Biocompatibility of dielectric Ta$_2$O$_5$ coatings in $in$ $vitro$ tests

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Abstract. A study was performed on the impact of the structure and properties of e-beam evaporated Ta$_2$O$_5$ films on the cell/material response. The surface properties and structure the films were investigated by means of XPS and XRD. The cyto toxicity and cyto compatibility were estimated by $in$ $vitro$ tests. Other parameters, such as the surface free energy (SFE) and fractional polarity, were determined by means of the Wu, and Owens-Wendt-Rabel-Kaelble methods. The dependence was followed of the cells adhesive behavior on the coatings surface composition and parameters. The best biological response parameters (total cell number, detached cells percentage, proliferation ratio) were obtained in the case of annealed Ta$_2$O$_5$ coatings with stoichiometric composition and the largest values of the SFE polar part and the of the fractional polarity.

1. Introduction

The interest in electret materials and coatings applications has considerably increased in various areas of science and technology in recent years. The implants applied now for surgical treatment with dielectric coatings in an electret state create a normal biopotential in the osteosynthesis area that prevents atrophy and necrosis formation, bone tissue deformation and surface strains of large joints, thus reducing the treatment period and minimizing the postoperative complications. Electret coating deposition necessitates ensuring high purity and a definite stoichiometric composition of the dielectric coatings in the electret state. Thus, the major factors are the optimum regime of fabrication and precise control of the technological process.

Tantalum and tantalum-based compounds are promising for biomedical applications. Ta-based implants exhibit high fracture toughness, corrosion and wear resistance, and chemical stability. The results of test on implantation of Ta in both soft and hard tissue of rats showed the good biocompatibility and osteogenesis of this metal [1]. TaC and TaN materials posses relatively high hardness due to the covalent nature of their bond [2] and demonstrate high thermal stability and superior corrosion resistance [3]. The blood compatibility of TaN films was shown to be better than that of Ta [4].

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In vitro tests of cell adhesion on material and coating surfaces are the basic tools to determine the material surface/tissue response on a cellular level [5, 6]. The effects of materials composition, surface chemistry and surface topography on cell adhesion and proliferation have been studied extensively [7, 8]. The surface energy is another fundamental material property that can influence the cell behavior [9]. In this study, Ta pentoxide ceramic coatings were found to be promising materials for various biomedical applications.

2. Materials and methods

The samples were formed on glass substrates. The main parameters of the process were described in our previous study [10]. The evaporation process was carried out at initial vacuum of $9.3 \times 10^{-3}$ Pa, operational vacuum of $4 \times 10^{-3}$ Pa, anode current of 50 mA and calculated evaporation power of 350 W [11]. The deposition rate under these conditions was 28 nm/min. The layer thickness and the deposition rate were controlled by a digital thin-film deposition monitor MSV-1843/H MIKI-EEV operating at 6 MHz. The surface properties and structure of the e-beam evaporated Ta$_2$O$_5$ films were investigated by means of XPS and XRD. The X-ray photoelectron spectroscopy measurements were carried out using ESCALAB MkII (VG Scientific) electron spectrometer at a base pressure in the analysis chamber of $5 \times 10^{-10}$ mbar (during the measurement $1 \times 10^{-8}$ mbar), using an AlK$_\alpha$ X-ray source (excitation energy $h\nu = 1486.6$ eV). The instrumental resolution measured as the full width at half maximum (FWHM) of the Ag3d5/2 photoelectron peak was 1 eV. The energy scale was corrected to the C1s - peak maximum at 285 eV for electrostatic charging.

Analysis was carried out of surface parameters, such as the surface free energy and the fractional polarity. The contact angles were measured by means of a tensiometric technique. Prior to contact angle measurements, the samples were ultrasonically cleaned in acetone and deionized water and dried. The advancing contact angle was measured by means of the Wilhelm’s technique (Kruss K12) at temperature 20 °C [10]. Standard liquids with well-known values of surface tension, component of dispersion and polar interaction were used. The surface free energy (SFE) and its polar and dispersion components were determined by means of the Wu [12] and the Owens-Wendt-Rabel-Kaelble [13] methods.

