# THE INFLUENCE OF SURFACE PARAMETERS OF COATINGS DEPOSITED BY VARIOUS VACUUM-PLASMA METHODS ON THE CELL/MATERIAL INTERACTION IN VITRO TESTS

*A. Zykova, V. Safonov[1], V. Luk'yanchenko[2], J. Walkowicz[3], R. Rogowska[3]*

*Institute of Surface Engineering, Kharkov, Ukraine, e-mail: zykov@bi.com.ua;*
*[1]NSC "Kharkov Institute of Physics and Technology", Kharkov, Ukraine;*
*[2]N.I.Sitenko Orthopedy and Trauma Surgery Institute Design Bureau, Kharkov, Ukraine;*
*[3]Institute for Sustainable Technologies, National Research Institute, Radom, Poland*

The surface of an implant plays a basic role in determining biocompatibility and integration due to its direct contact with the adjacent tissues. There is now the good understanding that life time of orthopedic implants and the design of the novel tissue-engineering medical products may be associated with local and remote biological tissue response and cell-material interaction. Material properties such as implant surface composition, roughness, topography can influence events at bone –implant interfaces and cell response to implant material.
PACS: 87.80 Rb

## 1. INTRODUCTION

In order to accelerate tissue-engineering research and to obtain artificial implants with enhanced physical, chemical, mechanical properties it's necessary to combine the advantages of materials nature with biocompatibility, bioactivity, high wear and corrosion resistance, low elastic modulus and friction coefficient. The way to the solution of the problem of load-bearing prosthesis producing is the deposition of multifunctional coatings on their working parts. $TiO_2$ titanium dioxide is widely used in biomedical application because its excellent biocompatibility [1]. TiN titanium nitride has been used for orthopedic and dental implants due to its high hardness, wear and corrosion resistance [2]. 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 [3,4]. The effects of materials composition, surface chemistry and surface topography on cell adhesion and proliferation have been largely studied [5].The material composition always influences cell attachment [6]. The roughness of substrate also significantly effects on cell attachment, adhesion, proliferation and differentiation [7]. Attachment is generally increased on rough surfaces compared to smooth ones [8] but sometimes no effects are shown [9]. The surface energy is also the fundamental material property that can influence on cell behavior [10]. The aim of the present study was the comparative analysis of cell adhesion on the surface of load bearing metal materials with ceramic coatings in vitro tests.

## 2. MATERIALS AND METHODS

The substrates for deposited coatings were titanium (Ti, Fe- 0,2% Si- 0,1%) samples. The substrates were cleaned in ultrasonic bath with standard technology. Various types of coatings were deposited: a) nitride coatings TiN, b) oxide $Al_2O_3$ by Plasma Spray Method (PS), c) oxide- films $TiO_2$ by electrochemical method, d) oxide $Al_2O_3$ by means of Magnetron Sputtering Method (MS). The TiN coatings were deposited by means Arc-PVD Method. The main parameters of the process were described in our previous study [11]. The method of ceramic $Al_2O_3$ (PS) coating deposition was typical: on the treated by means of sand-blasting and anodized methods titanium surface the corundum powder was sprayed at room temperature. The electrochemical treatment of titanium samples in salt solution of disubstitiuted sodium sulphate medium at different stabilize voltage values from 10 to 100 V in DC regime was made. $Al_2O_3$ (MS) coating deposition was performed in high vacuum pumping system with the base pressure about $10^{-5}$ mBar. A schematic description of the magnetron and ion source in the sputtering chamber is shown in Fig.1.

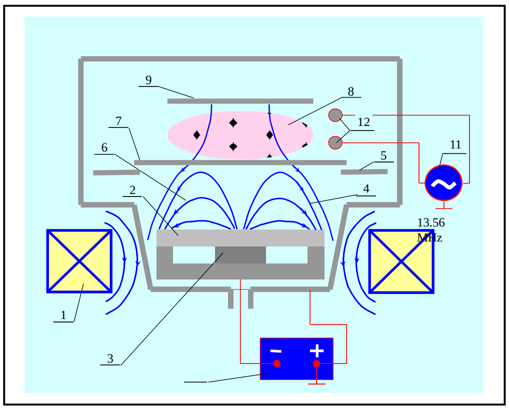



*Fig.1. Scheme of the magnetron installation: 1-coil of the magnetic field; 2 – target; 3- permanent magnet; 4- magnetic force lines; 5-protective screen; 6- magnetron plasma; 7- swivel damper; 8- RF-ECR plasma; 9- substrate holder; 10- DC voltage source; 11-RF generator; 12- inductive coil*

