BULLETIN OF THE POLISH ACADEMY OF SCIENCES
TECHNICAL SCIENCES, Vol. 62, No. 3, 2014
*DOI: 10.2478/bpasts-2014-0061*

# Contribution of TiN/Ti/a-C:H multilayers architecture to biological and mechanical properties

L. MAJOR[1]*, J.M. LACKNER[2], M. KOT[3], M. JANUSZ[1], and B. MAJOR[1]

[1] Institute of Metallurgy and Materials Science; Polish Academy of Sciences, 25 Reymonta St., 30-059 Cracow, Poland
[2] Joanneum Research- Materials- Institute for Surface Technologies and Photonics; Leobner Strasse 94; 8712 Niklasdorf, Austria
[3] AGH University of Science and Technology, 30 Adama Mickiewicza Ave., 30-059 Cracow, Poland

**Abstract.** Complex microstructure analysis of TiN/Ti/a-C:H multilayer coatings, subjected to mechanical and biological tests, were performed by means of transmission electron microscopy (TEM) and confocal scanning laser microscopy (CSLM), respectively. Influence of interface numbers and phase ratios on coating properties was studied. Thin films were fabricated by a hybrid PLD technique (PLD supported by magnetron sputtering). The a-C:H phase was characterized by an amorphous structure, while TiN was built of columnar crystallites. Multilayer coatings contained sequentially deposited TiN and a-C:H layers with thin metallic Ti inter- layers deposited at each interface. Mechanisms of mechanical wear of analyzed systems were presented focusing on the cracking propagation revealed in the scratch test. Biological tests were done basing on smooth muscle cells adhesion to coating surfaces.

An increase of TiN phase in the coating led to improvement of mechanical properties, while the carbon phase improved the biological behavior. Coatings comprising a higher rate of the carbon (a-C:H) revealed brittleness and were prone to delaminating. Optimal properties from the mechanical and biological point of view were stated for 8xTiN/Ti/a-C:H coating with 1:1 phase ratio (TiN to a-C:H).

**Key words:** microstructure, smooth muscle cells adhesion, cracking.

## 1. Introduction

Multilayer structures are used extensively in every thin film application, because they provide performance not achievable by a single layer system. They combine two or more materials designed and engineered with a required performance. For multilayers with two alternate phases, the resistance to plastic deformation increases and hardness rises as the thickness of bilayer thickness decreases. This effect of the bilayer thickness has been studied extensively for a large number of multilayer systems, and is now considered as a critical parameter for designing and fabrication of multilayer coatings. The electrical, optical, biological, mechanical, and tribological properties as well as the microstructure of thin film materials can vary over wide ranges, and are mostly dependent on the deposition process used. A number of books describe various deposition processes [1–4] and many publications describe properties of thin films [5–11]. Each process has strengths and weaknesses affecting film properties. Hybrid deposition combines two or more different techniques to take advantage in an optimum performance of each constituent of thin film layers. An attempt in this work was to characterize coating properties from the mechanical and biological point of views, simultaneously. The goal of the present paper was to describe an influence of the layers number and phases ratio in TiN/Ti/a-C:H multilayer coatings, on mechanical and biological properties. The potential application for examined coatings could be for surface protection of medical tools. Wear is a critical issue for prostheses, implants, and other medical devices and may lead to significant loss of material and/ or failure of a medical device. Even a relatively tiny wear can lead to the significant degradation of medical devices. An unique attempt of the authors was to join the coating properties with a detailed microstructure description. The optimisation was based on: the microstructure description of deposited coatings and cracking mechanisms, the description of coatings after scratch tests and study of smooth muscle cells adhesion.

