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Biocompatible Nb_2O_5 thin films prepared by means of the sol–gel process

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Thin biocompatible oxide films with an optimised composition and structure on the surface of titanium and its alloys can improve the implant integration. The preparation of these thin oxide layers with the intended improvement of the surface properties can be realised by means of the sol–gel process. Nb_2O_5 is a promising coating material for this application because of its extremely high corrosion resistance and thermodynamic stability. In this study, thin Nb_2O_5 layers (< 200 nm) were prepared by spin coating of polished discs of cp-titanium with a sol consisting of a mixture of niobium ethoxide, butanol and acetylacetone. The thickness, phase composition, corrosion resistance and the wettability of the oxide layers were determined after an optimisation of the processing parameters for deposition of oxide without any organic impurities. The purity of the oxide layer is an important aspect in order to avoid a negative response to the cell adhesion. The biocompatibility of the oxide layers which was investigated by *in vitro* tests (morphology, proliferation rate, WST-1, cell spreading) is improved as compared to uncoated and TiO_2 sol–gel coated cp-titanium concerning the spreading of cells, collagen I synthesis and wettability.

1. Introduction

For the long-term success of skeleton implants such as artificial hip or knee joints, the stable compound between the biomaterial and the surrounding tissue is the most important requirement. To achieve a strong adhesion of the cells to the biomaterial, the implant surface with its structure and composition plays an outstanding role [1, 2]. The presence and an additional deposition of thin films of a biocompatible ceramic on the surface of prostheses can improve the osseointegration of the implant [3, 4]. These composite materials combine the favourable mechanical properties of metals (e.g. titanium alloys) tolerating tensile stresses and the excellent physico-chemical, biocompatible and corrosion properties of thin ceramic layers (e.g. Nb_2O_5). The surface layer should be thin because the maximum tension stress appears, for example, during bending in the outermost fibre. Thin oxide films can be produced by means of the sol–gel process [5, 6]. This technique offers some advantages including the precise control of the chemical composition and microstructure of the coating, the preparation of homogeneous films, the low densification temperature of the ceramic and the ability to produce alternatively amorphous or crystalline layers. As compared to other coating methods, the sol–gel process requires considerably less equipment and is therefore comparatively inexpensive [7, 8].

In this study, discs of cp-titanium were coated with Nb_2O_5. Because of its extremely high corrosion resistance and thermodynamic stability, Nb_2O_5 is a promising material for application as an oxide coating for metallic implants. The aim was the preparation of biocompatible Nb_2O_5 oxide layers and the determination of oxide properties which guarantee a good adhesion of cells. Because of the organic components contained in the starting sol, it is essential to ensure that no organic residuals remain in the oxide layer which may have a negative influence on the biocompatibility [6].

2. Materials and methods

Polished discs of commercially pure titanium (cp-Ti, grade 2, ISO 5832) were used ($\varnothing$20 mm, thickness = 5 mm) as substrates for the coating procedure. All samples were abraded using SiC emery paper and mechanically polished using a mixture of SiO_2 suspension and distilled water. After metallographic preparation, an ultrasonic cleaning in ethanol and a subsequent drying followed.

In the first step of the sol–gel process, a sol was prepared consisting of a mixture of a metal-organic precursor (niobium(V) ethoxide, NEO, $Nb(OC_4H_9)_5$), a solvent (butanol, But, C_4H_9OH) and a chelating agent (acetylacetone, Acac, $CH_3COCH_2COCH_3$) in the molar ratio of NEO : But : Acac = 1 : 20 : 1. The solution was stirred for 24 h. With this sol the discs were spin-coated

