# Industrially-scaled pulsed laser deposition based coating techniques for the realization of hemocompatible surfaces for blood contact applications

Juergen M. Lackner[*a], Wolfgang Waldhauser[a], Roman Major[b], Boguslaw Major[b], Elzbieta Czarnowska[c], Franz Bruckert[d]
[a] JOANNEUM RESEARCH Forschungsgesellschaft mbH, Laser Center Leoben, Leobner Strasse 94, A-8712 Niklasdorf, Austria;
[b] Polish Academy of Sciences, Institute of Metallurgy and Metallurgy, ul. W. Reymonta 25, 30-059 Krakow, Poland;
[c] Children's Memorial Health Institute, Department of Pathology, Al. Dzieci Polskich 20, 04-730 Warszawa, Warsaw, Poland;
[d] Institut National Polytechnique de Grenoble, UMR 5628 CNRS-INPG, 3 L.Neel, 38016 Grenoble Cedex I, France

## ABSTRACT

Non-thrombogenic blood contacting surfaces and appropriate blood flow characteristics are essential for clinical application. State-of-the-art coatings are based on heparin and struggle with the problem of bleeding. Thus, there is increasing demand for developing new coating materials for improved human body acceptance. Materials deposited by vacuum coating techniques would be an excellent alternative if the coating temperatures can be kept low due to the applied substrate materials of low temperature resistance (mostly polymers). Under these circumstances, adequate film structure and high adhesion can be reached by the Pulsed Laser Deposition at room temperature (*RT*-PLD), which was developed to an industrial-scaled process at Laser Center Leoben. This process was applied to deposit Ti, TiN, TiCN and diamond-like carbon (DLC) on polyurethane, titanium and silicon substrates to study the biological interactions to blood cells and the kinetic mechanism of eukaryote cell attachment. Besides high biological acceptance, distinct differences for the critical delamination shear stress were found for the coatings, indicating higher adhesion at higher carbon contents.

**Keywords:** Pulsed laser deposition, Industrially-scaled coating, Room temperature coating, Cell adhesion, Cell attachment, Titanium, Titanium nitride, Titanium carbonitride, Diamond-like carbon

## 1. INTRODUCTION

### 1.1 Cell attachment control on biomaterials

Materials used in blood-contact devices (e.g. heart valves, artificial hearts, stents, capillary tubes) have often been chosen based on their physical characteristics, such as flexibility or rigidity, mechanical strength, transparency, degradability, etc. [1]. Moreover, cost effectiveness, ease of processing, and sterilization have also been important considerations when selecting a particular material. Thus, optimal thrombogenicity (the tendency to encourage blood clotting) may not always be achieved. Thrombus formation can occur, if blood is exposed to foreign materials, such as the biomaterial. The first clinically manifested process in this contact is the activation of hemostasis (clotting). The hemostasis involves the adsorption of blood proteins, followed by platelet adhesion and activation. Many types of eukaryotic cells (to which all blood cells belong to) are force sensible, especially in the context of cell adhesion. Shear stresses triggered by flowing blood strongly influence all cell adhesion processes on vessel walls in human body or the biomaterials surface.

The resulting mechanical tension affects the receptor-ligand bonds at the contact areas. E.g. fibroblasts adhere to the extracellular matrix through focal contact areas, whose number and surface increase with the mechanical tension applied to the cell [2,3]. Detachment of cells occurs at hydrodynamic stresses above a threshold, statistically following apparent

[*] email: Juergen.Lackner@JOANNEUM.at, phone: +43 3842 81260 2304, fax: +43 316 8769 2305, joanneum.at/lzl

High-Power Laser Ablation VII, edited by Claude R. Phipps
Proc. of SPIE Vol. 7005, 70050Q, (2008) · 0277-786X/08/$18 · doi: 10.1117/12.778176

first-order kinetics. The threshold stress for each cell depends on cell size, the nature of the substrate and the presence of adhesion proteins at the plasma membrane surface [4].

From the last twenty years experimental and theoretical works have been dedicated to cell adhesion [5]. From the biological point of view central importance gathers the understanding of molecular mechanisms of cells to adhere, roll, or slide on passive or reactive surfaces, since many functions of living cells depend on these properties. From the physical point of view even the passive (non-reactive) response of cells to external forces is of great significance e.g. to study the contact forces in bio-adhesion phenomena, which are influenced by the discrete region of the contact regions, the weak or non-covalent cell bonding on the substrate, or the multi-component variety of the cytoplasmic membrane and its extracellular matrix. Measuring the strength of these bonds is one major challenge in cellular biology, since it allows the identification of different species in adhesion phenomena.

