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Long-term stable surface modification of DLC coatings

Abstract: The use of coatings based on diamond like carbon (DLC) for medical applications was established during the last years. Main advantages of these coatings are its high hardness, good wear and friction behavior and its biocompatibility. Using low-energy electron-beam treatment, we addressed the surface modification of DLC coatings. The aim was to generate new biofunctional surface characteristics that are long-term stable.

Electron-beam modification resulted in significantly increased surface hydrophilicity, giving rise to the conclusion, that biological reaction on these surfaces will also be influenced by the modification. Furthermore, the stability of the surface modification was investigated. Therefore, the modified samples were stored for 8 weeks under ambient conditions. Additionally, the samples were stored in physiological saline solution at 37 °C for 8 weeks. The stability of the modification was analyzed by contact angle measurement confirming no changes over the whole period of storage. In addition, the stability against standard cleaning and sterilization procedures was investigated. The durability of the modification to withstand these cleaning procedures was also proven.

With these findings, the low-energy electron-beam modification seems to be a suitable tool for surface modification of DLC coatings. Thereby, the very good long-term stability is a great improvement in comparison to conventional surface modification methods like plasma treatment. In order to investigate the suitability of the modified coatings for biomedical applications, the cellular response was investigated using human fibroblasts, revealing a significantly reduced cell count on modified surfaces while maintaining their biocompatibility. By modification of the DLC surfaces, it is possible to adapt the cell adhesion on the treated surface areas. These findings demonstrate electron-beam treatment to be applicable for partial surface modification and functionalization within biomedical applications.

Keywords: DLC, surface modification, low-energy electron-beam, long-term stability, cell adhesion

https://doi.org/10.1515/cdbme-2017-0072

1 Introduction

The investigations of diamond like carbon (DLC) coatings for biomedical applications increased strongly during the last years. The advantages of these coatings are the high hardness, good wear and friction behaviour and their well known biocompatibility (1, 2). DLC coatings enable the combination of characteristics of carbon and diamond. These characteristics depend on the hydrogen content and the different bonding structures of the carbon atoms. There are many deposition methods available for DLC coatings. Mostly, the basic physical principle is vapor deposition. Currently, cathodic-arc deposition, plasma-activated chemical vapor deposition and magnetron sputtering represent the most utilized technologies (3, 4).

The further adaption of the capabilities of DLC coatings is often realized using surface modification technologies. The methods therefore vary from post-treatment with ionizing radiation (5, 6) to doping with different elements (7–11). The modification of sole surface areas is also under investigation. Therewith, the cell adhesion could be controlled by scribing patterns or adaption of particular surface characteristics (12).

Using plasma surface treatment allows the exclusive adaption of surface functionalities, leaving the bulk characteristics of a coating and the coating substrate unaltered. However, with this technology the surface modification results in time-dependant alterations of the surface characteristics, as the effects generated by plasma treatment are usually not long-term stable (6, 13, 14).

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2 Material and methods

2.1 Sample preparation

Stainless steel samples (material number: 1.4301 according to DIN EN 10088-3) were used as coating substrates. Besides round discs with a diameter of 10 mm, samples with a geometry adapted from tissue culture well plates were coated and used for biological tests. This specialized sample geometry has been previously described [4].

The samples were coated with DLC using a reactive DC magnetron sputtering process. The process gas was Ar/C_2H_2 (chamber pressure $1*10^{-2}$ mbar). An adhesion promoting Chromium-interlayer was used, following this Cr-layer, a graduated a-C:Cr was deposited. As top layer, pure a-C:H was deposited. The final coating thickness was adjusted to approximately 1 µm.

The DLC surface modification was done using the REAMODE electron-beam equipment at the Fraunhofer FEP, Dresden. The used technology is a low-energy electron-beam technology, allowing surface modification with accelerated electrons at atmospheric conditions and insignificant heat input. The electron acceleration voltage was set to 150 keV and the beam current was 5 mA. The working distance between the samples and the Lenard-window was kept constant at 45 mm.

The applied dose was determined by exposure time to the electron-beam and checked using radiochromic film dosimeters (Risø B3 dosimeter from Risø High Dose Reference Laboratory, Denmark). Under ambient conditions, the samples were modified with a dose of 500 kGy, which was applied in 10 single irradiation steps with 50 kGy.

