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EXPERIMENTAL STUDY

Cytocompatibility of implants coated with titanium nitride and zirconium nitride

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Abstract: *Introduction:* The positive cell response to the implant material is reflected by the capacity of cells to divide, which leads to the tissue regeneration and osseointegration. Technically pure titanium and its alloys are mostly used for implant manufacturing. These alloys have the adequate mechanical, physical and biological properties; nevertheless, the superior biocompatibility of bioceramics has been proven. With the arrival of new coating techniques, surface modification of materials used for implants has become a widely investigated issue. *Methods:* The paper studied properties of titanium nitride (TiN) and zirconium nitride (ZrN) coatings deposited by PVD (Physical Vapour Deposition). Coatings were applied to substrates of pure titanium, Ti6Al4V, Ti35Nb6Ta titanium alloys and CoCrMo dental alloy. Different treatments of substrate surfaces were used: polishing, etching and grit blasting. Cytocompatibility tests assessed the cell colonization and their adherence to substrates. *Results and conclusion:* Results showed that TiN layers deposited by PVD are suitable for coating all substrates studied. The polished samples and those with TiN coating exhibited a higher cell colonization. This coating technique meets the requirements for the biocompatibility of the implanted materials; furthermore, their colour range solves the issue of red aesthetics in oral implantology as the colour of these coatings prevents titanium from showing through the gingiva. This is one the most important criteria for the aesthetic success of implant therapy *(Tab. 5, Ref. 18)*. Text in PDF *www.elis.sk.*
Key words: titanium nitride and zirconium nitride coatings, cytocompatibility, bioceramics.

Introduction

In implantology, physical and chemical properties of materials play an important role. The strength of the material in the given dimension with respect to the reduced diameter of an implant while keeping the mechanical and physical properties is very important. Biocompatibility and in this case namely cytological properties are extremely important. In addition, the interaction between cells and the surface of the tested material plays an important role together with the question whether the basic physiological functions, i.e. capacity of the cytoskeleton to regenerate after the inoculation and capacity of cells to divide, have not been damaged. The character of the cell-material interaction may be manifested variously, not only as described in the relevant testing standards (10). The material may be neither cytotoxic nor it exhibits any mutagenic behaviour (clastogenic effect or positive gene tests) and still it may be unsuitable due to the disrupted relationship between the cells and the tested material. It depends on how the cells accept the material or material surface, how the cytoskeleton cells respond in terms of the cytoskeleton regeneration, and if the cell response to the material is positive, and to what extent cell division is maintained or limited. These are the principle parameters roughly indicating the osseointegration capacity.

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Further, we have a possibility to modify the surface of implant materials (2, 8, 11). In terms of biocompatibility, bioceramics has been confirmed to be a more suitable material than widely used technically pure titan or its alloys (1, 4, 6, 7). The material used previously was, for example, hydroxyapatite applied on metal implants. In a long-term perspective, the adhesion of the hydroxyapatite coating layer was not sufficient (3). The method for depositing coatings has been innovated. The PVD (Physical Vapour Deposition) method is one of possible techniques for coating biocompatible materials. This technique enables to form a nearly perfectly adhering layer of a material (adhesion 1), which does not disintegrate nor affects the surface topography (so important in implantology) (9, 12, 16). This methods allows to form a layer or layers in orders of microns, in case of hydroxyapatite, a degradable layer accelerating bone healing – osseointegration, can be formed. A number of materials based on hydroxyapatite, carbon, nitrides and other materials exist. This surface modification can change the core, i.e. the material forming the basis for the implant, to a biologically improved material achieving higher criteria of implant healing, i.e. upgrade osseointegration to biointegration. Titan and zirkon nitrides belong to the materials already tested for their mechanical and physical and biological properties. The issue to be solved currently is how to use these materials for different "cores" of materials (13, 17, 18).

Methods

Methods for sample surface modification: Samples were prepared from several materials: technically pure titanium, titanium

alloy Grade V (Ti6Al4V), beta-titanium alloy (Ti35Nb6Ta) and chrome-cobalt alloy (CrCoMo) as cylinders with a diameter of 8 mm and height 3 mm. Surfaces of the experimental cylinders were then modified. Sample surface modifications are shown in the Table 1. Front areas of substrates were ground and then from all sides modified as required according to experimental variants: by polishing, etching, or grit blasting with aluminum oxide (Al2O3) grit 120 mesh. The individual variants were split into two groups: one coated with the layer of titanium nitride (TiN), the other with zirconium nitride (ZrN). TiN / ZrN coatings were deposited with the PVD (Physical Vapour Deposition) using the CAE method (Cathodic Arc Evaporation) (Tab. 1).

Cellular material: The cell line MG 63 (ECACC – European collection of cell cultures - cat. no. 86051601) from osteosarcoma is usually used for testing the implant materials. Its morphology is epithelial without more pronounced cell locomotion. It was cultivated in DMEM supplemented with foetal bovine serum (10%), antibiotics and antimycotics. Cultures were maintained at 37 °C in a 5% CO_2 air atmosphere.

The aim of the tests was to compare the capacity of the cell line to colonize the surface of the tested materials after 48 hours of cultivation. The cell population was planted directly on the tested material placed in wells of the cultivation plate (NUNC). The whole experiment was conducted in the same 24-well plate. Table 2 shows the scheme of the plate arrangement. After cultivation, the cells were fixed with acetic acid-alcohol and stained to distinguish a border between the cell and material. Then the area occupied by cells in 32 fields of view in the incident light microscope was determined. Fields of view were chosen randomly by scanning. The evaluation was performed from photographic records (Tab. 2).