*Dictyostelium discoideum* is often used as a model organism to investigate cellular response to force, so called shear-flow-induced cell motility (SFICM). This simple unicellular eukaryote cell is a genetically and biochemically tractable model organism that is used extensively to study cytoskeleton organization, chemotaxis, cell differentiation, and development [6]. The lifestyle of *D. discoideum* consists of two phases: During the vegetative phase, the small (8 µm diameter) ameba feeds upon bacteria and yeast by phagocytosis. Plasma membrane adhesion is therefore directly related to the phagocytic properties of the cell. In the development phase, cell-cell adhesion is more important, and specific contact proteins are expressed [7]. Axenic strains showing an enhanced fluid-phase endocytosis were obtained and are able to grow in suspension in nutritive medium. Several mutant studies suggested the existence of three kinds of adhesion proteins [4,8,9]. Only the vegetative phase including the phagocytic process is a good model to describe adhesion of human fibroblasts to vessel and biomaterial surfaces.

For *D. discoideum* the detachment is not affected by depolymerisation of the actin or tubulin cytoskeleton [10]. The detachment rate is strongly affected by the presence of an intact actin cytoskeleton and by the cell-substrate adhesion energy. An interpretation of the detachment process is partly possible by an adhesive belt model describing the passive behavior of the cell edge under external or internal forces [5].

The current work shows the application of the biological and SFICM characterization for pulsed laser deposited (PLD) titanium (Ti), titanium nitride (TiN), titanium carbonitride (TiCN), and diamond-like carbon (DLC) coatings grown at room temperature in an industrially-scaled PLD coating machine (*RT*-PLD process).

### 1.2 Industrially-scaled coating by PLD (*RT*-PLD process)

The PLD technique has remarkable advantages, which are important for depositing biocompatible coatings [11], including the deposition of nearly all solid materials including refractory metals. PLD is relatively clean, and the average deposition rate is relatively high [12,13]. The ablated atomic material, both ions and neutrals, is significantly more energetic than for the majority of other thin film deposition techniques, leading to smoother, denser thin films as a result of increased adatom mobility [12] without using substrate heating. Nevertheless, there is one detrimental effect inherent to the PLD technique, which, presumably, limited the application of PLD in industrial style: The flux of atomic material leaving the target surface is characterized by a highly polar, forward-peaked distribution [14,15]. This results in thickness uniformity problems when depositing thin films over large areas, slowing down the way of PLD into the mainstream of industrially-applied coating techniques.

Besides the continuously decreasing investment costs for PLD laser systems mainly the lack of knowledge to deposit large-area films industrially-scaled and reproducibly limits the commercial use of PLD. The weak point in the PLD technique is the “point” (or “spot”) target vaporization compared to the “linear” vaporization in evaporation or sputtering techniques. Thus, the thickness uniformity according to the angular distribution of the laser-induced plasma flux requires special equipment of target or substrate movement to provide a relative motion between the substrate and the beam to scan the selected deposition area across the entire surface of the substrate [16]. High-rate coating is only guaranteed, if several laser systems are applied simultaneously.

Multi-spot PLD evaporation is based on an optimized superposition of plumes, which are simultaneously ablated from several evaporation spots and allow the imitation of a line evaporator [17] (see insert in Fig. 1c). Applying this concept thickness variation in the coatings can be kept lower than 5%. Fig. 1c shows a typical variation of the deposition rate determined from coatings made from a four-spot PLD evaporation source (*RT*-PLD). The deposition rate is only slightly lower in the overlapping zone, but it decreases rapidly to the left and to the right. The uniformity of the thickness achievable by superposition of plumes is sufficient for many applications [18].

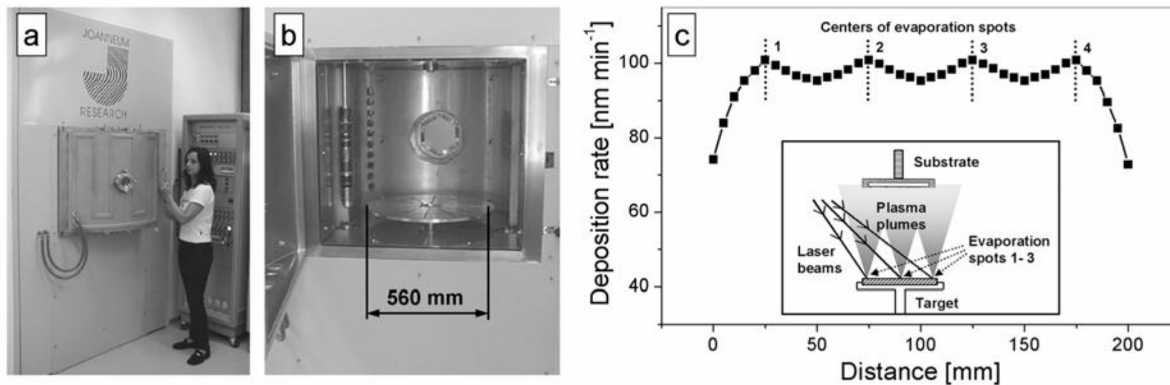


Fig. 1. (a, b) *RT*-PLD coater at Laser Center Leoben. (c) Experimentally determined deposition rates determined from titanium coatings produced by the four-spot PLD evaporation source at Laser Center Leoben, JOANNEUM RESEARCH Forschungsgesellschaft mbH (principle: see insert, target-substrate distance: 10 cm) [17]

Based on the principle of multi-beam evaporation from a static target position onto moved substrates an industrially-scaled PLD coating facility (*RT*-PLD) was built at Laser Center Leoben recently (Fig. 1a). *RT*-PLD coating is performed from rotating (metal) targets using a pulsed Nd:YAG laser system of four laser beams of 1064 nm wavelength, operating at a repetition rate of 50 Hz and providing 10 ns pulses of 600 mJ pulse energy [19]. To achieve reproducible coatings, the plasma analysis equipment contains a plasma emission monitor for plasma spectroscopy and a time-of-flight mass spectrometer for gas analysis. Stabilized laser power is reached by the application of laser power meters. These devices allow the creation of reproducible reactive deposition in oxygen, nitrogen, and hydrocarbon atmospheres. Additionally, inert gas atmosphere (argon, helium) allows the deposition of pure metals.