The analyses of surface parameters such as surface roughness, wettability, surface free energy were made. The surface roughness was measured by means of profilometer Hommel T-2000. Advancing water contact

 Problems of Atomic Science and Technology. 2007, № 1. Series: Plasma Physics (13), p. 200-202

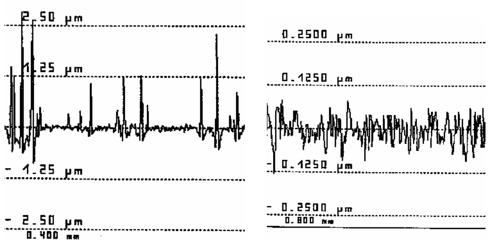
angle was measured by Wilhelm's method in Kruss K12 Tensiometer at temperature 20° C [12]. For next calculations of surface free energy (SFE) were used the values of advancing water contact angle by Roberson equation [13,14], which is in a good agreement with values obtained by means of Owens-Wendt-Rabel-Kaeble' and Van Oss' methods for TiN surfaces [12].

The experiments on study of cell/material interaction *in vitro* – in culture of fibroblasts were carried out. Rat hypodermic cellular tissue was extracted for initial fibroblast culture obtaining. The suspension of extracted cells was centrifuged at 750 orb/min during 15 min. The estimation of the total cell number and detached cell number was made by means of 0,1% trypan solution. Sowing cell area was $2x10^4$ cell/ml density of cultural medium. The fibroblast cultivation was made by methods of mono layer culture at thermostat condition (temperature 37° C) during 7 days. The isolated cells were seeded onto the sample surface after sterilization. After incubation for 1,3,5,7 days samples were removed and assayed for next cell account study. The terms 5-7 days are the step from fibroblast stabile culture growth to culture degeneration stage. After cultivation the adhered cells were trypsinized using trypsin- EDTA. The data were elaborated by standard variation statistical methods. Experiments were independently triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1. SURFACE STRUCTURE AND PROPERTIES

The characteristic features of coating roughness were presented at Fig .2 for TiN and $Al_2O_3$ (MS) coatings. The roughness data for all samples are presented in the table bellow. The increasing of oxidation potential from 20 V to 100 V for $TiO_2$ coatings results in the roughness parameter rise.




*a* *b*

*Fig.2. Surface roughness coatings of: a) TiN (Arc PVD), b) $Al_2O_3$*

Advancing water contact angle was measured by Wilhelm's method at temperature 20° C [12]. For next calculations of surface free energy (SFE) were used the values of advancing water contact angle by Roberson equation [13,14], which is a good approximation for hydrophobic surfaces. The advancing water contact angles of the samples were in the range 60-70 degrees at standard condition and surface energies values change from 40-50 mN/m according to the material and surface properties (see the table).

### 3.2. CYTOCOMPATIBILITY IN VITRO

The adhesive activity of cells in vitro test was investigated for next modeling cell /coating interaction.

*The surface properties of $TiO_2$, TiN, $Al_2O_3$ coatings and glass*

| Coatings type | Coating properties | | Surface properties | |
|---|---|---|---|---|
| | Thick-ness, µm | Rough-ness, Ra, µm | Water contact angle, degree | Surface free energy, SFE, N/m |
| $TiO_2$ (20 V) | 0,5 | 0,1 | 65.91 | 42,12 |
| $TiO_2$ (30 V) | 0,8 | 0,15 | 64,80 | 42,80 |
| $TiO_2$ (50 V) | 1,0 | 0,22 | 58,44 | 46,58 |
| $TiO_2$ (70 V) | 1,2 | 0,26 | 57,85 | 46,91 |
| $TiO_2$ (100 V) | 1,5 | 0,3 | 55,85 | 48,07 |
| Glass | | 0,02 | 84,8 | 29,9 |
| TiN | 1,5 | 0,09 | 67,15 | 41,43 |
| TiN | 1,8 | 0,12 | 69,50 | 39,98 |
| $Al_2O_3$ (PS) | 35 | 2,4 | 76,74 | 35,31 |
| $Al_2O_3$ (PS) | 30 | 2,2 | 77,85 | 34,5 |
| $Al_2O_3$ (MS) | 2,2 | 0,04 | 58,7 | 46,42 |
| $Al_2O_3$ (MS) | 1,9 | 0,03 | 61,15 | 44,98 |

The ratio of the detached cell number $N_d$ to the total cell number $N_t$ may be approximated by equation $ct^b$, (where t- time in culture, c – scale coefficient and b- kinetic coefficient b ≈ 0,5). The model of cell/material interaction and the analytical expression for $t_d$ (time taken by the trypsin to detach the cell) which finally describes the adhesion of cells on biomaterials after a time t in culture (without proliferation) was proposed in [15].

