The Influence of the Mechanical Properties of a-C:H Based Thin Coatings on Blood-Material Interaction

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Abstract. An interaction of blood with artificial materials is an important aspect in designing cardiovascular tissue analogues. The processes occurs on liquid/solid interface depends both on structural and mechanical properties of biomaterials. Main goal of the work was to develop novel blood contacting materials in the form of thin coatings with antithrombogenic properties by reduction of shear stress improving sufficient washing of biofunctional-adapted surfaces. Preliminary studies and simulations led us to carbon based thin coatings considered as silicon doped amorphous carbon. The rigidity of the surface design of materials dedicated for the biomedical purpose is of particular importance. The paper presents an analysis of the impact of material stiffness and mechanical for interaction with blood cells. Material analysis of in the context of the mechanical properties showed changes in stiffness depending on the thickness of the coating. The Young modulus and hardness of materials were examined by indentation test using Berkovich indenter geometry. Cell-material interactions were assessed using the cellular components of blood. Shear stress on the between the cell-and material were considered taking into account red blood cells and platelets concentrates.

Keywords: Hemocompatybility · Shear stresses · Blood-material interaction

1 Introduction

In blood-material interfacial the outermost layer of biomaterial is the most crucial for hemocompatibility properties of implant. Thus, an intensive research

has been focused on surface modification by controlling their physico - chemical properties [1]. There is growing interest in application of carbon based materials, in particular diamond like carbon (DLC) due to its bio-haemocompatible nature [2]. Carbon- based coatings exhibit attractive tribological, electrical, chemical and optical properties for blood contacting materials [3]. Many studies show that ultrathin a-C:H and a-C:H:Si films can be used to improve the surface features necessary for improved biocompatibility both metals and polymers [4]. Krishnan et al. [5] showed that DLC coating on titanium substrate exhibit a lower platelet adhesion in comparison to non-modified titanium. The blood compatibility of DLC coating depends on the deposition conditions which was confirmed by Alanazi et al. [6]. They have also proved that surface wettability and chemical composition is independent of film thickness. DLC coatings show very well adherent properties as well as the ability to protect biological implants against corrosion or serve as diffusion barriers [7]. Thus, DLC coatings have found many potential biological application for intra-coronary stents [8], prosthetic heart valves [9] or rotary blood pump [10]. Over the past decades evolution in the field of biomaterial engineering has shifted from developing materials that were merely tolerated by the body to creating those that elicit a specific response. When it comes to blood-material interaction it is necessary to design fully atrombogenic surface which do not adverse interact with any blood components [11,12]. This represents a really complex task due to a variety of processes occurring within this interface including plasma protein adsorption, cell adhesion, and activation followed by thrombus formation [1]. Interaction of blood cells with artificial surfaces plays a major role in host response and determine the material hemocompatybility [13]. Shear stresses as a consequence of blood flow can also improve cell activation and aggregation. Blood cascade activation can lead to a serious consequences including formation of blood clots followed by unhindered in blood flows and resulting in implant failure [14]. Thus, the blood -material interaction under physiological conditions is critical when designing new materials for blood interface. This interactions can be influenced by factor such as surface charge, hydrophobicity, topography and material strength. The work is focused on the in vitro interaction of red blood cells and platelets with artificial surfaces under physiological conditions. a-C:H and Si-DLC coatings with different thickness were deposited on the surface of silicon wafers by magnetron sputtering. The coatings adhesion strength, Young modulus, hardness and wettability were analysed. The protein adsorption from fatal bovine serum solution were performed in order to analyse surfaces affinity to albumin. Cell-materials interaction were analysed using dedicated radial flow chamber which gives ability to apply different values of shear stresses. Mechanical interaction between substrates and platelets/erythrocytes were analyzed. In the frame of the work, the radial detachment chamber was described in details.

2   Materials and Methods

2.1   Coatings Preparation

The silicon wafer (diameter 14 cm) were modified by ultrathin carbon based thin films using magnetron sputtering in direct current (DC), unbalanced mode. For the work the following thin coatings were prepared: a-C:H 100 nm, Si-DLC 500 nm, Si-DLC 300 nm, Si-DLC 200 nm, Si-DLC 125 nm and Si-DLC 15 nm thick. Carbon targets (the latter for Diamond Like Carbon-DLC) were used to deposit films on silicon wafer substrates at room temperature in an argon atmosphere. To ensure homogenous film thickness over the entire coated surfaces, substrates were rotated during deposition at a speed of 5.4 cm s^{-1} through the plasma plumes. A detailed description of the deposition arrangement is given elsewhere [15].

