Surface parameters modification by multilayer coatings deposition for biomedical applications

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Abstract. Studies are presented of the surface parameters of various multilayer coatings, namely, TiN, CrN, (Ti,Cr)N, TiN/TiC10N90, TiN/TiC20N80 deposited by means of Arc-PVD on stainless steel (1H18N9), as well as of the same coatings with an additional Al2O3 film deposited by reactive magnetron sputtering (RMS). The surface thickness, roughness and topography are estimated. Other parameters, such as the surface free energy (SFE) and fractional polarity are determined by means of the Wu and the Owens-Wendt-Rabel-Kaelble methods. Experiments are carried out on the in vitro cell/material interaction (in a fibroblasts culture) in order to determine the materials biomedical response. The results show some correlation between the surface properties and cell adhesion. The best biological response parameters (cell number, proliferation function, morphology) are obtained in the case of coatings with the highest values of the polar part component of the SFE and the fractional polarity, such as TiN, TiN/TiC10N90 and oxide coatings.

1. Introduction
Nearly all materials used for dental or orthopedic prostheses and coronary stents are pure metals or metal alloys. One way of solving the problem of improving the characteristics of metal prostheses is the deposition of multifunctional coatings on their working parts. Oxide and nitride multilayer coatings have been used for orthopedic and dental implants due to their high hardness and wear and corrosion resistance. The biocompatibility of these biomaterials is tested before surgical implantation. One criterion for the suitability of a given material for bio-applications involving close tissue contact is the physicochemical reactivity of the surface. In vitro studies of cell adhesion on various material and coating surfaces are the basic tools to determine the material surface/tissue response on a cellular level [1,2]. The effects of the materials composition, surface chemistry and surface topography on the cell adhesion and proliferation have been largely studied. The substrate roughness affects significantly
the cell attachment, adhesion, proliferation and differentiation [3]. The surface energy is also a fundamental property that can have influence on the cell behavior [4]. The aim of the present study was to perform a comparative analysis of the surface parameters of various types of multilayer coatings with respect to the direct cell adhesion, spreading and proliferation on the tested surface in in vitro tests.

2. Materials and methods
The substrates used for coatings deposition were stainless steel 1H18N9 samples. The substrates were cleaned in an ultrasonic bath by means of a standard technology. The TiN, CrN, (Ti,Cr)N, TiN/TiC10N90, TiN/TiC20N80 coatings were deposited by Arc-PVD. The process was described in detail in our previous study [5]; its main parameters were as follows: reactive atmosphere N2, deposition pressure 8×10⁻² Pa, arc current 100 A, substrate bias 150 V, deposition temperature 593 K, thickness of deposited coatings 2 - 5 μm. The Al2O3 coating deposition was performed by reactive magnetron sputtering (RMS) in a high-vacuum pumping system at a pressure of about 10⁻⁵ mBar. The main process parameters were: magnetron discharge power 1-8 kW, oxygen source power 1 kW, deposition rate 8 μm/hour [6]. The surface topography and adhered cell morphology was observed by scanning electron microscopy (SEM, S-450, Hitachi, Japan) and atomic force microscopy (AFM Q-Scope-250, Quesant Instrument Corporation, USA).

An analysis of was made of the surface parameters, namely, surface roughness, surface free energy, fractional polarity. The surface roughness was measured by means of a Hommel T-2000 profilometer. The roughness and thickness values of the coatings investigated are presented in table 1.