2.2 Surface characterization

The wettability of the DLC coated samples was evaluated using the contact angle measurement system OCA 20 from Data Physics Instruments GmbH, Filderstadt, Germany (software: SCA22, version 3.12.11).

The sessile drop method with a drop volume of 1 µL was chosen to determine the contact angle of water on the sample surfaces. A hollow needle with an inner diameter of 260 µm was used.

The contact angle (CA) was also used as measure to determine the long-term stability of the DLC modification after storage under varying conditions and after cleaning and sterilization of the modified samples.

2.3 Cell culture

The biological response to the modified DLC surfaces was investigated using human fibroblast primary cells (AG 01522D, Coriell Institute, USA). The cells were cultured in Eagle's MEM (supplemented with 10% fetal calf serum, 1% L-glutamine (Biochrome) and 1% non-essential amino acids (Biochrome)) at 5% CO_2 and 37 °C to near confluency. By trypsinization and subsequent centrifugation (5 min, 150 g), the cells were harvested. The determination of the cell density was carried out with a Neubauer counting chamber. With culture media (same as described above), the cells were diluted to a final concentration of $3*10^4$ cells/mL. Afterwards, a volume of 217 µL/cm^2 of the cell suspension was seeded into the DLC coated well plates (described above) and cultured for 72 h. Standard cover glasses were used as reference (VWR).

The cell number on these surfaces was determined after cell fixation with formaldehyde (4% in PBS) and permeabilization with TritonX (0.5% in PBS) by fluorescence staining of the cell nuclei with DAPI and examination by fluorescence microscopy (OlympusBX61, ex/em 350/470nm).

The cell viability was determined by flow cytometric analysis using propidium iodide (PI) staining as described by Zamai et al. (15). The analysis was conducted with a FACScan (ex 488 nm, em 585 nm, 5,000 events per sample). As negative reference for flow cytometric analysis, cells were treated with ice-cold ethanol for induction of apoptosis/necrosis.

2.4 Investigation of the modification stability

For the investigation of the long-term stability of the electron-beam modified DLC coatings, the samples were stored up to 8 weeks under varying conditions. During storage under ambient conditions (at air and room temperature), the contact angle of the samples was determined directly after the electron-beam modification as well as in time intervals of 1 week, 4 weeks and 8 weeks. To determine the long-term stability when stored in physiological medium, the contact angle was determined directly after the modification and after 8 weeks of storage in physiological saline solution (PBS) at 37 °C and 5 %CO_2.

The stability of the electron-beam modified DLC samples against standard cleaning and sterilization procedures was investigated by mechanical cleaning of the samples with ethanol (70% in distilled water) and subsequent autoclaving (20 min at 134 °C). The contact angle was determined directly after the electron-beam modification and subsequently after the cleaning and sterilization process.

3 Results and discussion

3.1 Sample characterization

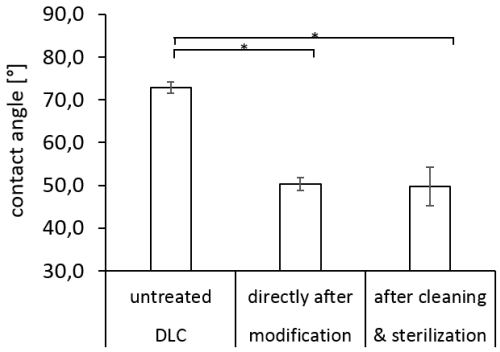

Figure 1: Contact angle on untreated and modified DLC and after cleaning and sterilization of the DLC surface.

The wetting behavior of the DLC samples before and after the electron-beam surface modification is illustrated in Figure 1. The treatment leads to hydrophilization of the DLC surfaces.

In previous studies, it was demonstrated that the improved wettability is due to the increase of the polar component of the surface free energy. In parallel, increased amounts of surface bound functional groups containing oxygen and nitrogen were observed (4). These observations give rise to the assumption, that cell adhesion on these surfaces could be influenced by electron-beam modification. In order to use these capabilities for biomedical applications, the biocompatibility of the electron-beam modification had to be assessed.