2.2   Radial Flow Chamber

Cell-material interactions were analysed using dedicated radial flow chamber (Fig. 1A). For analysis red blood cells concentrate and platelets concentrate were used irrespectively. The radial flow chamber allows to apply different values of shear forces in cell-material interface. The detachment of the cells from the surface depends on the value of applied shear forces (Fs) and adhesion forces (Fa), which determine the strength of cell adhesion (Fig. 1B). When the Fs is less or equal to Fa, cell detachment is not observed (Fig. 1Ba). In other cases, cells are removed from surface one by one (Fig. 1Bb) or randomly (Fig. 1Bc). In radial flow chamber, hydrodynamic stresses are applied on material surface by liquid flow along the radius of disc-shape sample (Fig. 1C). A workspace of chamber consists of a liquid reservoir with stand in a central location. A sample was placed on stand when liquid (phosphate buffered saline (PBS, pH 7.4)) level was below its surface. Concentrate of human erythrocytes and platelets (diluted 4000x by using a phosphate buffered saline (PBS, pH 7.4) was uniformly applied to the surface of the tested materials. The covered samples by cell solution were left for a 10 min in order to cells adhesion to the surface. After that time, the PBS level in reservoir was raised until the draft of the sample. On the top of sample, disc-shape tripod was placed leaving a 150 µm gap. A constant volumetric flow of PBS was pumped through the whole in center of the tripod and flows radially to the disc-shape sample edges. The distance between the surface of the investigated material and the flow chamber had the strong influence on the value of the shear stress. It was set on the level of 250 µm. The flow rate was constant during the entire experiment and depended individually on the tested material. In the center of the radial flow chamber, in the place of injection of the elution medium, the direction of flow of the liquid is perpendicular to the surface of the sample located under the chamber. At this point, the likelihood of the cell detachment is the lowest. At the edge of the hole the direction of flow of elution medium changed from perpendicular to parallel to the surface analyzed. At this point,
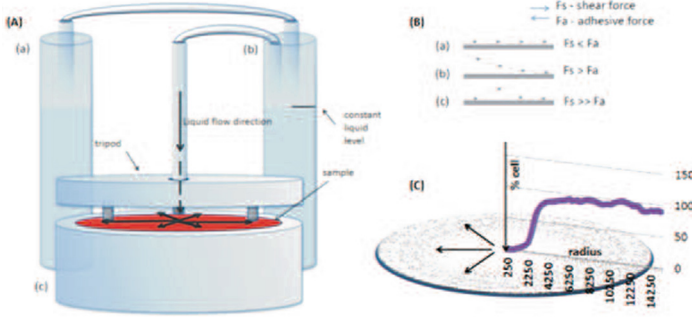
Fig. 1. Schematic illustration of radial flow test; (A) Radial flow chamber assembly (Aa) working reservoir (Ab) stress determining reservoir (Ac) radial flow chamber with sample; (B) Influence of shear forces into cell detachments (Ba) no cell detachment, (Bb) detachment one by one, (Bc) selective cell remove from substrate; (C) Distribution of shear forces on the sample and exemplary of cell detachment from surface. The arrows represent the liquid flows. The graph shows standard example of the percentage of cells on surface along radius (Color figure online)

the likelihood of cell detachment is the greatest. The applied shear stress can be calculated using Eq. (1).

$$\sigma = \frac{3D\eta}{\pi re^2} \tag{1}$$

where D is the flow rate, which depends on the material, η is the dynamic viscosity of the fluid $(10\,\mathrm{g/(cm^*s)})$, and e is the distance between the disk and the plate set at $150\,\mu\mathrm{m}$ for this experiment.

3   Results and Discussion

3.1   Cell Detachment

The percentage of adhered erythrocytes and platelets in the function of applied stresses is presented in Figs. 2 and 3, respectively. The values of shear stresses were calculated using equation (1). Generally, the strength of cell-material inter-action depends on the coating composition, thickness as well as on cell type. The differences in erythrocytes and platelets affinity to substrates were observed. The platelets-material interaction reaches plateau at higher stresses than for erythrocytes except SI-DLC 300 nm film. Si-DLC 300 nm exhibit strong affinity to both, platelets and erythrocytes. The 85,5 % of attached erythrocytes were removed from the Si-DLC sample after exposure to 73 Pa. Comparing to other samples, at plateau the smallest number of detached cells at the highest value of applied stresses was observed. Si-DLC 500 nm, Si-DLC 200 nm and Si-DLC 15 nm films show weak affinity to erythrocytes but strong to platelets. For Si-DLC 15 nm coating, cell detachment at plateau is about 90 % for erythrocytes