Fig. 3 shows the proliferation kinetics of fibroblast cells. The proliferation ratio (PR, number of cells after 7 days in culture/ number of cells after 1day) was used for most statistical analysis. The best results were obtained in the case of oxide coatings: $TiO_2$ at 100V, $Al_2O_3$ (PS) and $Al_2O_3$ (MS).

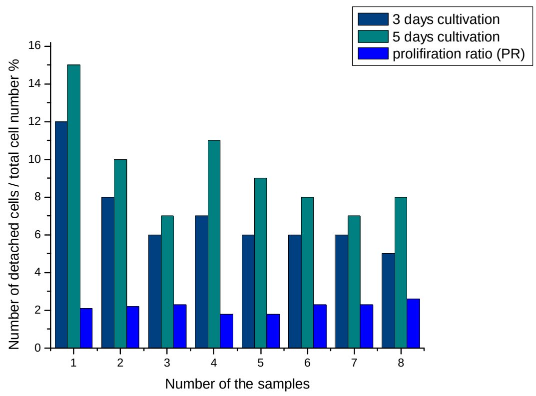



*Fig.3. Ratio of the number of detached cell to the total cell number after 3 and 5 days cultivation (%) and cell proliferation ratio PR (the total cell number after 5 days cultivation/the total cell number after 1 day cultivation) for the samples 1,2,3 –$TiO_2$ 20V,50V,100V, 4,5- TiN (PVD), 6 $Al_2O_3$ (PS),7-$Al_2O_3$ (MS),8 –glass*

## 4. CONCLUSIONS

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 which adjust cell growth and function. The analysis of cell adhesion on the surface of the samples with ceramic coatings and study of surface parameter influence on cell/ material interaction in vitro tests was made.

The results show some correlation between the surface properties and cell adhesion. The best biological response parameters (total cell number, PR) were obtained in the case of oxide coatings deposited by various methods in comparison with $TiO_2$ at 20V potential and TiN coatings (Fig. 4). The dependence between surface parameters (roughness, SFE) and adhesive behavior has been observed only in the case of oxidation process parameter changes. The increasing of the process potential from 20 to 100 V leads to roughness and SFE parameter rise (see the table above). The greater surface roughness, higher surface energy results in greater total number of attached fibroblast cells , higher cell activity and proliferation ratio (Fig. 3)

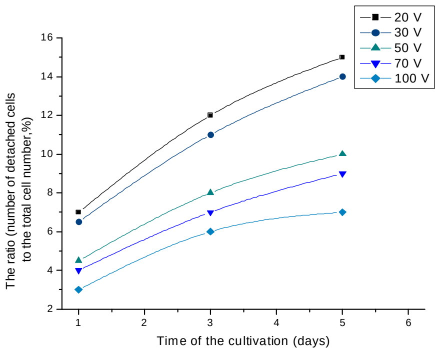



*Fig.4. The ratio of the detached cell number to the total cell number (%) for the $TiO_2$ coatings after cultivation time (days)*

Use of modern advancing methods of multifunctional coating deposition allows improving the biocompatibility of implanted materials and prolonging prosthesis service life in the patient organism.

## REFERENCES

1. C. Lin, S. Yen //*J. Electrochem. Soc.* 2004, v.51, p.127-133.

2. S. Mandl, B. Rauchenbach // *Surf. & Coating Technol.* 2002, v.156, p.583-589.

3. U. Meyer, A. Bushter, H.P. Wiesmann, U. Joos, D.B. Jones // *Eur. Cells Mater* . 2005, N 9, p.39-49.

4. K. Anselme // *Biomaterials.* 2000, v. 21, p.667-681.

5. A. Hunter, C.W. Archer, P.S. Walker, G.W. Blunn. Attachment and prolifiration of osteoblasts and fibroblasts on biomaterials for orthopedic use // *Biomaterials.* 1995, v.16, p.287-295.