The deposition of the evaporated species takes place on moveable and rotatable substrate manipulators mainly at room temperature, but, if necessary, substrate heating is possible. The whole equipment was designed for industrially-scaled coating and possesses the largest vacuum recipient used worldwide for PLD at the moment (height of coating area: 500 mm, diameter of substrates up to 560 mm), and is a very versatile tool for the development of coatings as well as for job lot coating (Fig. 1b). Pumping is performed by a combination of a turbomolecular and a mechanical pump, allowing recipient background pressures down to the $10^{-6}$ mbar range.

## 2. EXPERIMENTAL

### 2.1 Film deposition by *RT*-PLD

Before starting deposition the chamber was evacuated to pressures below $2x10^3$ Pa. High-purity titanium and graphite targets were used to deposit 50 nm thick titanium films on polyurethane (PU, ChronoThane P) substrates and 300 nm thick films on silicon wafer and grade-2 titanium substrates at room temperature by the pulsed Nd:YAG laser system described above. To obtain the different coatings, pure argon atmosphere was used for DLC deposition from graphite and for Ti deposition from titanium targets. Ti targets were also used for deposition of TiN films in nitrogen and TiCN films in $N_2$-$C_2H_2$ atmosphere with low (0.5 sccm) and high (2.5 sccm flow) $C_2H_2$ content resulting in lower ($TiCN^l$) and higher ($TiCN^h$) carbon content. To provide homogenous film thicknesses over the whole coated surfaces, the substrates were moved with a relative speed of 54 mm $s^{-1}$ through the plasma plumes during deposition.

### 2.2 Biocompatibility characterization with human fibroblasts

The biocompatibility was tested for human fibroblasts obtained from healthy donors in 48-hours tests. The cells in a concentration of $1.5x10^5$ $ml^{-1}$ in Dulbecco culture medium (Sigma-Aldrich Co.) were moved on the surface of materials (standard glass as control material, PU coated with Ti, TiN and $TiCN^h$), which were sterilized in plasma and washed in a physiological salt buffered solution. Adhering cells were fixed by a solution of 4% paraformaldehyde and 70% methanol. These fixed cells were washed with 0.5% BSA serum (ATTC Co.), marked with several antibodies (CD49E (Serotec Co.), Alexa Fluor 488 (Molecular Probes Co.)), and colored by 7AAD (Merck AG). These cells were analyzed by laser confocal microscopy (Olympus FV-500 device) and by laser scanning cytometry (ComuCyte device). Fixing of the adhering cells for the confocal microscopy was achieved by the treatment in a solution of 4% parformaldehyde and 70%

methanol. Cell nuclei visualization was performed by incubation with 7-amonioactinomycin D (Merck AG). The amount of interleukine IL-1β was marked by the immunoensimatic method in the incubation fluids of the cell cultivation. The colored reaction was achieved by OPD hydrochloride and analyzed by a single-channel reader-assay system (ELX 800, Biotek Instruments Inc.) at a wavelength of 492 nm.

For light microscopical investigations the cells were detached from the sample surfaces by nonenzymatic cell dissociation solution (Sigma-Aldrich Co.), analyzed with Trypan blue staining and counted using a Burcker's camera.

### 2.3 Shear flow testing with Ax-*2 D. discoideum* cells

Several techniques are used as setup for studying SFICM [20]. Among them, the applied radial flow detachment assay based on the setup used by Lauffenburger et al. is often used for studying cell adhesion to solid substrates [21-25]. Cells are attached to a horizontal disc manufactured from the biomaterials (coated titanium and silicon discs) (Fig. 2a). A second, upper dish (stainless steel) with a centre hole (1.5 mm diameter) is mounted in distance *e* of 0.1 mm for Ti, TiN, and DLC and 0.25 mm for TiCN films. Through the hole liquid (21°C) is supplied forming a radial hydrodynamic flow of Sörensen buffer. Thus, the shear flow depends on the distance from the centre hole (see finite element simulation in Fig. 2b). When the forces exerted are sufficient, cells are removed from the solid surface and taken away in the laminar bulk flow. After testing the samples are observed in a fluorescence microscope in order to characterize and count the detached cells (detailed description see [24], shown e.g. for DLC coating on silicon wafer in Fig. 2c). The percentage of detached cells was then redrawn as a function of the wall shear stress ($\sigma(r)$ dependent on the radius $r$) calculated using the relation

$$\sigma(r) = 3*D*\eta / (\pi * r * e^2) \tag{1}$$

in which $D$ is the flow rate and $\eta$ is the dynamic viscosity of the Sörensen buffer fluid. An amount of 50% detached cells was assumed for the critical shear stress.