## 2. Materials and methods

The hybrid PLD system (Pulsed Laser Deposition connected with magnetron sputtering) equipped with a high purity titanium (99.9% Ti) and carbon (graphite) targets were used for multilayer coatings deposition. By application of the magnetron sputtering in PLD coating plants, higher deposition rates can be reached as well as good film adhesion even at room temperature. Coatings were produced by sequential deposition of amorphous carbon (a-C:H) and titanium nitride (TiN). Coatings were deposited on the austenitic steel substrates (DIN 1.4301). To increase adhesion coatings to substrates, Ti buffer layer (first layer from the substrate) was deposited in each case. Pure titanium (Ti) layers were deposited in the argon (non- reactive) atmosphere, while for TiN deposition the atmosphere was gradually switched to nitrogen. Additionally to reduce a residual stress concentration, thin metallic titanium (Ti) layers were deposited at each interface. More details of the deposition process can be found in [12, 13]. A set of multilayer coatings with a different number of constituting layers and phases ratio at the constant total coating thickness of 1.5 $\mu$m was produced. Additionally, a-C:H

*e-mail: l.major@imim.pl

and TiN single layer coatings were deposited as references. The list of different coatings architecture is presented below:

- a-C:H single layer
- TiN single layer
- 2xTiN/a-C:H (4 layers with TiN to a-C:H phases ratio 1:1)
- 8xTiN/a-C:H (16 layers with phases ratio 1:1)
- 32xTiN/a-C:H (64 layers with phases ratio 1:1)
- 8xTiN/a-C:H (16 layers with phases ratio 1:2)
- 8xTiN/a-C:H (16 layers with phases ratio 1:4)
- 8xTiN/a-C:H (16 layers with phases ratio 4:1)

Bio- medical tests were carried out using smooth muscle cells (SMC) [14, 15], which were purchased from Lonza. Each vial had a concentration of 500 000 cells/mL. Cells were stored in liquid nitrogen before use. Before adding cells, the medium was warmed up to 37°C in a water bath. Cells were taken from the liquid nitrogen container and placed for 2–3 min into a 37°C water bath. SMC were deposited directly on the surfaces of coatings. After three days the cells were fixed in 4% paraformaldehyde. Then the cells were permabilised in a detergent Triton X-100 0.2% for 4 min. The actine cytoskeleton was marked with AlexaFluor Phalloidin, the nucleus with DAPI (fluorescent staining of the DNA content and nuclei for cellular imaging techniques- diamidino-2-phenylindole). Fluorescent dyes were excited with an appropriate laser wavelength, namely, 488 nm for actin cytoskeleton visualization, 405 nm to visualize nucleus of the cell. The cells adhesion to the coatings surface was observed by the confocal microscopy (Carl Zeiss Exciter 5).

Mechanical properties of coatings were investigated by means of the scratch adhesion test using the Rockwell C indenter with the curvature radius of 200 $\mu$m. The length of a scratch path was 5mm. Uploading for each coating was gradually increased from 0 to 30 N. The indentation tests were carried out using the Berkovich indenter with 2 and 5 mN of the applied load. The microstructure of the as- deposited coatings and after the mechanical tests, was studied using the Scanning (SEM) (QUANTA 200 3D) and the Transmission (TEM) (TECNAI G$^2$ F20 FEG (200 kV)) Electron Microscopes, which allows microstructure observation in the smallest scale. Chemical composition was analyzed by Energy Dispersive X-ray Spectroscopy technique (EDS). Thin foils for the TEM analysis were prepared using Focused Ion Beam technique (FIB- Gallium Ions) (QUANTA 200 3D Dual Beam), together with the OmniProbe in- situ lift out system. The procedure allowed to prepare foils directly from places of interest, namely, from mechanically deformed areas within scratch tracks.

## 3. Results

Tests on smooth muscle cells showed that the satisfactory cells adhesion was found for a-C:H single layer coating as it is presented in the image (Fig. 1).