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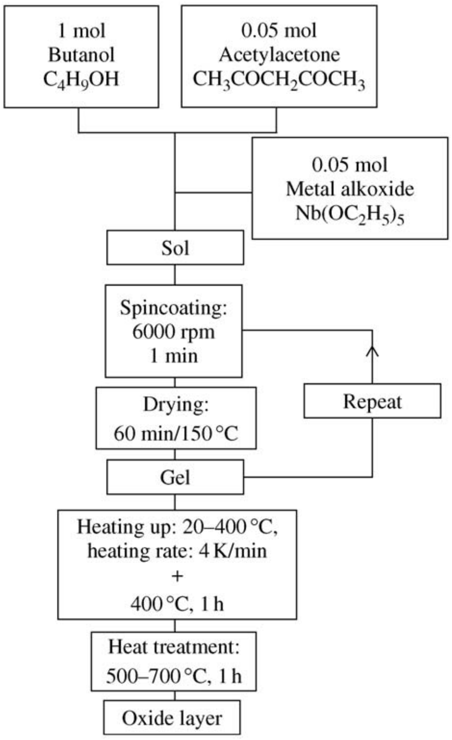

Figure 1 Schematic description of the preparation of Nb_2O_5 layers by means of the sol–gel process.

(6000 rpm, 60 s) and then dried for 30 min at 150 °C. In order to achieve thicker oxide layers, cycles of coating and subsequent drying can be repeated several times. After drying, the samples were heated up to 400 °C in air at a rate of 4 K/min and held at this temperature for 1 h to remove organic residuals. Finally, the samples were annealed at temperatures between 450 and 700 °C. Fig. 1 shows schematically the preparation of the sol–gel oxide layers. The temperature of the heat treatment necessary to produce an organic-free oxide layer was determined by means of differential thermal analysis, thermogravimetry (DTA–TG) and CHN analyses. The powder samples for the DTA–TG and CHN analyses were prepared by adding water to the sol to start the hydrolysis–condensation reaction resulting in oxide precipitation. After cleansing in ethanol and distilled water, the suspension was dried at 150 °C until the solvent had evaporated and the powder remained.

DTA–TG were carried out with dried oxide powder in a synthetic air flow at a heating rate of 1 K/min. For DTA analysis, α-alumina was used as a reference. The carbon of potential organic residues in oxide powder heat treated in a range from 200 to 500 °C for 1 h have been detected by means of CHN analysis. The thicknesses of the prepared oxide layers were determined by means of variable angle spectroscopic ellipsometry (VASE), which is possible because of the transparency of the films in the range of the visible light. To determine the phase compositions of the oxide layers, X-ray diffraction (XRD) patterns of the coated substrate discs were recorded by means of glancing angle XRD (Cu K_α, $\theta = 1°$). The identification of the XRD patterns was achieved by the use of the JCPDS. The corrosion properties of the coated samples were determined by measuring current density-potential curves under physiological conditions (37 °C, 0.9% NaCl in H_2O, saturated with oxygen). The potential between the working electrode (sample) and the reference electrode (Argenthal Ag/AgCl, + 207 mV to SHE) was varied from − 1 to + 3 V at a scan rate of 0.1 mV/s. The contact angle of bidistilled water on the oxide layer was determined by means of the sessile drop method. To remove impurities and adjust reproducible surface qualities for the measurement of the contact angle, the samples were cleansed in cyclohexane, acetone, ethanol and twice in distilled water.

The samples designed for biocompatibility testing were sterilised by an autoclave. The materials used for cell viability tests (proliferation, cell volume and metabolic activity) consisted of cp-titanium, cp-titanium coated with TiO_2 (sol–gel), cp-titanium coated with Nb_2O_5 (sol–gel) and Co35Ni20Cr10Mo. The cell function tests (spreading, collagen I production and cell migration) were carried out on the same materials except Co35Ni20Cr10Mo. For the cell migration test, the cell monolayer (which was formed after seven days on the samples) was detached from cells within a gap of 0.5 mm width by a cell scraper. After 24, 36 and 48 h the migration of the cells from the bordering monolayer in the cell-free gap was observed. The spreading of the cells was analysed after staining the cells on the samples with May–Grünwald solution. The collagen I synthesis of the osteoblasts on the samples was compared by colourising the collagen I by Sirius Red stain.