For testing *D. discoideum* Ax-2 cells were chosen, which were grown in axenic medium [26] in agitated suspension (180 rpm). The vegetative cells were harvested during exponential phase at a density of 2 - $4 \times 10^6$ $ml^{-1}$, pelleted by centrifugation and washed twice in Sörensen phosphate buffer (2 mM $Na_2HPO_4$/14.5 mM $KH_2PO_4$, pH = 6.2). Cell pellets ($10^7$ cells) were stored on ice and used within 8 h.

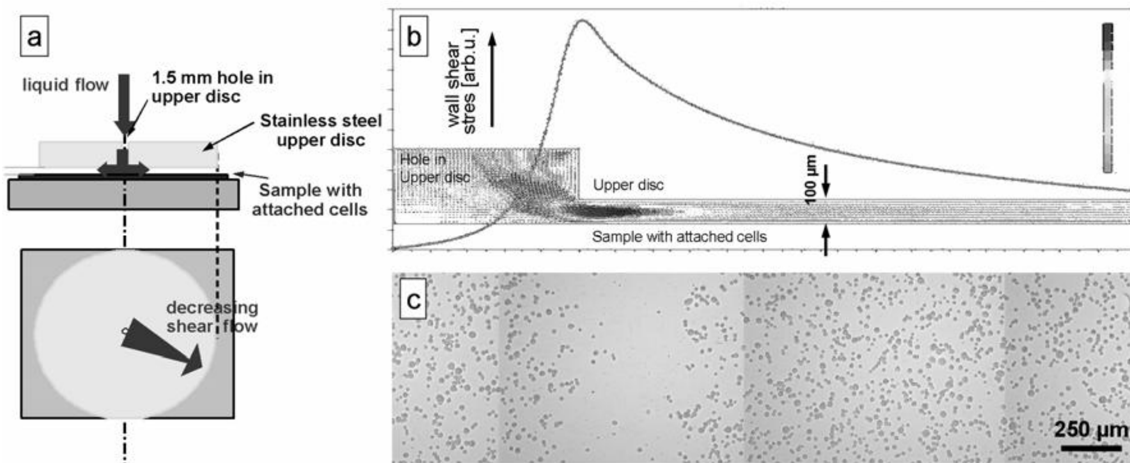


Fig. 2. (a) Experiment setup of the applied shear-flow-induced cell motility test (based on [21]): A radial hydrodynamic flow is generated between the upper stainless steel disc and the lower sample (coated) plate on which cells adhere. (b) Finite element simulation of the shear flow in the gap between the upper and lower disc and the wall shear stress on the (coated) sample plate. The shear stress induced by the flow on the plate decreases as *1/r*. (c) Dark-field light microscopy images of the cell attachment on a DLC coated silicon wafer sample disc in dependence on the distance from the middle axis of the SFICM test device (correlated to Fig. 2b), indicating no cell adhesion in the regions of very high wall shear stresses.

## 3. RESULTS AND DISCUSSION

### 3.1 Biocompatibility characterization with human fibroblasts

To investigate the biocompatibility to fibroblasts it was first ensured, that the number of adhering species was equal on the glass and the Ti, TiN, and $TiCN^h$ *RT*-PLD coated PU substrate surfaces. Subsequently, the expression of internal and external fibronectin was investigated by confocal microscopy. Fibronectin is a glycoprotein existing extracellular and on the cell surfaces in blood, body fluids and connective tissue. This protein associates with the other proteins of the extracellular matrix like fibrinogen, collagen, glicozaminoglicans and with suitable receptors of the cell membrane. The analyses in confocal microscopy (Fig. 3a-d) reveal the creation of bands with dense fibres laying on the axis of the adhered cells. On the control glass substrates and – a bit less developed – on $TiCN^h$ coated PU the net of the fibronectin create irregular connections in all directions.