The in vitro experiments on cytotoxicity and cytocompatibility were carried out in a culture of fibroblasts. During cell cultivation on coated samples, the cell cytology, morphology and proliferation activity were determined by means of optical microscopy after 24 h and 3 and 5 days cultivation. Rat hypodermal cellular tissue was extracted to obtain the initial fibroblast culture. The suspension of extracted cells was centrifuged at 750 rev/min for 15 min. The sowing cell area was $3 \times 10^5$ cell/ml density of cultural medium. The fibroblast cultivation in a 3 ml of Dulbecco Modified Eagle’s Medium (DMEM, Sigma) was made by using a monolayer culture in a thermostat (temperature 37 °C for 5 days). After cultivation, fixation in an acetic acid and methyl alcohol (1:3) solution and azure-eosin coloration, the cellular proliferation on the cover glasses was determined by optical microscopy (Micros). The experiments were triplicated.

3. Results and discussion

XPS survey spectra of the e-beam evaporated Ta$_2$O$_5$ films (initial, non-annealed sample; sample annealed at 450°C in O$_2$) were obtained. All spectra consist of well-defined XPS lines of Ta 4f, 4d, 4p and 4s; O 1s; C 1s. All binding energies of the high-resolution spectra were calibrated with the C 1s binding energy of 285.0 eV. Figure 1 shows the high-resolution Ta 4f and O 1s XPS spectra of the structures investigated. Ta 4f doublets are typical for e-beam evaporated Ta$_2$O$_5$ and have two peaks: Ta 4f7/2 at ~ 26.3-26.6 eV and Ta 4f5/2 whose binding energy higher by 1.9 eV. The Ta 4f lines of the films deposited agree well with the Ta 4f doublet representative of the Ta-0 bond in Ta$_2$O$_5$. The Ta4f7/2 peak is located at 26.5 and 26.6 eV, and the Ta 4f5/2 one is at 28.4 and 28.5 eV for the annealed and non-annealed films, respectively. These results clearly demonstrate that annealing improves the stoichiometry of the Ta$_2$O$_5$ films. The O1s spectra further support this assumption. The O 1s peaks of the deposited layers are centered at binding energies of 530.9 and 530.8 eV for the annealed and non-annealed films, respectively, which is consistent with data reported for Ta$_2$O$_5$. The
FWHM of both peaks is 1.9 eV. The O/Ta ratio estimated from the spectra is ~ 3 for all samples. The annealing process changes the Ta$_2$O$_5$ coatings structure from amorphous one (for as-deposited films) to an orthorhombic phase, the surface topography, from smooth to nano-crystalline (up to 20 nm) and improves the crystallinity of the Ta$_2$O$_5$ films. At the same time, the dielectric constant of the films increases from 17 to 28 with the increase of the annealing temperature due to the improvement in the film crystallinity and packing density [14].

![Figure 1. High-resolution XPS spectra of Ta 4f and O 1s spectra of e-beam evaporated Ta$_2$O$_5$ films.](image)

The calculation of the surface free energy of solids using contact-angle measurements is based on the surface energy balance condition: The values of the surface free energy and its polar and dispersion components, as calculated by the Wu method for two liquids and the Owens-Wendt-Rabel-Kaeble method for the liquid system α-bromonaphthalene- formamide-ethylene glycol-diiodomethane-glycerol-water were determined from contact angle measurements at 20°C (table 1).