All biocompatibility tests (cell viability and cell function tests) were carried out with MC3T3-E1 murine calvaria osteoblast like cells which were seeded on polished 15 mm diameter slices in 12 well plates with 3 ml RPMI 1640 medium with 15% foetal calf serum and cultured for seven days on the material samples.

3. Results and discussion

The heat treatment for the preparation of pure oxide layers has to be carried out at temperatures high enough to release all organic contamination. The DTA–TG curves providing information about the required temperature are illustrated in Fig. 2. In the TG curve, the weight loss of 17.5% occurs in two steps up to a temperature of 380 °C, which is thought to be due to the release of organic residuals from the gel [9]. The DTA measurement shows two superposing exothermic peaks (the first one from 40 to 200 °C and the second, stronger one from 200 to 400 °C) which are caused by the

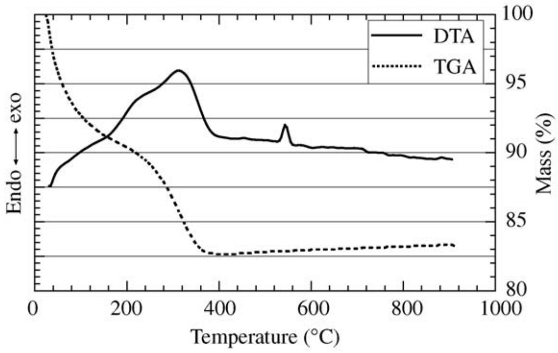

Figure 2 DTA–TG curves of dried Nb_2O_5 sol–gel powder.

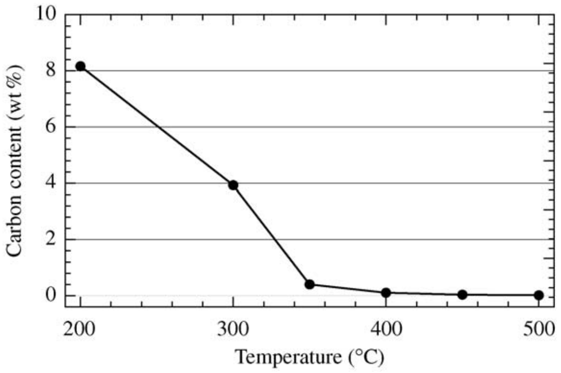

Figure 3 Carbon content of Nb_2O_5 sol–gel powder heat treated at different temperatures for 1 h.

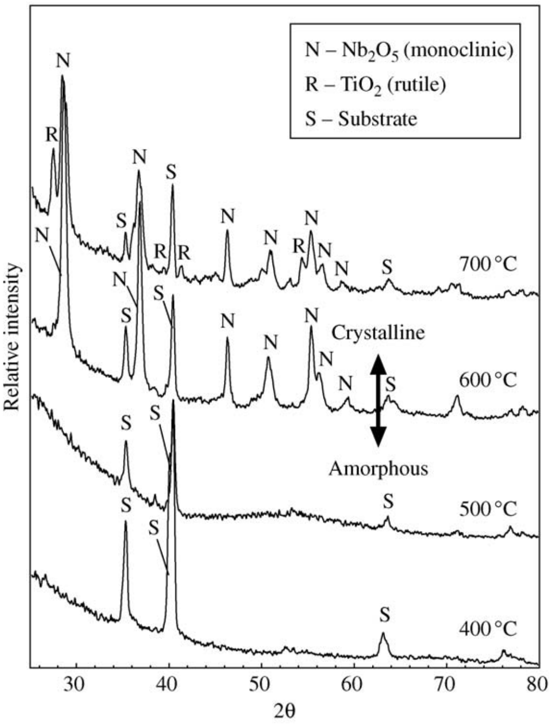

Figure 4 XRD pattern of Nb_2O_5 sol–gel oxide layers heat treated at different temperatures for 1 h.