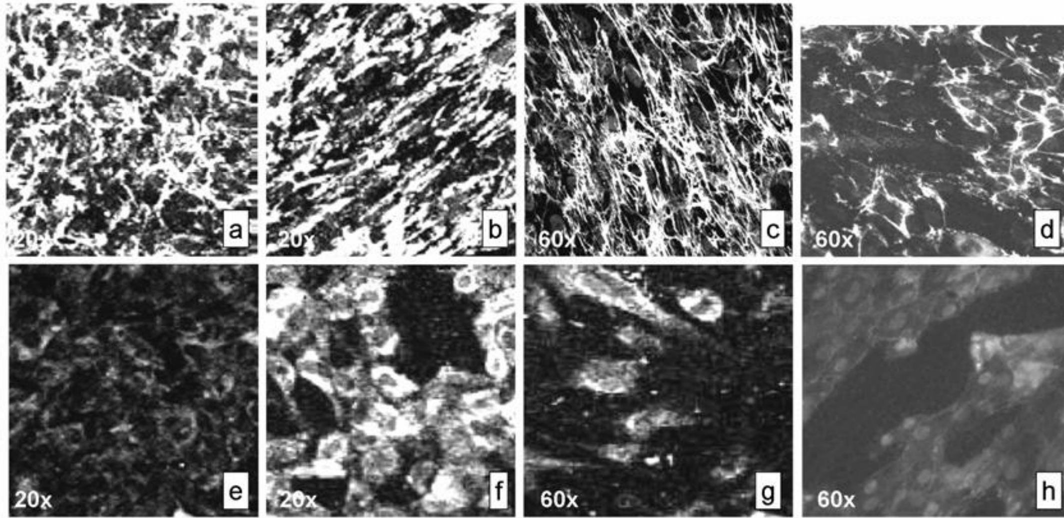


Fig. 3. Selected results of the biocompatibility tests: (a-d) Expression of internal and external fibronectin in the fibroblast cultivation. (e-h) Winculin expression in the fibroblasts. Substrate material: (a,e) control glass, (b,f) 50 nm Ti coating on PU, (c,g) 50 nm TiN coating on PU, (d,h) 50 nm $TiCN^h$ coating on PU.

Winculin is a polypeptyd connecting the proteins of the cell membrane with an active cytoskeleton of the cytoplasma cortex at the connection between cell and extracellular matrix – the place of adhesive plate formation. A huge expression proves strong adhesion of the cells to the substrate. By analyzing the winculin expression in confocal microscopy, such huge expression is eveident for Ti and $TiCN^h$, while the expression is lower on the glass substrate and the TiN film on PU (Fig. 3e-h). By using the Burcker camera the viability of the cells was observed, revealing 99% alive cells and only individual dead cells. Additionally, the proof for missing interleukin IL 1β showed no evidence of its formation in the 48-hours cultivation liquid gathered from the tested surfaces. This strongest immunostimulator is one of the basic factors for examining the immunogenicity of a biomaterial being formed in the cultivated cells, if the biocompatibility is low. Its missing reveals the high acceptance of all investigated materials by fibroblasts, which is in high accordance to Breme et al. [27].

### 3.2 Shear flow testing with *D. discoideum* cells

Initially, the static adherence of *D. discoideum* cells – attached on the coating surface from Sörensen phosphate buffer solution – was tested for referencing the subsequent dynamic testing. The attachment was found to be initially time dependent, but leading to stable, time independent cell densities on the surfaces after ~5 min for $TiCN^l$ and $TiCH^h$, ~10 min for DLC and ~15-20 min for TiN and Ti *RT*-PLD films. As higher the carbon content in the content the higher density was found – the maximum with ~1400 *D. discoideum* cells/mm² for $TiCN^h$, followed by DLC, while Ti and TiN surfaces lead to only ~600 adhering cells/mm². For all coatings an agglomeration of cells is evident, which develops during the static cell attachment, but with decreasing tendency with increasing cell density.

Performing SFICM tests generally leads to dependencies of the percentage of detached cells and the hydrodynamic stress due to the forced flow of Sörensen buffer solution as shown in Fig. 4a. At low shear stress, quite close to the edge of the disc, nearly all cells are still attached after testing. Increasing the stress by investigating regions closer to the disc center leads to a linearly to slight exponential increase of the detached cell percentage, which logarithmically approximates to 100% close to the centre hole, through which the buffer solution is pumped.

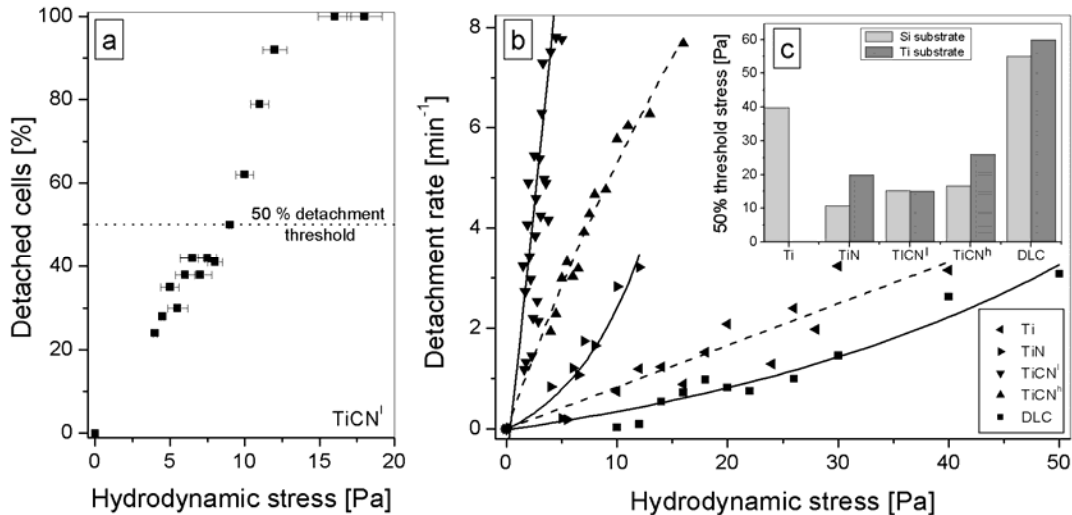


Fig. 4. Results of SFICM studies with *D. discoideum* cells on *RT*-PLD coatings: (a) Dependency of detached cells on the hydrodynamic stress for a $TiCN^l$ film on Si wafer substrate under stable conditions. The evaluation of the 50% threshold stress (50% cell detachment) is marked. (b) Detachment rate in dependency on the hydrodynamic stress of all coatings on Si wafer substrate. (c) 50% threshold stress for all coatings on Si and Ti substrates.