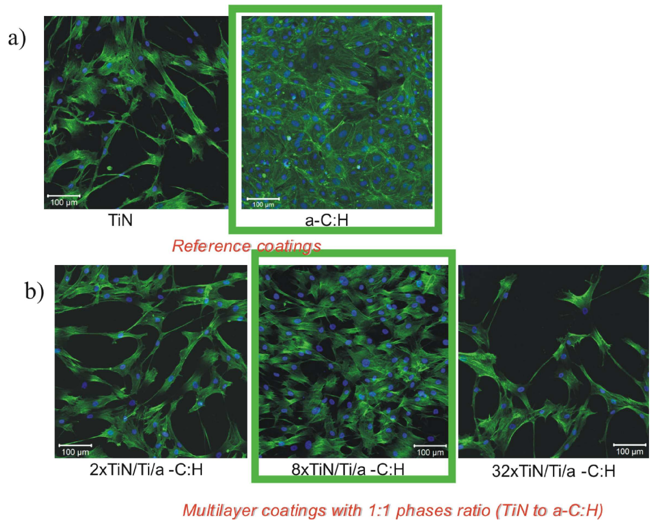


Fig. 1. Smooth muscle cells adhesion test: a) single layer coatings, b) multilayer coatings

The 8xTiN/Ti/a-C:H coating was characterised by the best smooth muscle cells adhesion among the investigated multilayers.

Basing on the presented results, the application of such complicated multilayer structures seems to be unnecessary due to the fact that the single- layer a-C:H revealed better properties. However, dedication of analysed coatings was not such as the one of biocompatible films, but they also should withstand mechanical uploading.

The hardness of the a-C:H single layer coating was at the level of 19 GPa, while the TiN has the hardness of 33 GPa, while the modulus of elasticity was 200 and 390 GPa respectively. Hardness and the modulus of elasticity of multilayer coatings were close to the a-C:H single layer one. In case of different phases ratio, hardness decreased with a-C:H phase increase.

The detailed results has been presented in the Table 1.

Table 1
Results of micro- hardness and elastic modulus

| Coating | 2 mN | | 5 mN | |
|---|---|---|---|---|
| | Hardness | Elastic modulus | Hardness | Elastic modulus |
| a-C:H single | 18.70±1.57 | 206±15 | 19.32±1.65 | 198±7 |
| TiN single | 33.46±0.88 | 388±12 | 25.87±0.82 | 300±12 |
| 2xTiN/a-C:H | 14.63±0.29 | 199±8 | 15.43±1.71 | 207±8 |
| 8xTiN/a-C:H | 16.76±1.54 | 204±11 | 13.59±1.27 | 206±13 |
| 32xTiN/a-C:H | 11.49±1.45 | 184±12 | 11.01±0.81 | 191±9 |
| 8xTiN/a-C:H r 1:2 | 17.03±0.6 | 222±7 | 14.95±1.28 | 217±12 |
| 8xTiN/a-C:H r 1:4 | 13.31±1.1 | 194±13 | 12.60±0.88 | 175±9 |
| 8xTiN/a-C:H r 4:1 | 23.36±0.64 | 254±13 | 17.86±0.59 | 249±8 |

Coatings would potentially find an application as medical tools thus their adhesion to a metallic substrate was crucial (Fig. 2).

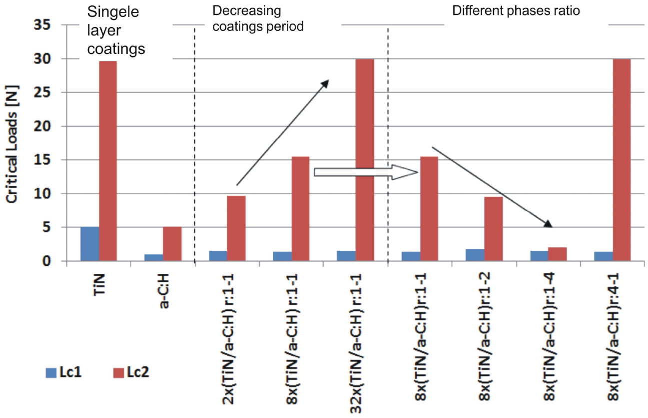


Fig. 2. Scratch adhesion test results of multilayer coatings with different number of layers and phases ratio at the constant coating thickness ($L_{C1}$- load under which first cracks appeared; $L_{C2}$- critical load under which coatings delaminated)

