European Cells and Materials Vol. 5. Suppl. 1, 2003 (pages 44-45) ISSN 1473-2262

HEMOCOMPATIBLE DIAMOND-LIKE CARBON (DLC) SURFACES

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INTRODUCTION: Diamond-like carbon (DLC) coatings are attractive because of low friction coefficient, high hardness, chemical inertness and smooth finish which they provide to biomedical devices. Silicon wafers (Si_{waf}) and silicone rubber (Si_{rub}) plates were coated using plasma-enhanced chemical vapor deposition (PE-CVD) techniques. Human blood was used for the *in vitro* assessment of hemocompatibility. This study has taken into account three events of the blood activation: coagulation, platelet activation and inflammatory process. Tests are based on the observation of platelet adhesion and activation, thrombin generation and complement convertase production induced by the sample surface.

METHODS: Hydrogenated DLC (a-C:H) films were fabricated on Si_{waf} and Si_{rub} using magnetron sputtering techniques with a graphite target and acetylene as reactive gas. Both sides of the samples were coated with a typical thickness of 1µm. Coated surfaces were compared to uncoated materials and to the following reference materials: polymethylmethacrylate (PMMA), polyethylene (LDPE), polydimethylsiloxane (PDMS) and medical steel (MS). The thrombin generation assay (TGA) and complement convertase assay (CCA) were provided by HaemoProbe bv as ready-to-use test kits.

TGA can be briefly described as a measurement via an enzymatic colorimetric assay of the amount and the speed of thrombin formation when samples are incubated in a modified plasma. The thrombin generation kinetic for each material is obtained by plotting the thrombin concentrations versus the incubation time.

The complement activation results in the generation of complement factors contributing to the initiation of inflammation process. CCA allows to evaluate the samples for complement factor binding ability.

Platelet adhesion, aggregation and spreading are processes by which platelets form thrombus. Samples were incubated in a freshly prepared human platelet-rich plasma (PRP) according to the method developed by Frank et al [l]. After incubation, glutaraldehyde was used to fix the platelets on the material surfaces, and imaging was done by scanning electron microscopy (SEM).

RESULTS: Generation of thrombin is the critical event of the coagulation process. Thrombin plays a key role in the formation of blood clot through diverse activities. Figure 1 shows the amount of thrombin generated by the samples in a citrated plasma.

Fig.1 : Thrombin generation curves

PMMA, PDMS, MS and Si_{waf} native surfaces led to the activation of coagulation with a significant increase of thrombin generation after 1 min in contact with plasma. Si_{waf} was the most activating surface. On this substrate, thrombin generation reached a maximum of approximately 200mU/ml after 2 min of incubation in plasma. For the LDPE and Si_{rub} samples, thrombin generation was dramatically delayed and reduced. These samples did not seem to propagate and amplify the blood clotting process by the thrombin generation event. Thrombin formation was much more retarded in plasma exposed to DLC-coated than to native Si_{waf}. The time to onset of thrombin generation was about 4min after which an increase to a maximal amount of 174mU/ml was observed. In the case of the Si_{rub} sample, the DLC coating seemed to stimulate a higher thrombin formation than on the native sample. On the other hand, although reaching the same final maximum levels, DLC coatings on Si_{waf} delayed significantly the induction of thrombin activity and might delay the blood-clotting process.

Platelet activation results in platelet shape changes, spreading, aggregation and secretion of their granule content. Platelet surface coverage was very prominent on PMMA and MS, with a strong aggregation (Figure 2). On these surfaces, the platelets were flat and widely spread and seemed to have reached complete cytoskeletal reorganization, with a tendency to achieve complete circumferential and confluent filamentous areas around aggregates. LDPE samples showed platelets with a dense formation of pseudopodia and few developed platelets. Platelets adhered to the Si_{waf} surface to a lesser extent than to PMMA and MS surfaces, but they were in an activated state with the presence of long pseudopodia. In the cases of Si_{rub}, DLC-Si_{waf} and DLC-Si_{rub}, the few adherent platelets remained in discoïd form. Some filamentous areas were observable on the DLC-Si_{rub} surface.

Synthetic surfaces do not only activate the coagulation cascade but do also provoke inflammatory response involving the complement system. PDMS, MS, PMMA, LDPE, Si_{waf} all provoked a complement convertase adsorption whereas DLC-coated surfaces and Si_{rub} sample could be qualified as inactive for complement factor binding (Figure 3).

CONCLUSIONS: DLC coating delayed clotting time and tended to suppress platelet and complement convertase activation, in contrast to PMMA, LDPE, PDMS, MS and Si_{waf} native substrates. The inert nature and the smoothness of the DLC-coated surfaces appeared to dominantly explain the good *in vitro* hemocompatibility.

REFERENCES: [1] M. Jones, I. McColl, D. Grant, K. Parker, T. Parker (2000) *J Biomed Mater Res* **52**:413-21. [2] R. Frank, H. Dresbach, H. Thelen, H. Sieberth (2000) *J Biomed Mater Res* **52**:374-81.

ACKNOWLEDGEMENTS: This work is supported by the COST Action 527 (Contract n° BBW/OFES-NBC01.0051) from the Swiss government, which is acknowledged.

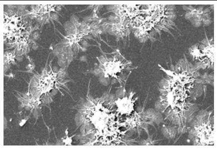
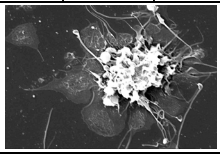
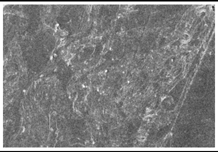
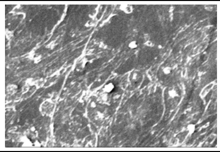
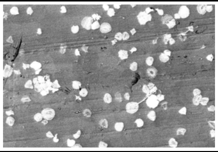
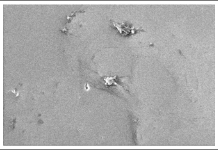
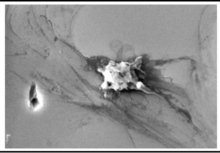
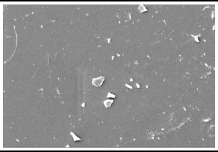
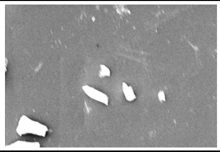
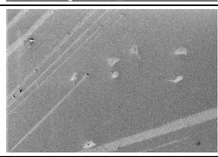
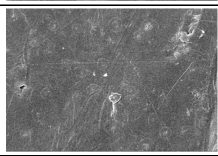
Samples	140µm x 92µm	36µm x 25µm
PMMA		
LDPE		
MS		
Si_{waf}		
Si_{rub}		
DLC-Si_{waf}		-
DLC-Si_{rub}		-

Fig. 2: SEM of adherent platelets on samples.

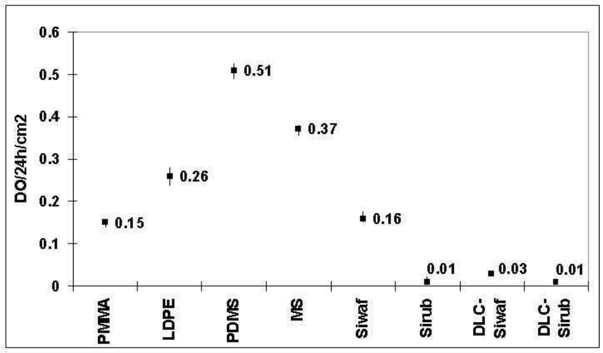


Fig.3:Amount of complement convertase bound to the sample surface.