an average diameter of about 80 nm. The voltage of 40 V caused rather unordered pores of a diameter of roughly 100–200 nm. All these results were reported for samples treated for 600 s. A sample was also treated for 300 s, and un-ordered surface morphology was observed. Lu et al. [34] also reported that pitting appeared on this type of stainless steel when the electrolyte temperature was about 40 °C.

Unlike previously cited authors, Lu et al. [34] also reported on the structure of the surface film. They used X-ray photoelectron spectroscopy (XPS) to evaluate the composition and chemical bonding at two different anodisation times. The chemical compositions were measured at various depths of 3 nm, 15 nm, 30 nm, 45 nm and 60 nm. They found little difference in the composition at a depth of 3 nm and 15 nm, indicating the formation of a rather compact surface film. The depth profiling enabled estimation of the thickness of the oxide film, and according to XPS results, it was roughly 10 nm. The surface film was rich in chromium and perhaps iron oxides, and the concentration of nickel was close to the detection limit of the XPS. The original nickel content of 904L stainless steel is about 25%, therefore, more than Cr, which is 20%. Obviously, the anodisation enabled the preferential formation of chromium oxide film on the surface. From high-resolution XPS peaks of iron and chromium, the researchers managed to conclude that the surface film actually consisted of Cr_2O_3 and Fe_2O_3. These are the most stable forms of oxides, so one can conclude that anodic oxidation causes complete oxidation, which is also preferential since the composition of the oxide film does not reflect the composition of the alloy.

Top-quality nanopores were also synthesised on SS316L substrates by Ban et al. [35]. They used a dielectric container and a couple of electrodes. The workpiece was the anode, and the cathode was a Pt foil. The samples were first electropolished for 10 min at a temperature of 80 °C using a water solution of phosphoric and sulphuric acids. As-polished substrates were then exposed to anodisation using anhydrous ethylene glycol containing perchloric acid at 5 wt.%. They used various voltages and treatment times in order to find the optimal conditions. They synthesised pores with a diameter of 50 nm using a voltage of 40 V and treatment time of 10 min. Top-quality pores of a diameter of 80 nm were synthesised at the voltage of 50 V and treatment time of 35 min. They tested such materials for antibacterial properties, and they reported outstanding results for the case of *Listeria monocytogenes*.

Namely, a smooth surface enabled the log/CFU concentrations of 3.6 cm^{-2}, whereas the concentration was about 1.6 and 1.4 cm^{-2} for the cases of 50 nm and 80 nm pores, respectively. The nanoporous surfaces were therefore

found to be very antibacterial (similarly as it was shown in Ref. [36]) as the density of bacteria dropped for over 100-times as compared to smooth surfaces.

Farrag et al. [37] used various voltages, treatment times, and electrolyte temperatures in order to synthesise nanopores on the surface of stainless steel 316L materials. They also provided some characterisation of the materials using XPS and X-ray diffraction spectrometry (XRD). The aim of their study was to probe such materials as a photoanode for visible light photo-electrolysis. They performed anodisation using a mixture of ethylene glycol and 5 vol. % of perchloric acid to obtain highly oriented nanopores with an average diameter of about 120 nm. The concentration of perchloric acid of 3 vol. % or 10 vol. % did not enable the formation of the nanopores. Furthermore, the pores were much smaller at lower voltages; about 40 nm at 30 V and about 70 nm at 35 V. Prolonged treatment times caused degradation of the porous structure, and the same applied to elevated electrolyte temperatures. These authors also probed admixtures of methanol as well as inorganic acids. Such admixtures prevented the formation of ordered nanopores but facilitated an extremely porous surface finish, which is useful for photocatalysis.

Bae et al. [38] synthesised nanoporous 316L stainless steel surfaces to study the platelet adhesion, cellular response and drug delivery capability of such materials. Unlike previously cited authors, Bae et al. [38] used an electrolyte composed of 1 M H_2SO_4 and 1.0 wt.% hydrofluoric acid solution with pH 2–3. A stainless steel substrate was the anode, and the cathode was a platinum plate. The anodic oxidation process was performed at room temperature. Such substrates were probed for a biological response using a variety of techniques, including SEM for estimation of the blood platelet adhesion on the surface, a cell migration assay and an in vitro cell proliferation assay. The substrates were also loaded with everolimus, and the drug amount, as well as the release rate, was measured. The surface morphology changed significantly after the electropolishing for 10 min at a voltage of 10 V, an electrolyte temperature of 50 °C and additional anodic oxidation at room temperature. Namely, evenly distributed nanopores of a diameter of about 100 nm were observed both by SEM and AFM. The latter technique also enabled the estimation of the average roughness, which was found to be close to 10 nm. The concentration of blood platelets was decreased by five times as compared to the untreated sample, while the density of smooth muscle cells decreased by about 30%.

The surface morphology of nanoporous stainless steel enabled a small increase in the drug amount, but the release rate dropped significantly as compared to the untreated samples. About 75% of the drug was released on the first day in the case of an untreated sample, but the samples prepared according to the methods developed by Bae et al. [38] enabled much slower release, i.e., 18% till the end of the first day, 40% till day 4 and 62% after a week. The surface finish with dense pores enabled gradual drug release, which was measurable even a month after deposition.

An advanced approach regarding previous attempts of anodisation of stainless steel was disclosed by Rodriguez-Contreras et al. [39]. They used a mixture of sulfuric acid and 30% hydrogen peroxide as the electrolyte. They limited the experiments to 96% of sulfuric acid. The electrolyte was allowed to heat continuously during the anodisation process. The starting temperature was 40 °C, and the ultimate was 80 °C. The ultimate temperature

was reached after 12 min of anodisation. Unfortunately, the anodisation voltage was not specified; only the current density of 700 A/m^2 was disclosed. The treatment caused a rich surface morphology of unevenly distributed semi-circular pores of diameter below 20 nm. High-resolution XPS spectra revealed both chromium oxide (Cr_2O_3) as well as hydroxide ($Cr(OH)_3$). Several iron oxides were detected, with FeO and Fe_2O_3 being the most predominant. The concentration of nickel compounds was also above the detection limit. An outstanding antibacterial property of the samples treated according to the innovative methods was proved for *E. Coli* and *B. Subtilis*. The proliferation of osteoblast cells improved significantly, but the concentration of fibroblast cells did not change significantly as compared to untreated stainless steel samples. Osteogenic cell filopodia morphology was found to be beneficial on the surface of nanostructured anodised samples. Unlike the above-cited authors, Rodriguez-Contreras et al. [39] also reported on the surface energy. The anodisation caused an increased polar component of the surface energy from about 5 to 8–10 mN/m (depending on the type of stainless steel, either 304 or 316), and the static water contact angle decreased from about 78 to 69 and 64 for SS 304 and 316, respectively. The anodisation, therefore, didn't cause a large change in the surface hydrophilicity. Here, it is worth mentioning that the researchers haven't reported the storage time between the anodisation and the wettability measurements, which may be crucial due to the hydrophobic recovery typical for such materials.

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