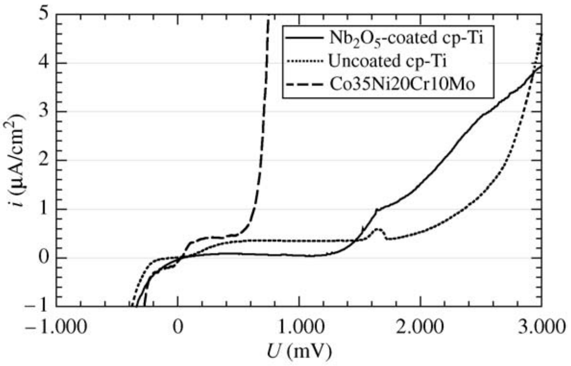

Figure 5 Current density-potential curves of coated and uncoated cp-titanium as compared to Co35Ni20Cr10Mo.

exothermic reactions due to the combustion of H and C from the organic constituent [10]. The small exothermic peak at 550 °C, which is correlated with no weight loss, can be described by the phase transformation from amorphous to crystalline oxide. Above a temperature of 400 °C, no further exothermic peak and weight losses appear demonstrating that the removal of the organic contamination is completed. To confirm this conclusion, oxide powder was investigated by means of CHN analysis in order to detect the carbon content as a function of the annealing temperature (Fig. 3). The carbon content decreases from 8.1 wt % at 200 °C to 0.2 wt % at 400 °C attesting the purity of the oxide. The remaining low carbon content at this temperature might be due to hydrocarbons which are always present at the outermost oxide surface in normal atmosphere [11]. These results demonstrate that a heat treatment of at least 400 °C is necessary to prepare pure oxide layers without organic impurities.

Determined by means of ellipsometry, the oxide thickness amounts to 52 nm per coating cycle after drying and decreases to 38 nm after annealing at 500 °C for 1 h because of a densification. For the following investigations the samples were coated three times. The XRD measurements were carried out on coated samples heat treated at 400, 500, 600 and 700 °C for 1 h. As shown in Fig. 4, the diffraction patterns for the samples annealed at 400 and 500 °C show peaks only from the substrate indicating an amorphous structure of the oxide layer. After a heat treatment at 600 and 700 °C, besides the substrate peaks, X-ray reflexes of crystalline oxide, monoclinic Nb_2O_5 [12], are observed. After annealing at 700 °C, additional TiO_2 (rutile) peaks appear which might be the result of thermal oxidation. At elevated temperatures, oxygen from the air diffuses through the coating and causes oxidation of the substrate metal.

The corrosion resistance was investigated by means of current density-potential curves. In Fig. 5 the corrosion behaviour of Nb_2O_5-coated (600 °C, 1 h) and uncoated cp-titanium are compared to Co35Ni20Cr10Mo. The range of passivity of Nb_2O_5-coated and uncoated cp-titanium extends from 0 to about 1600 mV. The passive current density in this range amounts to 0.05 and 0.39 $\mu A/cm^2$, respectively, showing the improved corrosion resistance achieved by the oxide film of Nb_2O_5. For the corrosion samples of cp-titanium the region of transpassivity occurs above 1600 mV. The breakdown potential of Co35Ni20Cr10Mo (about 600 mV) is comparatively low and can easily be reached in the body in cases of an inflammation. The consequence would be an elevated, harmful current of ions to the surrounding tissue. This behaviour, which corresponds to the results of the literature, shows the superior corrosion properties of titanium and titanium alloys [13].

A high wettability of the implant surface is an important factor for a good spreading of the attached cells and therefore for an osseointegration [14]. The contact angle of the Nb_2O_5-coated cp-titanium (600 °C, 1 h) amounts to 24°, whereas the uncoated cp-titanium shows a higher value of 42°. In comparison, the contact angles of TiO_2 sol–gel coated cp-titanium and polished Co35Ni20Cr10Mo were measured to be 37° and 48°, respectively. The measured contact angles show that the Nb_2O_5-coated samples are more hydrophilic as compared to the other materials and therefore a better spreading of the cells can be assumed.