The performed test showed that the critical adhesion force for the single a-C:H reference coating was relatively low (small $L_{C2}$). It delaminated fast, while TiN single layer coating adhesion was high. In the case of multilayer coatings, the scratch test revealed utility to possible steering of properties. The higher amount of interfaces (the higher amount of layers) at the constant coating thickness, the better adhesion. The adhesion test was done on coatings with different phase ratios as well (i.e 1:2, 1:4, 4:1). The scratch test on coatings with the different phase ratios showed that the higher amount of carbon phase (1:4) led to the lower critical load (lower adhesion to substrate), while the higher amount of TiN phase the better properties.

The 8xTiN/Ti/a-C:H structure with the 4:1 phases ratio (TiN to a-C:H) had better scratch properties than the same coating with 1:1 phases ratio. A good mechanical adhesion was confirmed by the detailed TEM microstructure analysis which presented stages of coating delamination during mechanical uploading (Fig. 3).

The another thin foil prepared for TEM observation allowed to study the earlier deformation stage in the cohesive cracking (Fig. 4).

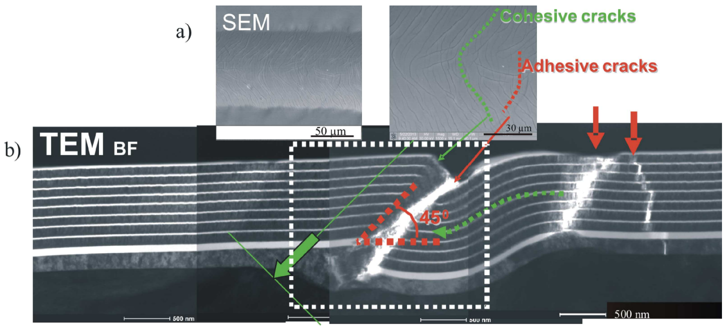


Fig. 3. Microstructure analysis of 8xTiN/Ti/a-C:H multilayer coating with 4:1 (TiN:a-C:H) phases ratio, after scratch adhesion test: a) topography analysis by SEM technique, b) microstructure analysis on cross- section by TEM technique (bright field mode)

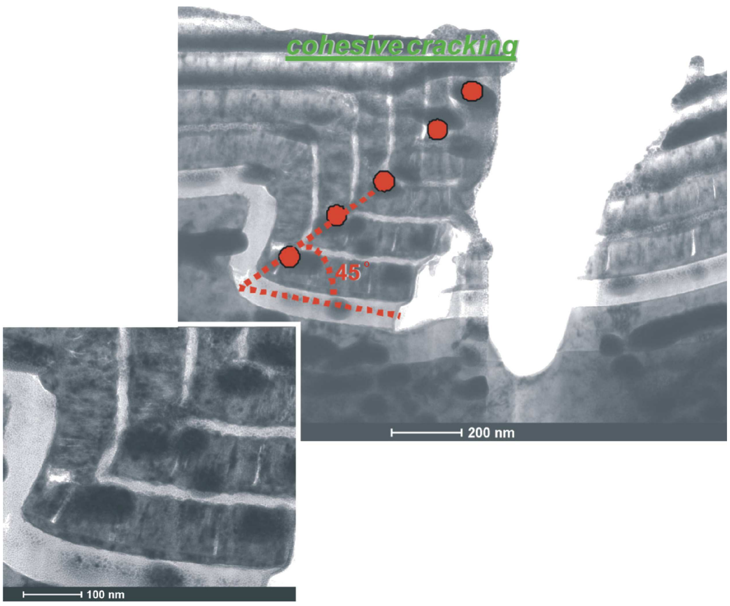


Fig. 4. Microstructure analysis of 8xTiN/Ti/a-C:H multilayer coating with 4:1 (TiN:a-C:H) phases ratio, after scratch adhesion test- earlier stage of cohesive cracking than presented in Fig. 3