| Component of surface free energy [mN/m] | Glass substrate | Ta$_2$O$_5$ non-annealed | Ta$_2$O$_5$ (annealed) |
|----------------------------------------|-----------------|--------------------------|-----------------------|
| Total $\gamma$                         | 61.7            | 34.9                     | 54.5                  |
| Dispersion part $\gamma^d$             | 30.9            | 26.8                     | 39.7                  |
| Polar part $\gamma^p$                  | 29.8            | 8.1                      | 14.8                  |
| Fractional polarity $\gamma^p/ (\gamma^d + \gamma^p)$ | 0.48            | 0.23                     | 0.27                  |

Breaking the surface bonds with oxygen by either thermal or plasma treatment results in shear in the more chemically stable hydrophilic surface region. After a-three-day stay in the culture, the fibroblast cells were well spread on both the control and coated surfaces. The cell morphology was typical for cells on a coated surface. The cell structural organization corresponded to the initial fibroblast. After five days of cultivation, the cell density increased for all samples. Most of the cells were ripe fibroblasts with strongly marked phenotype. The effect of the surface properties on cell proliferation was investigated by counting the cells attached to the glass and coating surfaces after incubation (table 2).

Previous studies have examined the effect of surface energy on cell functions such as adhesion, proliferation and differentiation. In some cases, the cell functions are enhanced on hydrophilic surfaces, in other cases, on hydrophobic. In our study, the SFE values were in the range 35 - 55 mN/m. The annealing process results in shear in the chemically stable hydrophilic surface region.

The more detailed study of the effect of the surface free energy on cell spreading and proliferation requires that one should account for the dispersive and polar components of surface free energy and
the fractional polarity [15]. The polar part of SFE changes from 8.1 for non-annealed to 14.8 for annealed films, while the fractional polarity changes from 0.23 to 0.27, respectively (table 2).

Fibroblast cells were well spread both on the glass (control) and the coated surfaces. Differences in the cell attachment and spreading on e-beam evaporated Ta$_2$O$_5$ coating surfaces was observed (table 2).

**Table 2.** Total cell number and detached cell percentage on the surface of Ta$_2$O$_5$ (non-annealed and annealed) coatings after 3 and 5 days of cultivation.

| Type of coatings            | 3 days Total number of cells | Number of detached cells and percentage | 5 days Total number of cells | Number of detached cells and percentage |
|-----------------------------|------------------------------|----------------------------------------|------------------------------|----------------------------------------|
| Glass (control)             | $2.34 \times 10^4 \pm 2.35 \times 10^3$ | $1.17 \times 10^3 \pm 1.77 \times 10^2$ | $3.77 \times 10^4 \pm 1.97 \times 10^3$ | $3.06 \times 10^3 \pm 2.15 \times 10^2$ |
| Ta$_2$O$_5$ (non-annealed)  | $2.18 \times 10^4 \pm 2.32 \times 10^3$ | $1.57 \times 10^3 \pm 1.53 \times 10^2$ | $3.48 \times 10^4 \pm 1.72 \times 10^3$ | $3.94 \times 10^3 \pm 3.36 \times 10^2$ |
| Ta$_2$O$_5$ (annealed)      | $2.62 \times 10^4 \pm 2.67 \times 10^3$ | $1.78 \times 10^3 \pm 2.05 \times 10^2$ | $3.95 \times 10^4 \pm 2.02 \times 10^3$ | $2.92 \times 10^3 \pm 2.49 \times 10^2$ |

4. Conclusion
The biocompatibility of Ta$_2$O$_5$ films was estimated by in vitro tests. The results demonstrated the good cyto compatibility of e-beam evaporated Ta$_2$O$_5$ coatings especially in the case of annealed films with good stoichiometric Ta$_2$O$_5$ composition (table 2). The best biological response parameters (cell number, proliferation function, morphology) were obtained in the case of materials with the largest values of the SFE polar part component and of the fractional polarity. The results show that the surface properties are strongly influenced by the preliminary treatment. By varying the deposition and treatment conditions, one can control the surface parameters of the e-beam evaporated Ta$_2$O$_5$ films and the cell response.

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