The results of the cell viability tests are shown in Fig. 6. The osteoblast migration after 48 h is represented in

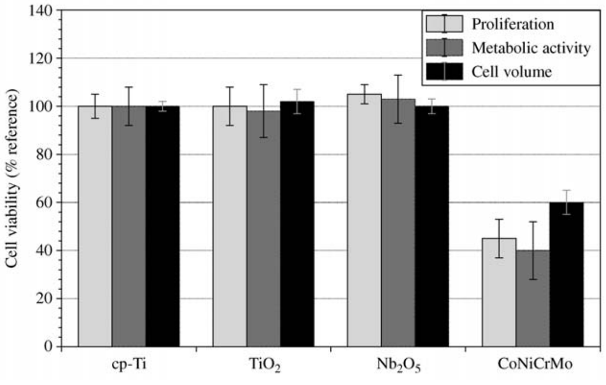

Figure 6 Viability of MC3T3-E1 osteoblasts on cp-titanium, TiO_2- and Nb_2O_5 coated cp-titanium as compared to Co35Ni20Cr10Mo ($n = 6$ tests).

Fig. 7 and the cell spreading is shown in Fig. 8. The cell viability tests resulted in a very good compatibility of the Nb_2O_5 layers produced by means of the sol–gel process. The proliferation and the metabolic activity is even slightly increased on the Nb_2O_5 oxide layer as compared to uncoated cp-titanium. The investigation of the cell migration, spreading and collagen I production show a lower migration rate on the Nb_2O_5-coated samples but a increased spreading of the osteoblast-like cells on the Nb_2O_5 coatings. Additional tests showed that the collagen I production of the osteoblasts is slightly increased on TiO_2 and highly increased on Nb_2O_5 as compared to uncoated cp-titanium. It takes the cells longer to cover a gap in a monolayer in contact with Nb_2O_5 as compared to the cell migration on uncoated cp-titanium and TiO_2 coated slices but the MC3T3-E1 cells show a better spreading and an increased collagen I production on the Nb_2O_5 coatings. Whether an influence on cellular adhesion and cell bonding on Nb_2O_5 coatings arises from these cell function tests must be verified with further investigations such as cell adhesion tests, adhesion kinetics assays and examination of the cytoskeleton.

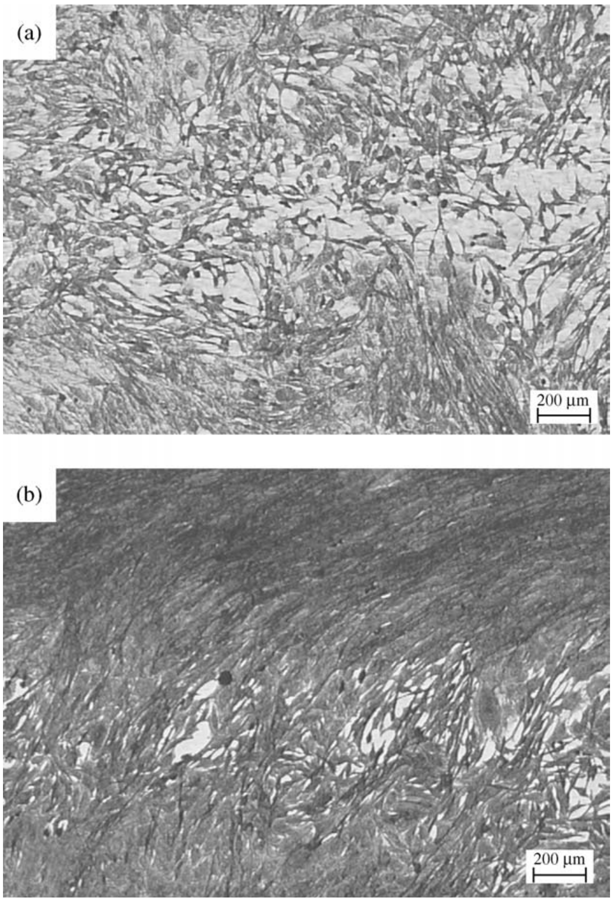

Figure 7 MC3T3-E1 migration on Nb_2O_5- (a) and TiO_2 (b) coated cp-titanium ($n = 3$ tests) after 48 h.