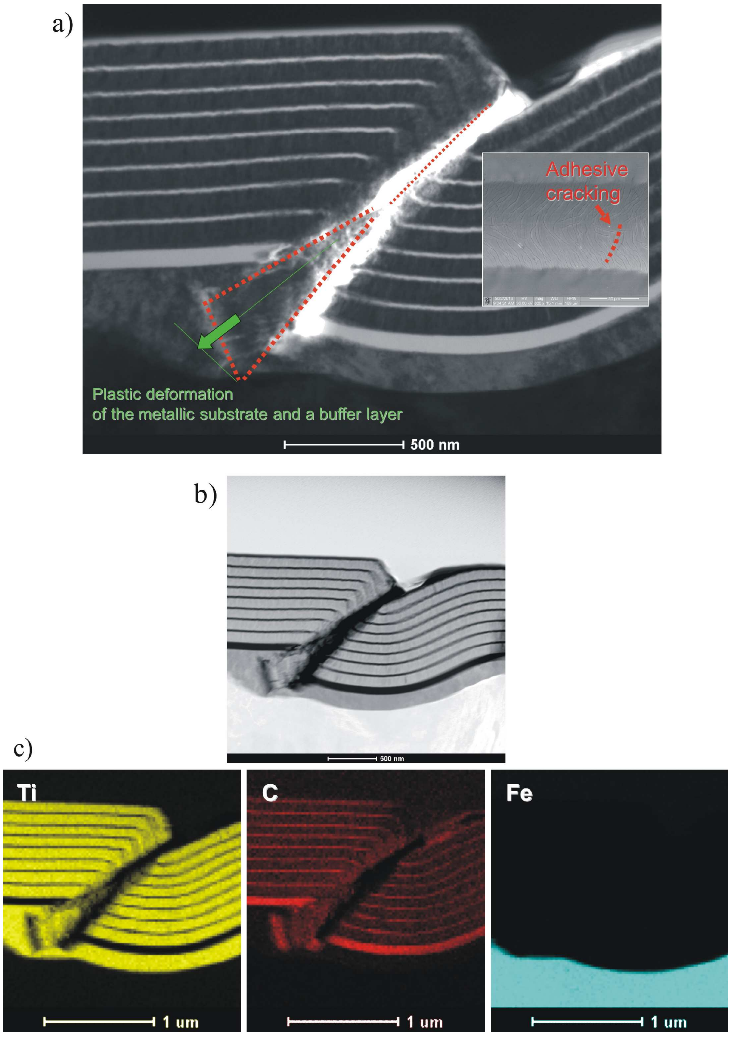


Fig. 5. Microstructure analysis of 8xTiN/Ti/a-C:H multilayer coating with 4:1 (TiN:a-C:H) phases ratio, after scratch adhesion test- adhesive cracking: a) TEM bright field image, b) image in the Z- contrast (contrast dependent on atomic number Z), c) maps of selected elements obtained in qualitative chemical analysis EDS

In cohesive cracking ceramic TiN and a-C:H layers brittle cracked, while the crack propagation was fixed by very thin Ti layers at each TiN/a-C:H interfaces, as it is presented in Fig. 4. The red dots were placed on points where individual layers were bended. In the subsequent stage, with higher uploading (when adhesive cracking started to dominate), the crack was formed along line at 45°, however, the coating adhesion to the substrate was so strong that the plastic deformation of the metallic substrate pulled down the coating fragment, which is well seen in the image (Fig. 5).

Such behavior is a proof of a very good coating adhesion to the substrate. Unfortunately, the smooth muscle cells adhesion test showed that coating could be toxic (Fig. 6), similar like single TiN coating, which revealed a poor cell proliferation.

Relatively weak mechanical properties of another coatings with a higher amount of a carbon phase (namely: 8xTiN/Ti/a-C:H with 1:4 (TiN to a-C:H) phases ratio), was probably connected with a high residual stress, which was confirmed by the smooth muscle adhesion test (Fig. 7).