6. R.K. Sinha, F. Morris, S.A. Shah, R.S. Tuan. Surface composition orthopedic implant metals regulates cell attachment, spreading and cytoskeletal organization of primary human osteoblasts in vitro // *Clin. Orthop. Relat. Res.* 1994, v. 305, p.258-272.

7. J. Links, B.D. Boyan, C.R. Blanchard, C.H. Lohmann, Y. Liu, D.I. Cochran et al. Responce of MG63 osteoblast-like cells to titanium and titanium alloy is dependedon surface roughness and composition // *Biomaterials.* 1998, v. 19, p.2219-2232.

8. S.P. Xavier, P.S. Carvalho, M.M. Beloti, A.L. Rosa. Response of rat bone marrow cells to commercially pure titanium submitted to different surface treatments // *J. Dent.* 2003, v. 31, p.173-180.

9. K. Mustafa, J. Wroblewski, K. Hultenby et al. Effect of titanium surfaces blasted with TiO2 particles on the initial attachment of cells derived from human mandibular bone // *Clin. Oral Implant Res.* 2000, N 11, p.116-128.

10. P. Van der Valk, A.W.J. van Pelt, H.J. Busscher, H.P. de Long et al. Interaction of fibroblasts and polymer surfaces, relation between surface free energy and fibroblast spreading // *J. Biomed. Mater. Res.* 1983, v. 17, p.807-817.

11. A.V.Zykova, V.V. Luk'yanchenko, V.I. Safonov. The corrosion properties of implanted materials with protective coatings in isotonic physiological solution // *Surf. & Coating Technol.* 2005, v. 200, p.90-93.

12. R. Rogowska. Surface free energy of thin-layer coatngs deposited by means of the arc-vacuum method // *Problemy Eksploatacji* (*Maintenance Problems*). 2006, v. 61, N2, p.193.

13. S.B. Kennedy, R.N. Washburn, C.G. Simon, E.J. Amis. Combinatorial screen of effect of surface energy on fibronectin-mediated osteoblast adhesion, spreading and proliferation // *Biomaterials.* 2006, v. 27, p.3817-3824.

14. S.V. Roberson, A.J. Fahey, A. Sehgal, A. Karim. Multifunctional ToF-SIMS combinatorial mapping of gradient energy substrates // *Appl. Surf. Sci*. 2002, v. 200, p.150-164.

15. K. Anselme, M. Bigerelle. Modelling approach in cell/material interaction studies // *Biomaterials.* 2006, v. 27, p.1187-1199.

## ВЛИЯНИЕ ПОВЕРХНОСТНЫХ СВОЙСТВ ПОКРЫТИЙ, НАНОСИМЫХ РАЗЛИЧНЫМИ ВАКУУМ-ПЛАЗМЕННЫМИ МЕТОДАМИ, НА ПРОЦЕСС ИХ ВЗАИМОДЕЙСТВИЯ С КЛЕТОЧНЫМИ СТРУКТУРАМИ IN VITRO

***А. Зыкова, В. Сафонов, В. Лукъянченко, Я. Валькович, Р. Роговская***

Поверхностные свойства функциональных покрытий, широко применяемых в современной медицине, играют определяющую роль при оценке их биосовместимости. Срок службы существующих эндопротезов и дизайн новых биоинженерных разработок непосредственно связаны с локальными и отдаленными последствиями взаимодействия биоматериалов и биологических тканей. Поверхностные характеристики, такие как состав, шероховатость, поверхностная энергия оказывают прямое воздействие на процесс взаимодействия с тканями организма на клеточном уровне.

## ВПЛИВ ПОВЕРХНЕВИХ ВЛАСТИВОСТЕЙ ПОКРИТТІВ, ЩО НАНЕСЕНІ РІЗНИМИ ВАКУУМ-ПЛАЗМОВИМИ МЕТОДАМИ, НА ПРОЦЕС ЇХ ВЗАЄМОДІЇ З КЛІТИННИМИ СТРУКТУРАМИ IN VITRO

***Г. Зикова, В. Сафонов, В. Лук'янченко, Я. Валькович, Р. Роговська***

Поверхневі властивості функціональних покриттів, що широко застосовані у сучасній медицині, мають визначне значення при оцінці їх біосумісності. Термін використання існуючих ендопротезів та дизайн нових біоінженерних розробок безпосередньо зв'язані з локальним та віддаленим впливом взаємодії біоматеріалів та біологічних тканин. Поверхневі характеристики, такі як склад, шорсткість, поверхнева енергія мають прямий вплив на процеси взаємодії з тканинами організму на клітинному рівні.