| Symbol of coatings | Parameters of roughness [μm] | Thickness [μm] |
|--------------------|-----------------------------|----------------|
|                    | Rₐ                     | Rₜ                     | R₉                     |                      |
| (TiCr)N            | 0.140                  | 3.000                  | 1.370                  | 1.50                 |
| (TiCr)N/Al₂O₃      | 0.111                  | 1.723                  | 0.903                  | 3.70                 |
| CrN                | 0.060                  | 1.153                  | 0.533                  | 1.45                 |
| CrN/Al₂O₃         | 0.053                  | 1.263                  | 0.473                  | 3.40                 |
| TiN                | 0.097                  | 2.300                  | 0.853                  | 1.80                 |
| TiN/Al₂O₃        | 0.250                  | 4.043                  | 1.660                  | 3.90                 |
| TiN/TiC₁₀N₉₀     | 0.063                  | 1.837                  | 0.460                  | 1.50                 |
| TiN/TiC₁₀N₉₀/Al₂O₃ | 0.150                 | 2.550                  | 1.263                  | 3.50                 |
| TiN/TiC₂₀N₈₀     | 0.180                  | 4.343                  | 1.783                  | 1.57                 |
| Steel 1H18N9     | 0.040                  | 0.890                  | 0.237                  | 2.37                 |

The contact angles were measured by means of a tensiometric method. Prior to these measurements, the samples were ultrasonically cleaned in acetone and deionized water and dried. The advancing water contact angle was measured by Wilhelm’s method (Kruss K12) at a temperature 20°C [6]. Standard liquids were used with well-known values of the surface tension, and the dispersion and polar interaction components, such as water, formamide, diiodo methane, ethylene glycol, α-bromo naphthalene and glycerol. The surface free energy (SFE) and its polar and dispersion components were determined by means of the Wu [7] and Owens-Wendt-Rabel-Kaelble [8] methods.

The experiments on cytotoxicity and cytocompatibility were carried out in vitro, in a culture of fibroblasts. In process of cell cultivation with coated samples, the cell cytology, morphology and proliferation activity were determined after 24 h and 3 and 5 days of cultivation. Rat hypodermic cellular tissue was extracted to obtain the initial fibroblast culture. The suspension of extracted cells was centrifuged at 750 rev/min during 15 min. The sowing cell area was 3×10⁵ cell/ml density of cultural medium. The fibroblast cultivation with 3 ml of Dulbecco Modified Eagle’s Medium
(DMEM, Sigma) supplemented with 10% fetal calf serum (FCS), 80 mg/ml penicillin, 100 mg/ml streptomycin was carried out by a mono-layer culture method in a thermostat (temperature 37 °C in 5% CO₂ atmosphere for 5 days). The cell adhered to the samples were cleaned by a buffer solution (pH 7.2) and doubly distilled water and fixed at 2.5% glutaraldehyde on a 0.1 M buffer solution during 2 h and a 1% OsO₄ solution during 1 h. The samples were then dehydrated in a grade series of alcohol. The cellular morphology on the different coated samples was examined by SEM and AFM. Other samples with the adhered cells were trypsinized with 0.01% trypsin/ 0.5 mM EDTA. The cell proliferation on the different biomaterials was determined by haemocytometry. The experiments were triplicated independently.

3. Surface free energy (SFE) estimation

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

Table 2. The values of the total surface free energy, dispersion, polar components, and fractional polarity.

| Symbol of coatings | Component of surface free energy [mN/m] |  
|--------------------|------------------------------------------|
|                    | Disp. part \( \gamma^d \) | Pol. part \( \gamma^p \) | Total \( \gamma \) | Fractional polarity \( \gamma^p / (\gamma^d + \gamma^p) \) |
| (TiCr)N            | 41.2                       | 3.9                       | 45.1                   | 0.086 |
| (TiCr)N/Al₂O₃      | 33.0                       | 9.1                       | 42.1                   | 0.22  |
| CrN                | 38.6                       | 4.0                       | 42.6                   | 0.093 |
| CrN/Al₂O₃          | 30.8                       | 7.7                       | 38.5                   | 0.2   |
| TiN                | 39.0                       | 7.0                       | 46.0                   | 0.152 |
| TiN/Al₂O₃          | 34.8                       | 9.9                       | 44.7                   | 0.22  |
| TiN/TiC₁₀N₉₀       | 39.7                       | 8.2                       | 47.9                   | 0.17  |
| TiN/TiC₁₀N₉₀/Al₂O₃ | 31.6                       | 9.9                       | 41.5                   | 0.24  |
| TiN/TiC₂₀N₈₀       | 39.2                       | 4.7                       | 43.9                   | 0.107 |
| TiN/TiC₂₀N₈₀/Al₂O₃ | 33.2                       | 7.1                       | 40.3                   | 0.18  |