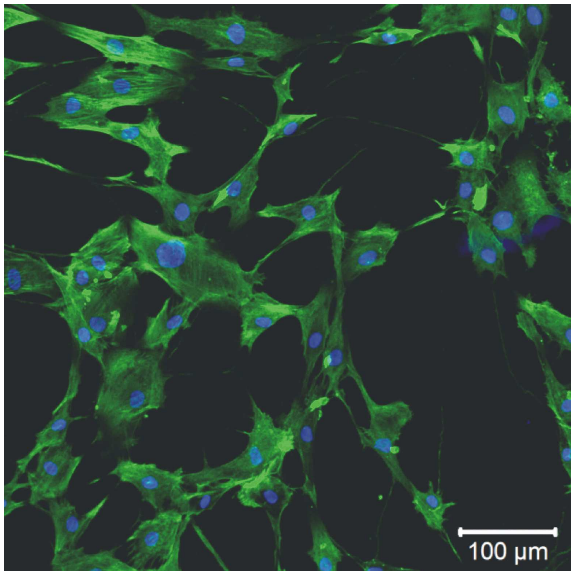


Fig. 6. Smooth muscle cells adhesion test on 8xTiN/Ti/a-C:H multilayer coating with 4:1 (TiN:a-C:H) phases ratio

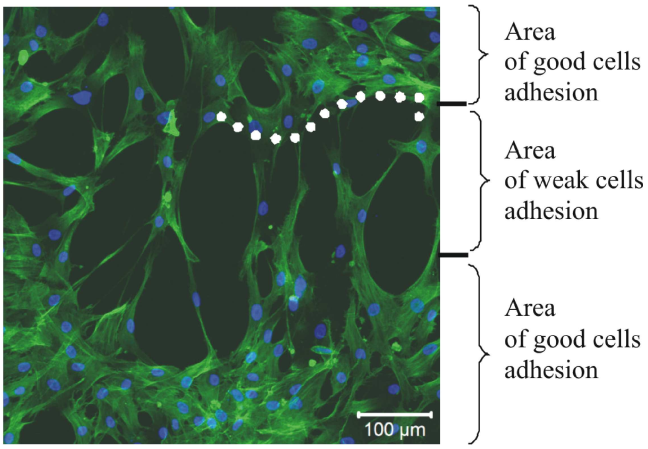


Fig. 7. Smooth muscle cells adhesion test on 8xTiN/Ti/a-C:H multilayer coating with 1:4 (TiN:a-C:H) phases ratio

It was possible to distinguish two areas on the coating surface. One area with a good cell adhesion. The adhesion was similar like in the case of the amorphous a-C:H single layer coating.

The second area had a weak cell adhesion. This effect may inform about the high residual stress and coating brittleness. Looking at the topography of the coating using SEM, the coating delaminated very fast even at the cohesive cracking stage (Fig. 8).

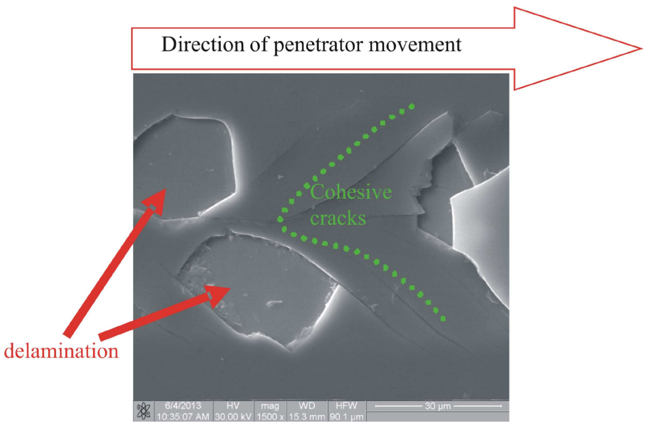


Fig. 8. Topography analysis of 8xTiN/Ti/a-C:H multilayer coating with 1:4 phase ratio (TiN to a-C:H), performed by SEM technique