As well as for the static attachment, the dynamic detachment is strongly dependent on time. Thus, reaching the stagnation in detachment rates is decisive for pointing out differences of the surfaces. If such stable conditions are found, the detachment rate (detached cells / unity of time) describes the detachment kinetics quite well for up to 50% detached cells. As shown in Fig. 4b, the detachment rates increase linearly or exponentially with increasing shear stress (except $TiCN^l$ due to the very high rates). Ti and DLC surfaces allow good binding / sticking of *D. discoideum* cells while TiN and TiCN coating promote apparently the cell detachment. As a result, the hydrodynamic stress for 50% detached cells ("50% threshold stress") is lower than for the other coatings on the silicon substrates. The quite similar threshold stress found for Ti substrates [28] (for comparison see Fig. 4c) reveals the high influence by the nature of the coating / surface which is directly in contact to the cells and the diminishing influence of the substrate itself. Extremely high hydrodynamic stress (> 60 Pa) is required to detach *D. discoideum* cells (in their vegetative phase) from DLC surfaces revealing the optimal behavior of DLC as a biomaterial shown in numerous studies in literature in the last years. The comparison of the cell shape before and after shear flow testing (Fig. 5a and b) reveals no visible damage of the adhering cells even after applying the highest shear flows on DLC.

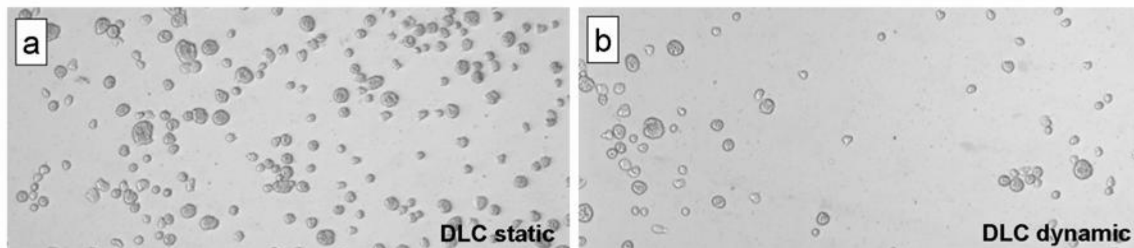


Fig. 5. Fluorescence microscopy images of the cell distribution on a DLC coating in (a) static attachment and (b) dynamic detachment of *D. discoideum* cells in the region of maximum shear stress in SFICM.

Based on earlier findings in shear flow testing of coatings on Ti substrates [28] the agglomeration of *D. discoideum* cells was comparatively investigated on silicon wafer substrates. Choosing such highly polished ($R_a < 1$ nm) substrates excludes all effects of differing substrate surface textures. In the previous work, the general tendency to agglomeration was found to be indirect proportional to the carbon content in the films – the highest tendency was found for TiN and $TiCN^l$ coatings, while in $TiCN^h$ and DLC the effect is strongly decreasing. A similar tendency was found in the current work: The higher the carbon content, the more regular the cell distribution on the surface is (see Fig. 6). As mentioned above in the comparison of the cell shapes (Fig. 5) neither an "accidental" (poisoning) or a "programmed" death of cells could be proved due to the missing of any modifications of the cell shape in the agglomerates. Because of the similar results on Ti and Si substrates effects of the disc or coating preparation can be ruled out, leading to a normal evolution behavior of the cells: Aggregation of *D. discoideum* amoebae relies on cyclic adenosine monophosphate (cAMP) signal molecules. The founder of a colony begins to secrete cAMP in response to "stress", like the shear stress in the SFICM. Other cells in the founder's surrounding detect the signal and respond by moving towards the signal and secreting more cAMP to boost the signal. Thus, a chain reaction is released with a movement of cells towards the area of highest cAMP concentration.