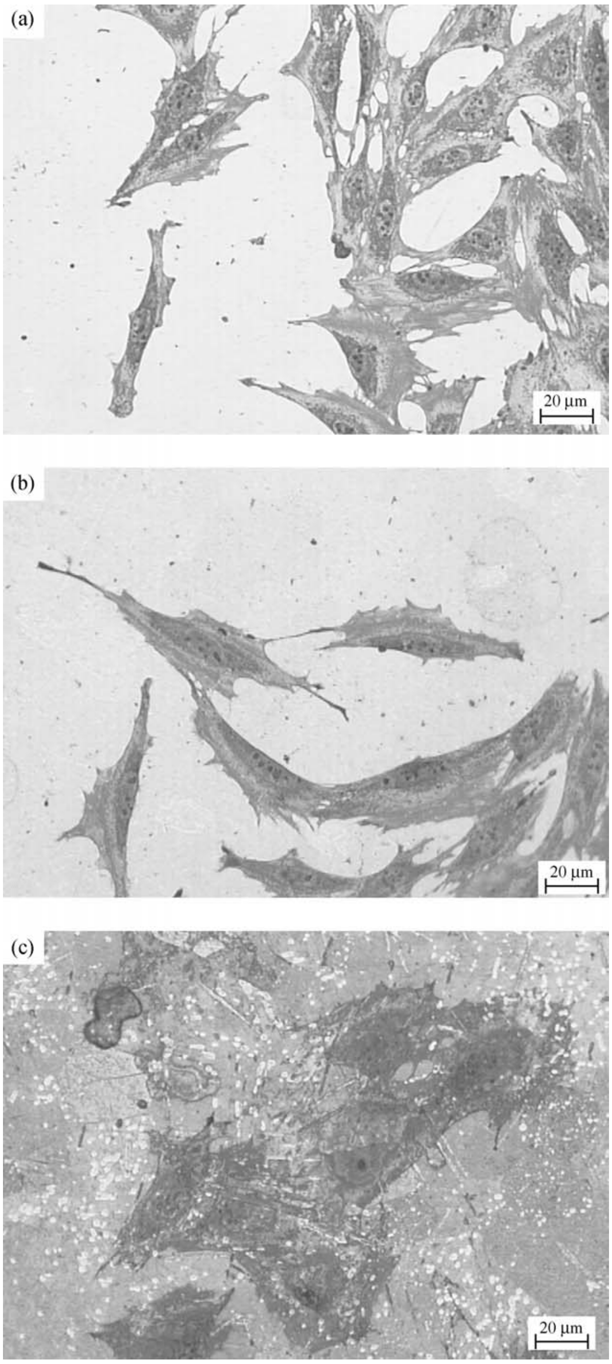

Figure 8 Osteoblast spreading ($n = 3$ tests) on cp-titanium (a), TiO_2- (b) and Nb_2O_5 (c) coated cp-titanium.

4. Conclusions

The implant performance can be improved by tailoring the surface properties relevant for a good adhesion of cells, achieving a stable anchoring to the bone. One such approach is the preparation of thin, biocompatible Nb_2O_5 layers on metallic biomaterials consisting of Ti prepared by means of the sol–gel process – a technique that offers many advantages such as a simple adjustment of the oxide properties and that of low costs. In this study, sol–gel-derived Nb_2O_5 layers were obtained with a high purity without organic impurities by optimising the process parameters. By repetition of the coating procedure the thickness of the layers can be varied. The structure (amorphous or crystalline) of the films can be adjusted by the heat treatment. The corrosion resistance of cp-titanium is enhanced by the Nb_2O_5 coating, leading to a reduced release of ions. The oxide layer improves the wettability of the surface as compared to uncoated cp-titanium and therefore, the spreading and adhesion of cells is improved. The biocompatibility of the oxide layers was confirmed by *in vitro* cell tests.

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