Thrombogenicity Ranking of Metal and Diamond-like Carbon Coated Polyurethane Surfaces under Dynamic Test Conditions

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Statement of Purpose: Improving the biocompatibility of blood contacting materials requires dynamic testing to simulate in-vivo conditions. Minimizing biomaterial impact to platelet (PLT) function is the final goal. However, PLTs respond to minimal stimulation of blood flow (shear stress) and vessel/biomaterial surface. The commercially available and clinically popular cone-and-plate(let) analyzer (CPA) is an option for PLT function in-vitro testing due to low donor blood consumption and its accepted principle for shear stress testing [1]. Goal of this study is its first time use for thrombogenicity ranking of biomaterial coatings – 20 nm thick thin films of titanium, titanium nitride (TiN), titanium oxide (TiO_x) and diamond-like carbon (pure a-C:H and doped with Ti (Ti:a-C:H), Si (Si:a-C:H) and Ti+N (Ti:a-C:H:N) deposited on polyurethane (PU) surfaces.

Methods: Films were deposited on PU (Elasthane 55D) by magnetron sputtering and pulsed laser deposition at room temperature from metallic targets in reactive atmosphere (O_2, N_2, C_2H_2) [2]. Films have either nanocrystalline (Ti, TiN) or amorphous structure and maintain the micro-topography of the substrates, while the nano-topography is roughened by stress-induced formation of 5 nm high hills, separated by 15 nm gaps [3]. The CPA device (Impact-R, DiaMed AG) consists of a PTFE cone of 2.45° point angle, rotating at 1800 s^{-1} for 5 minutes in a venous blood-filled (130µl, healthy male donor) polystyrene well that was modified for this study by introducing the (coated) PU base disc. Baseline control of platelet function occurred by static storage and full activation by adenosine diphosphate. Flow cytometry (EPICS XL, Beckman Coulter Inc.) was applied for analyzing PLT activation markers CD61 (conformational change of glycoprotein (GP) IIb/IIIb) and CD62 (P-selectin expression). PLT-PLT aggregates were investigated after lysis of erythrocytes by CD61, granulocyte-PLT aggregates after centrifugation and immunostaining with CD14+ and CD61. Testing the thrombogenic potential of blood plasma occurred by the Zymuphen MP-activity ELISA kit (Hyphen Biomed) for trapping phospholipid-rich microparticles derived from cell membranes.

Results and discussion: Hemostasis starts with primary, initially reversible PLT adhesion on damaged vessel walls (or biomaterials) [4]. If shear stress is too high or the surface is thrombogenic, this can result in PLT activation [5]. Indicators for PLT function are GPs at the PLT membrane, of which active GP IIb-IIIa complexes are the most important, controlling fibrinogen binding to PLTs in primary aggregation. In flow cytometry of the tested liquids after CPA testing, low concentrations of GP IIb-IIIa were found for pure PU and for a-C:H, Ti:a-C:H, TiN and Ti:a-C:H:N films, while concentrations for Si-a:C:H, Ti, and TiO_x are significantly higher. However, flow cytometry results of liquid derived in a-C:H and Ti testing are questionable, because confocal microscopy revealed high surface adhesion for PLTs for them. Although initial PLT binding by fibrinogen is reversible, it is followed minutes later by an irreversible stabilization in secondary aggregation, as expected for flow cytometry results due to ~10 min handling time (CPA and cytometry). For all non-PLT adhesive films and PU (except TiN and Ti-a:C:H:N), no statistically significant differences in PLT-PLT aggregate concentrations were measured in the liquid. Only these two and the PLT adhesive materials show lower concentrations. Aggregation is accompanied by Ca^{2+}, fibrinogen, and P-selectin release from PLT granules [5], being important in mediating adhesion of activated PLTs to neutrophils, monocytes and lymphocytes. However, no statistically significant differences were found for all non-PLT adhesive surfaces. In contrast, the activation and aggregation triggered PLT consumption is distinctly influenced by the films: Lowest consumption / removal from circulation is evident for Si:a-C:H, Ti:a-C:H:N, and TiN, having lowest PLT aggregate concentrations in liquid. Finally, shear stress can result in PLT fragmentation, release of cell membrane vesicles and final PLT-rich microparticle (PMP) formation, imposing thrombotic burden to circulating blood [6]. PMP concentration was generally found to be direct proportional to the PLT consumption of tested films.

Conclusions: Ranking the thrombogenic behavior of the investigated thin films in-vitro based on CPA test results leads to Ti:a-C:H:N and TiN to be optimally applicable and much better than state-of-the-art PU, possessing the least thrombogenic surfaces and inducing least impact to blood. Si:a-C:H is a further candidate, however, struggles with high GP IIb-IIIa activation. High surface adhesion of PLTs and worst behavior regarding PLT consumption were found for Ti and a-C:H. Similar ranking was found in albumin/fibrinogen protein adsorption studies of these materials presented elsewhere [7].

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