but only 70 % for platelets. For platelet-material interaction, Si-DLC 15 nm function reach plateau at stress equal to 60 Pa which is the highest value comparing to other samples. Comparing to other coatings, a-C:H 100 nm sample shows weak interactions with both, erythrocytes and platelets. About 90 % of attached cells were removed from the surface of a-C:H 100 nm film by applying the shear forces in the range of 20–25 Pa. For Si-DLC 125 nm film differences in materials interaction with erythrocytes and platelets are very gentle.
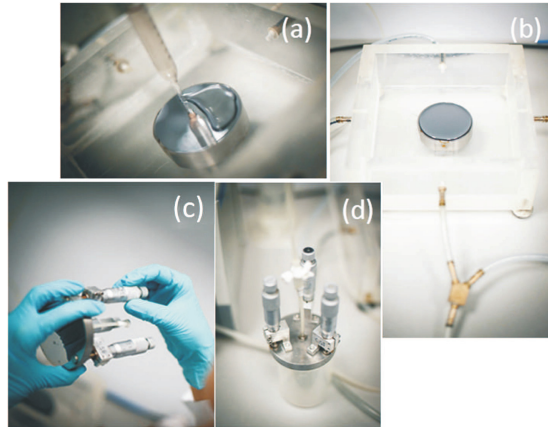

Fig. 2. The percentage of adhered erythrocytes to the surfaces in the function of applied shear stresses

In Figs. 2 and 3, 50 % of detached cells determine critical stresses in which the probability of cell detachment or their remain on the surfaces is the same. The values of critical stresses for each coating is presented on Table 1. In most cases critical stresses for erythrocytes are higher than for platelets except Si-DLC 300 nm and Si-DLC 15 nm. Generally, applying stresses between 4 Pa to 12 Pa remove half of adhered cells from surfaces. For all coatings detachment rates, which represent the number of detached cells per minute were presented in the function of applied stresses for erythrocytes (Fig. 4) and for platelets (Fig. 5). Exponential trends lines were adjusted to measurement points. The exponential function was extrapolated to 0 shear stress in order to determine the detachment rate under conditions where there is no shear force generated by flow applied. The values of the spontaneous shear rate are presented in (Table 1). For all materials the values of spontaneous detachment rate of platelets are much higher than derachment rate of erythrocytes.
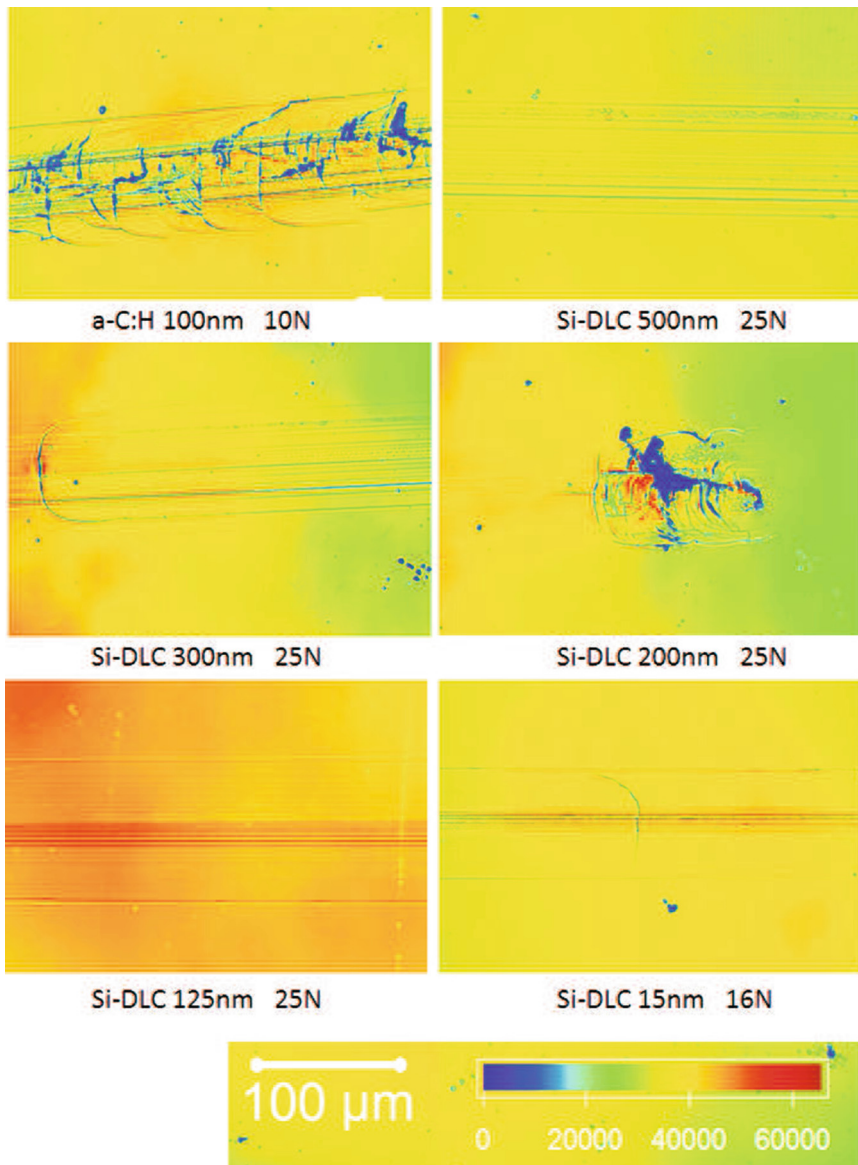
a-C:H 100nm   10N