## 4. Discussion

The smooth muscle adhesion cell test clearly determines the use of the suggested layered structures [15]. The goal of the presented multilayer structures was protective films for medical tools. Biological tests showed that the satisfactory properties had the a-C:H single layer coating. Basing on this test and using such complicated structures, like the presented multilayers, seems to be unnecessary, however, coatings should also withstand adequate mechanical uploading. Hardness and the modulus of elasticity of multilayer coatings were close to the a-C:H single layer. In the case of different phase ratios, hardness decreased with a-C:H phase increase. Coatings on medical tools should have relatively good mechanical adhesion to a substrate. The scratch adhesion test revealed that suggested multilayer structures allowed to steer their properties. The higher amount of layers the better properties.

Similar effect was noticed by authors in previous experiments on different type of multilayer coatings [10]. The influence of number of layers in Cr/CrN multilayer coatings, deposited also on austenite steel, on mechanical properties was studied. It has been described that the higher amount of layers on the constant coating thickness the better properties. However, it has been also noticed that there is an optimal number of layers (optimal individual layer thickness), over which the mechanical properties decrease [10].

The current test also showed that the higher amount of carbon phases the poorer properties. The decrease of properties with increase of amount of carbon phases, was probably connected with relatively high residual stress in carbon layers [16]. It was confirmed by the smooth muscle adhesion test. Studding the topography of the coating, using smooth muscle cells by means of the confocal microscopy technique, it was possible to distinguish two areas i.e one where cells adhered very well, and the second not covered by cells.

Multilayer coatings with higher amount of TiN phase had very good mechanical properties. The microstructure analysis of this coating allowed to observe deformation stages up to delamination during the mechanical uploading. The cohesive cracking ($L_{C1}$ – the first deformation stage in the scratch test-first cracks usually formed in the opposite direction to the penetrator), was connected with cracking in individual ceramic layers (TiN and a-C:H), while very thin metallic Ti layers deformed plastically forming at 45° a deformation line across the coating, which is an angle of plastic deformation of metallic polycrystalline materials. This is a proof of an important role of very thin Ti layers in toughness properties of coating. After cohesive cracking, subsequently adhesive cracks were presented ($L_{C2}$ – brittle cracking leading to coating delamination-formed in the direction of penetrator movement). They were connected with cracking along the deformation line (formed at the cohesive stage). Plastic deformation of a metallic substrate caused by mechanical uploading, pulled down a fragment of coating, was a proof of a very good adhesion of the coating to the substrate. Unfortunately, the smooth muscle adhesion test showed that coating was toxic. It contained too much amount of non- stoichiometric TiN phase. It has been well proved in the authors' recent papers, connected with this issue [17]. The cells respond is from the coating surface, but the chemical composition influence on the surface properties. Summing up, the increase of TiN phase influenced on the mechanical properties increase, however, from a biological point of view it caused that coating was more toxic.

The increase of carbon phase influenced on the biological properties increase, however, because of a high residual stress, the coating was very brittle and it delaminated fast. The optimum properties from mechanical and biological points of view were found for 8xTiN/Ti/a-C:H coating with the phases ratio 1:1 (TiN to a-C:H) among all coatings taken under consideration.

## 5. Conclusions

The research described in this work yields the following conclusions:

- biological and mechanical properties dependent on the multilayer coating architecture and phase ratio,
- establishment of the balance between mechanical and biological properties of multilayer coatings, defined an appropriate structure to be used for medical tools.

**Acknowledgements.** The research project was financed by the National Science Centre (Polish – Narodowe Centrum Nauki, abbr. NCN) No: 3066/B/T02/2011/40 - Polish - Austrian exchange project (2012–2014) 023/2012/2013/2014; 8548/R 12/R 14.

REFERENCES

[1] P.M. Martin, *Handbook of Technologies for Films and Coatings*, 3$^{rd}$ ed, Elsevier, Amsterdam, ISBN: 978-0-8155-2031-3, 2009.