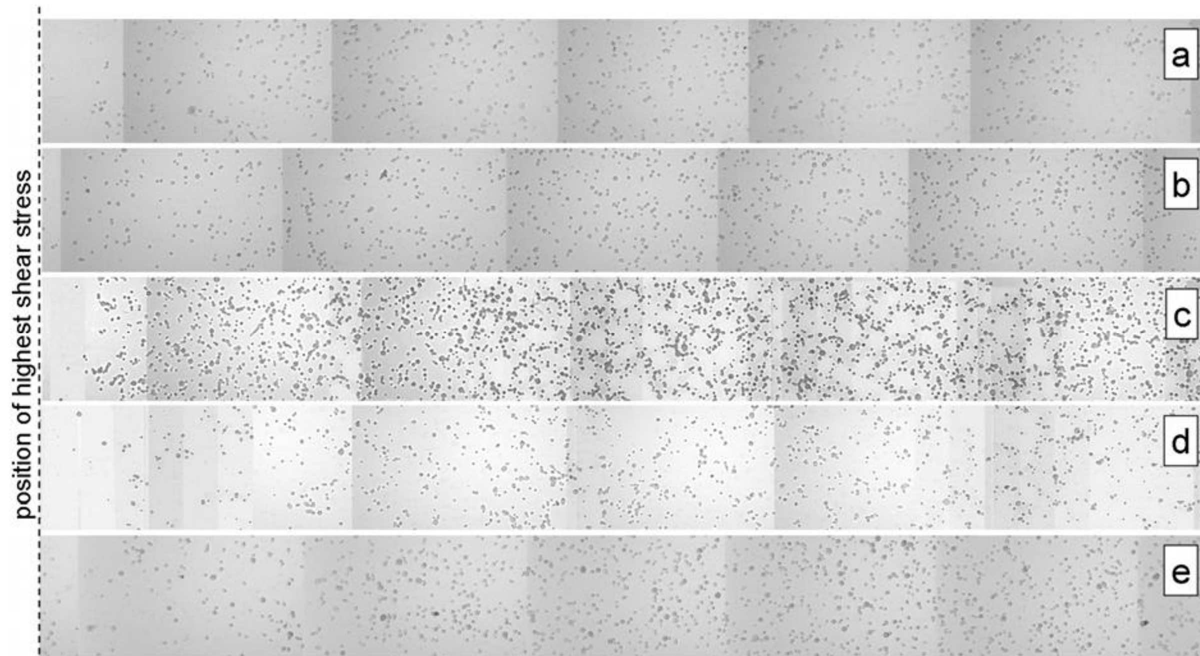


Fig. 6. Fluorescence microscopy images of the cell distribution and aggregate formation tendency under stable conditions in the SFICM test on coatings on Si wafers in dependence on the distance to the position of the highest shear stress (close to the disc centre): (a) Ti, (b) TiN, (c) $TiCN^l$, (d) $TiCN^h$, (e) DLC. For the TiCN films the decrease of the shear stress from left to right is due to the higher distance between the plates (0.25 resp. 0.1 mm) in the shear flow test about 7 times less than for the other coatings. The periodically brighter background is due to lightening in microscopy.

## 4. CONCLUSIONS

The current work showed, that the application of room-temperature coating be the pulsed laser deposition process (*RT*-PLD), industrially developed at Laser Center Leoben, allows high-adhesive deposition of Ti, TiN, TiCN and DLC coating on extremely soft polyurethane, but also on medical-grade pure titanium and silicon wafers. Testing the biocompatibility revealed comparable adhesion of fibroblast cells and cell death for these coatings and control glass substrates and a missing of the interleukin 1β immunostimulator. Thus, the *RT*-PLD coating let expect improvements in decreasing the tendency to thrombus formation in blood flowing on such surfaces. Additionally, as a strong tool for investigating the influence of the shear stresses to cells, the radial shear flow test results presents high critical stresses for

cell detachment for coating of high content of carbon, the highest for pure DLC. These tests were performed with *D. discoideum* cells in their vegetative phase allowing high comparability of the adhesion phenomena to human fibroblasts. Agglomeration tendency of these cells was found to be indirect proportional to the carbon content of the films, being a hint for the stress response of the cells.

## ACKNOWLEDGEMENTS

Financial support of this work by the Austrian Federal Ministry of Traffic, Innovation and Technology, the Austrian Industrial Research Promotion Fund (FFG) in the frame of the Austrian Nanoinitiative programme, the Polish Ministry of Science and Higher Education (PBZ, KBN-100/T08/2003, N 507 136 32/396)), the Government of Styria, and the European Union in the frame of the EFRE and EUREKA programme (E!2841 "Advanced PLD") is highly acknowledged. Further support for this work was given by the Knowledge-based Multicomponent Materials for Durable and Safe Performance KMM NoE project FP6.