The advancing water contact angles of the samples were in the range 60-70 degrees at standard condition and the surface energies values changed from 40-50 mN/m depending on the material and its surface properties (table 2). The polar part of SFE and the fractional polarity vary from 3.9 – 4.7 (0.086-0.107) for (TiCr)N and TiN/TiC₁₀N₉₀ to 7.0-8.2 (0.152-0.17) for TiN, TiN/TiC₁₀N₉₀ and 9.9(0.24) for oxide coatings.

4. Cytocompatibility in vitro

Figure 1 shows the adhered cell morphology for TiN, TiN/TiC₁₀N₉₀, TiN/TiC₂₀N₈₀ coatings after 5 days cultivation. The cell counts by hemocytometry demonstrated the characteristic features of cell spreading and proliferation on the control and sample surface after 1-, 3- and 5-day stay in the culture. The fibroblast cells were well spread both on the control and on the coated surfaces. The cell morphology was typical for cells on a coated surface. The fibroblast cells had polygonal forms - spindle and oblong with cytoplasm micro-filaments. The nucleus was large with chromatin. There existed a large number of cells at the mytios stage. Moreover, a large amount of naked nuclear cell clumps was observed for the TiN/TiC₂₀N₈₀ coatings. Differences were detected in the cell.
attachment and spreading on various coating surfaces. The mean cell counts per unit differed from the control \((n = 78)\), namely, the cell numbers increased on TiN \((n = 93)\), and decreased on TiN/TiC_{20N_80} \((n = 50)\).

![Figure 1. The cell morphology by AFM (a-TiN coatings, b-TiN/TiC_{10N_90}, c-TiN/TiC_{20N_80}) after 5 days cultivation.](image)

5. Results and discussion

Previous studies have examined the surface energy effect 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. The average cell area after 24 h has a maximum at a contact water angle of 60° [9]. In our study, the values measured of the water contact angle were in the range 60-70°, and the values of evaluated SFE, in the range 40-50 mN/m. This is the intermediate region between hydrophobic and hydrophilic surfaces. A possible explanation of the similar cell behavior on the various multilayer and oxide coating surfaces with close roughness parameters (table 1) may be in the fact of the intermediate values of SFE for such coatings. A more detailed study of the SFE effect on cell spreading and proliferation requires that one should account for the disperse and polar components of the SFE and the fractional polarity [10]. The polar part of SFE and fractional polarity changes from 3.9 – 4.7 (0.086-0.107) for (TiCr)N and TiN/TiC_{20N_80} to 7.0-8.2 (0.152-0.17) for TiN, TiN/TiC_{10N_90} and 9.9(0.24) for oxide coatings (table 2). The fibroblast cells were well spread both on the control and the coated surfaces. The cell morphology was typical for cells on surface for all coating types, except TiN/TiC_{20N_80}. Differences in the cell attachment and spreading on various coating surfaces were detected (figure 1). The cell number on TiN was the highest with respect to the control, whereas the cell number of TiN/TiC_{20N_80} was the lowest.

6. Conclusion

The initial cell behavior on the biomaterial interface will influence the cell differentiation, proliferation and extra cellular matrix formation. The surface topography, roughness, energy and chemistry are the main factors affecting the cell growth and function. We performed a comparative analysis of the cell adhesion on the surface of the samples with multilayer coatings and a study of the surface parameters influence on the cell/material interaction in in vitro tests. The results show some correlation between the surface properties and cell adhesion. The best biological response parameters (cell number, proliferation function, morphology) were obtained in the case of coatings with the highest parameters of the polar part component of SFE and the fractional polarity, such as TiN, TiN/TiC_{10N_90} and oxide coatings. Higher surface energy and fractional polarity result in a greater number of attached fibroblast cells, and higher cell activity and proliferation ratio.

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