Si-DLC 500nm   25N

Si-DLC 300nm   25N

Si-DLC 200nm   25N

Si-DLC 125nm   25N

Si-DLC 15nm   16N

100 µm

0   20000   40000   60000

Fig. 3. The percentage of adhered platelets to the surfaces in the function of applied shear stresses.

Table 1. Critical shear stresses and spontaneous cell detachment rate for platelets and erythrocytes.

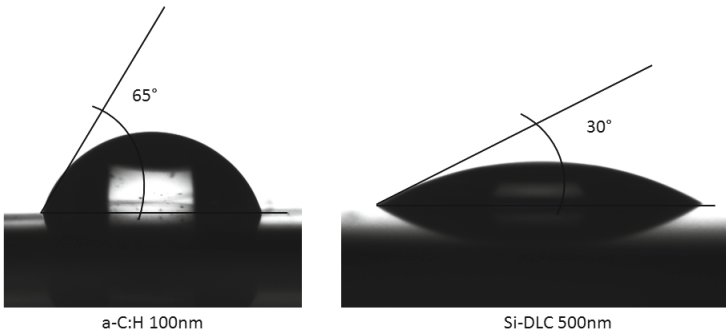| Material	Critical stress [Pa]		Spontaneous detachment rate [1/min]	
	Erythrocytes	Platelets	Erythrocytes	Platelets
a-C:H 100 nm	5.62	3.88	0.25	4.95
Si-DLC 500 nm	6.37	3.86	0.23	4.33
Si-DLC 300 nm	9.13	11.24	0.11	0.52
Si-DLC 200 nm	6.59	5.68	0.24	0.51
Si-DLC 125 nm	7.39	4.98	0.12	2.04
Si-DLC 15 nm	7,96	9.01	0.19	1.49

Fig. 4. Detachment rate in the function of applied stress for erythrocytes.
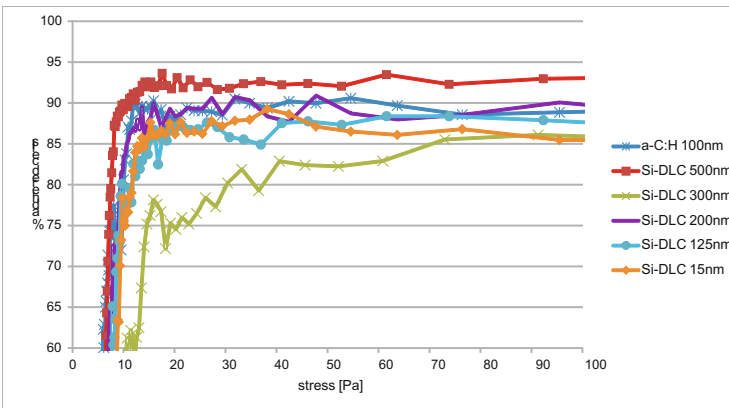

Fig. 5. Detachment rate in the function of applied stress for platelets.

4   Conclusions

Progress in developing materials for blood interfaces have been an area of active research over the last two decades. Thrombus formation as an effect of blood-biomaterial interaction represent one of the greatest risk factor of cardiovascular implant failure. Blood cells-material interaction is a key factor which has direct influence on surface hemocompatybility. Based on achieved results the following conclusions can be made: The surface modification by ultra-thin coatings deposition impacts on the interaction of materials with blood components

- The surface modification by ultra-thin coatings deposition impacts on the interaction of materials with blood components
- The interaction of Si-DLC coatings with platelets differs than for erythrocytes. Only Si-DLC 300 nm film exhibit strong interaction with both, platelets and erythrocytes (Table 1).

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