## REFERENCES

[1] Cao, W., and Hench, L.L., "Bioactive materials", Ceramics Int., 22 (6), 493 (1996).
[2] Choquet, D., Felsenfeld, D.P. and Sheetz, M.P., "Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages", Cell, 88, 39 (1997).
[3] Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B. and Bershadsky, A.D, "Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism", J. Cell. Biol., 153, 1175 (2001).
[4] Cornillon, S., Pech, E., Benghezal, M., Ravanel, K., Gaynor, E., Letourneur, F., Bruckert, F. and Cosson, P., "Phg1p is a nine transmembrane protein superfamily member involved in Dictyostelium adhesion and phagocytosis", J. Biol. Chem., 275, 34287 (2000).
[5] Garrivier, D., Decave, E., Brechet, Y., Bruckert, F. and Fourcade, B., "Peeling model for cell detachment", Eur. Phys. J. E, 8, 79 (2002).
[6] Kay, R.R., "Chemotaxis and cell differentiation in Dictyostelium", Curr. Opon. Microbiol., 5, 575 (2002).
[7] Bozzaro, S., and Ponte, E., "Cell adhesion in the life cycle of Dictyostelium", Experientia, 51, 1175 (1995).
[8] Vogel G., Thilo L., Schwarz H., and Steinhart, R., "Mechanism of phagocytosis in Dictyostelium discoideum: phogocytosis is mediated by different recognition sites as disclosed by mutants with altered phagocytotic propgerties", J. Cell Biol., 86, 456 (1980).
[9] Chia, C.P., "A 130 kDa plasma membrane glycoprotein involved in Dictyostelium phagocytosis", Exp. Cell Res., 227, 182 (1996).
[10] Decave, E., Garrivier, D., Brechet, Y., Bruckert, F., and Fourcade, B., „Peeling process in living cell movement under shear flow", Phys. Rev. Lett., 89, 108101 (2002).
[11] Lackner, J.M., Waldhauser, W., Major, R., Major, B., Kustosz, R., and Czarnowska E., "Extending the biocompatibility of polyurethane surfaces in blood contact by pulsed laser deposited thin films", 49th Annual Technical Conference Proceedings of the Society of Vacuum Coaters, 17 (2006).
[12] Metev, S., and Meteva, K., "Nucleation and growth of laser-plasma deposited thin films", Appl. Surf. Sci., 43, 402 (1989).
[13] Reichelt, K., "Nucleation and Growth of Thin Films", Vacuum, 38, 1083 (1988).
[14] Muenchhausen, R.E., Hubbard, K.M., Foltyn, S., Estler, R.C., Nogar, N.S. and Jenkins, C.,"Effects of beam parameters on excimer laser deposition of YBaCuO", Appl. Phys. Lett., 56, 578 (1990).
[15] Afonso, C.N., Serna, R., Catalina, F. and Bermelo, D.," Good quality Ge films grown by excimer laser deposition", Appl. Surf. Sci., 46, 249 (1990).
[16] Kreutz, E.W., "Pulsed laser deposition of ceramics–fundamentals and applications", Appl. Surf. Sci., 127-129, 606 (1998).
[17] Lackner, J.M., [Innovative Coating by Pulsed Laser Deposition], PhD thesis, University of Leoben / Austria (2003).
[18] Lackner, J.M., Waldhauser, W., Ebner, R., Stotter, C., Jakopic, G., Schöberl, T., "Laserzentrum Leoben: Innovative Raumtemperatur-Laserbeschichtung für Optik, Sensorik und Verschleißschutz", BHM, 149, 118 (2004).

[19] Lackner, J.M., [Industrially-scaled hybrid Pulsed Laser Deposition at Room Temperature], Orekop, Krakow / Poland (2005).
[20] Orsello, C.E., Lauffenburger, D.A., and Hammer, D.A., “Molecular properties in cell adhesion: a physical and engineering perspective”, Trends Biotechnol., 19, 310 (2001).
[21] Cozens-Roberts, C., Quinn, A., and Lauffenburger, D.A., “Receptor-mediated adhesion phenomena. I. Model studies with the radial-flow detachment assay”, Biophys. J., 58, 107 (1990).
[22] Cozens-Roberts, C., Quinn, A., and Lauffenburger, D.A., “Receptor-mediated cell attachment and detachment kinetics. II. Experimental model studies with the radial-flow detachment assay”, Biophys. J., 58, 857 (1990).
[23] DiMilla, P.A., Stone, J.A., Quinn, A., Albelda, S.M., and Lauffenburger, D.A., “Maximal migration of human smooth muscle cells on fibronectin and type IV collagen occurs at an intermediate attachment strength”, J. Cell Biol., 122, 729 (1993).
[24] Decave, E., Garrivier, D., Brechet, Y., Fourcade, B., and Bruckert, F., “Shear flow-induced detachment kinetics of Dictyostelium discoideum cells from solid substrate”, Biophys. J., 82, 2383 (2002).
[25] Mikhalovska, L.I., Santin, M., Denyer, S.P., Lloyd, A.W., Teer, D.G., Field, S., and Mikhalovsky, S.V. “Fibrinogen adsorption and platelet adhesion to metal and carbon coatings”, *Throm. Haemost.*, 92, 1032 (2004).
[26] Ashworth, J.M., and Waats, D.J., “Metabolism of the cellular slime mould Dictyostelium discoideum grown in axenic culture”, Biochem. J., 119, 175 (1970).
[27] Breme, F., Buttstaedt, J., and Emig, G., “Coating of polymers with titanium-based layers by a novel plasma-assisted chemical vapour deposition process”, Thin Solid Films, 377-378, 755 (2000).
[28] Lackner, J.M., Waldhauser, W., Berghauser, R., Kahn, M., Bruckert, F., Major, R., and Major, B., “Detachment Kinetics of Eukaryote Cells from Biocompatible PVD Coatings”, 50th Annual Technical Conference Proceedings of the Society of Vacuum Coaters, 113 (2007).