[2] M. Donald Mattox, *Handbook of Physical Vapor Deposition (PVD) Processing*, William Andrew, London, ISBN: 978-0-8155-2037-5. 1998.

[3] F. Rointan Bunshaw, *Handbook of Deposition Technologies for Films and Coatings*, Noyes, New Jersey, ISBN 0-8155-1438-7, 1994.

[4] David Glocker, Ismat Shaw, and Canthia Morgan, *Handbook of Thin Film Process Technology*, Taylor and Francis, New York, ISBN 0-8155-1442-5, 2001.

[5] S. Kumar, D. Zhou, D.E. Wolfe, J.A. Eades, and M.A. Haque, "Length-scale effects on fracture of multilayers", *Scripta Mater.* 63, 196–199 (2010).

[6] J. Kusinski, S. Kac, A. Kopia, A. Radziszewska, M. Rozmus-Gornikowska, B. Major, L. Major, J. Marczak, and A. Lisiecki, " Laser modification of the materials surface layer – a review paper", *Bull. Pol. Ac.: Tech.* 60, 711–728 (2012).

[7] L. Major, W. Tirry, and G. Van Tendeloo, "Microstructure and defect characterization at interfaces in TiN/CrN multilayer coatings", *Surf. & Coat. Technol.* 202, 6075–6080 (2008).

[8] J.M. Lackner, L. Major, and M. Kot, "Microscale interpretation of tribological phenomena in Ti/TiN soft-hard multilayer coatings on soft austenite steel substrates", *Bull. Pol. Ac.: Tech.* 59, 343–355 (2011).

[9] L. Major, J. Morgiel, J.M. Lackner, M.J. Szczerba, M. Kot, and B. Major, "Microstructure design and tribological properties of Cr/CrN and TiN/CrN multilayer films", *Adv. Eng. Mater.* 10 (7), 617–621 (2008).

[10] M. Kot, W.A. Rakowski, Ł. Major, R. Major, and J. Morgiel, "Effect of bilayer period on properties of Cr/CrN multilayer coatings produced by laser ablation", *Surf. & Coat. Technol.* 202 (15), 3501–3506 (2008).

[11] J.M. Lackner, W. Waldhauser, L. Major, J. Morgiel, M. Kot, and B. Major, "Nanocrystalline Cr/CrN and Ti/TiN multilayer coatings produced by pulsed laser deposition at room temperature", *Bull. Pol. Ac.: Tech.* 54 (2), 175–180 (2006).

[12] J.M. Lackner, W. Waldhauser, and R. Ebner, "Large-area high-rate pulsed laser deposition of smooth TiCxN1-x coatings at room temperature – mechanical and tribological properties", *Surf. & Coat. Technol.* 188–189, 519–524 (2004).

[13] J.M. Lackner, W. Waldhauser, A. Alamanou, C. Teichert, F. Schmied, L. Major, and B. Major, "Mechanisms for self- assembling topography formation in low- temperature vacuum deposition of inorganic coatings on polymer surfaces", *Bull. Pol. Ac.: Tech.* 58, 281–294 (2010).

[14] R. Major, F. Bruckert, J.M. Lackner, W. Waldhauser, M. Pietrzyk, and B. Major, "Kinetics of eucariote cells adhesion under shear flow detachment on the PLD deposited surfaces", *Bull. Pol. Ac.: Tech.* 56, 223–228 (2008).

[15] R. Major, J. M. Lackner, K. Gorka, P. Wilczek, and B. Major, "Inner surface modification of the tube-like elements for medical applications", *RSC Adv.* 3, 11283–11291 (2013).

[16] H. Ronkainen, S. Varjus, and K. Holmberg, "Friction and wear properties in dry, water and oil- lubricated DLC against alumina and DLC against steel contact", *Wear* 222, 120–128 (1998).

[17] L. Major, J.M. Lackner, and B. Major, "Bio-tribological TiN/Ti/a-C:H multilayer coatings development with built-in mechanism of controlled wear", *RSC Adv.* 4, 21108–21114 (2014).