Tab. 1. Experimental groups of samples and their modification.

Sample no.	Substrate	Modification	Coating
1	Ti	P	TiN
2		E	TiN
3		B	TiN
4		P	ZrN
5		E	ZrN
6		B	ZrN
7	Ti6Al4V	P	TiN
8		E	TiN
9		B	TiN
10		P	ZrN
11		E	ZrN
12		B	ZrN
13	Ti35Nb6Ta	P	TiN
14		E	TiN
15		B	TiN
16		P	ZrN
17		E	ZrN
18		B	ZrN
19	CrCoMo	P	TiN
20		E	TiN
21		B	TiN
22		P	ZrN
23		E	ZrN
24		B	ZrN

P – polished substrate, E – etched substrate, B – grit-blasted substrate

Tab. 2. Scheme of the experimental cultivation.

	Coating					
Substrate	TiN	TiN	TiN	ZrN	ZrN	ZrN
Ti	P	E	B	P	E	B
Ti6Al4V	P	E	B	P	E	B
Ti35Nb6Ta	P	E	B	P	E	B
CrCoMo	P	E	B	P	E	B

P – polished substrate, E – etched substrate, B – grit-blasted substrate

Tab. 3. Cell colonization of the modified sample surface with a studied coating in percents.

Sample no.	Cell colonization %
1	56.72
2	55.84
3	48.13
4	46.58
5	47.37
6	38.14
7	58.03
8	56.28
9	49.43
10	45.23
11	43.12
12	39.44
13	54.31
14	56.18
15	49.15
16	48.83
17	46.28
18	40.62
19	53.64
20	51.22
21	47.97
22	46.97
23	45.12
24	37.65

Results

Results are given in percentages for the individual alloys, TiN or ZrN surface coatings, and finally according to substrate modification before coating, i.e. polished, etched, or blasted substrate. The results are summarized in the Table 3 as a percentage of the area colonized by cells for the individual experimental variants. From tables it is evident that in the group with TiN coating, the lowest value (47.97 %) was recorded in the blasted surface of cobalt chromium (as anticipated) and the highest value (58.03 %) in the polished surface of titanium alloy Grade V. In the ZrN coating, the lowest value (37.65 %) was in the blasted surface of chromium cobalt alloy (again as anticipated), and the highest value (48.83%) was in the polished surface of beta-titanium alloy. The effect of coating of the modified substrate showed interesting results: the comparison of TiN (Tab. 4) and ZrN (Tab. 5) was in favour of the TiN coating.

Table 3 shows the summarized results of the areas colonized by cells in all samples according to sample numbers and surface modifications given in the Table 1. At first sight it is evident that the individual variants do not differ dramatically, the effect of the substrate modification is more pronounced. Therefore, the experimental variants were split both according to the substrates and their modification and according to the coating with the tested TiN or ZrN layers (Tabs 4 and 5). A more pronounced difference in the cell coloniza-

Tab. 4. Colonization of the sample area with cells in percents in titanium nitride coating.

	TiN coating		
Substrate	P	E	B
Ti	56.72	55.84	48.13
Ti6Al4V	58.03	56.28	49.43
Ti35Nb6Ta	54.31	56.18	49.15
CrCoMo	53.64	51.22	47.97

P – polished substrate, E – etched substrate, B – grit-blasted substrate

Tab. 5. Colonization of the sample area with cells in percents in zirconium nitride coating.

	ZrN coating		
Substrate	P	E	B
Ti	46.58	47.37	38.14
Ti6Al4V	45.23	43.12	39.44
Ti35Nb6Ta	48.83	46.28	40.62
CrCoMo	46.97	45.12	37.65

P – polished substrate, E – etched substrate, B – grit-blasted substrate

tion of the sample area was recorded in coating with the TiN, compared to ZrN. The tables also show the effect of substrate modification before coating. It is evident that the glossy surfaces are better accepted by cells than the rougher blasted ones (Tabs 3, 4 and 5).

Discussion

The materials used in implantology must meet the criteria for mechanical and physical resistance (1, 5, 15, 17). In addition, biological properties or biocompatibility are crucial (4, 5, 7, 9, 12, 16) for successful osseointegration (a fast connection between the material of an implant and bone) or biointegration ensuring a cell-material bond. Until recently, the situation was not fully optimal. The problem was not in a good choice of ceramics– hydroxyapatite – for coating but rather in the technique of adhesion of the material as the coating degraded after some years. Currently, there is an effort to achieve the adhesion of the material marked as 1 – i.e. the highest possible in scale of the assessment of the surface adhesion (9). The PVD method is one of possible techniques for depositing coatings while maintaining the supreme mechanical and physical properties of the surface layer. This method not only allows to achieve adhesion 1, it also ensures that surface modification before coating is not damaged (13, 14, 18) and the topography of the implant material is not altered by coating. The coating material exactly copies the surface and it can be deposited in only a few micrometers thick layers. These findings have also been proven within the grant project (Vaněk, Prachár, Bartáková) studying TiN and ZrN coatings on the chromium cobalt alloy.

Conclusion

We can conclude that the cellular adhesion is significantly affected by the type of the implant material and its surface modification. In the group of TiN coating, the best material was the titanium Grade V alloy in polished treatment and the worst was blasted chromium cobalt alloy. In the group of ZrN coating, beta-titanium alloy with polished modification was assessed as the best and chromium cobalt alloy in the blasted surface was the worst. Further important finding was the difference in cell colonization in favour of the polished surface versus the blasted surface and a higher cell colonization in samples with the TiN coating compared to those with ZrN coating. All these findings confirm suitability of the use of TiN and ZrN coating materials in implantology.

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