3.2 Cell count and biocompatibility

The cell count was determined by fluorescence staining of the cell nuclei. Figure 2 shows a significantly reduced cell number on electron-beam modified DLC samples (b) when compared to untreated DLC surfaces (a).

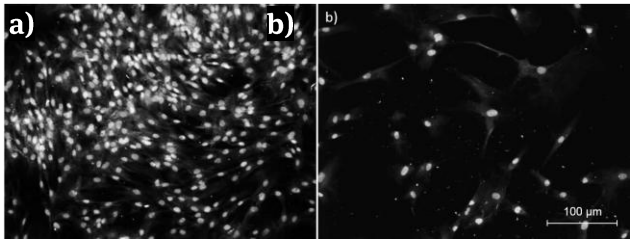

Figure 2: Cell nuclei on untreated (a) and modified DLC coatings (b).

Evaluation of the cell count revealed a significant reduction to 25 % compared to the standard reference (data not shown).

The cell viability was determined by flow cytometric analysis with PI staining. On untreated and on modified DLC coatings the vital cell amount when compared to the standard reference was 103.0±1.1 % and 94.8±3.9 % respectively (data not shown), which indicates no significant differences.

The cell count revealed a reduction of the adherent cell number, while the biocompatibility of the modified coatings is not impaired as demonstrated by the flow cytometric analysis of the cell viability. With these findings, it is possible to adapt the cell adhesion on DLC surfaces by electron-beam modification allowing varying surface functionalities for biomedical applications of the DLC coatings.

In order to investigate, whether the coatings could be modified long time before the application of the medical product, the long-term stability of the modification was evaluated.

3.3 Stability of the modification

The long-term stability of the electron-beam modified DLC coatings was investigated over a time period of 8 weeks. Therefore, samples were stored under ambient conditions or in PBS, respectively. The water contact angle of the surfaces was used as measure for any alterations. Samples were measured before and after the modification and after long-term storage.

As can be seen in Figure 3, whether the storage under ambient conditions nor storage in PBS have significant influence on the wetting behaviour of the modified samples. These results demonstrate the long-term stability of the modification for at least 8 weeks under varying conditions.

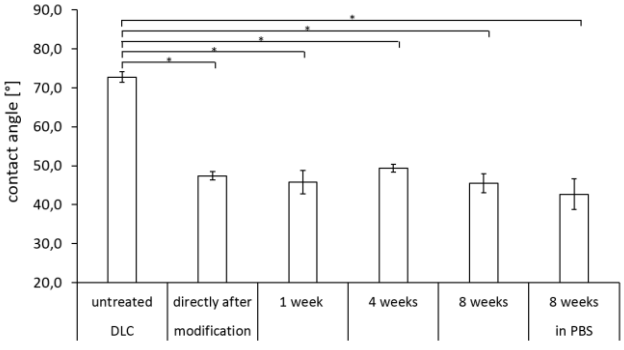

Figure 3: Contact angle on untreated and modified DLC during long-time storage under ambient conditions and in PBS.

Furthermore, the stability of the modified coatings against cleaning and sterilization procedures was investigated. The samples were subjected to mechanical cleaning with ethanol and subsequent sterilization at 134 °C. Despite this harsh cleaning procedure, no changes of the surface wettability were observed (Figure 1). The results demonstrate the very good stability of the electron-beam modified DLC coatings against reprocessing methods of medical devices.

4 Conclusion

DLC coatings obtained by DC-magnetron sputtering were modified by low-energy electron-beam treatment. This results in a surface hydrophilization with significantly reduced cell adhesion on modified surfaces. As the biocompatibility on the modified coatings is maintained, these surfaces can be used to adapt the biofunctionality by means of cell adhesion.

The modification is long-term stable under varying conditions and in addition, the modified coatings are able to withstand conventional cleaning and sterilization procedures.

Both, the good long-term stability and the durability during the harsh reprocessing for medical products allows using electron-beam modified DLC coatings for medical applications. The confirmed biocompatibility as well as the possibility to adjust the cell adhesion, enable the biofunctionality adaption of these coatings for biomedical purposes.

Acknowledgment: EC Europ Coating GmbH (Dresden, Germany) are acknowledged for preparing the DLC coatings within the presented investigations.

Author's Statement

Research funding: The European Union and the Free State of Saxony funded parts of this work, funding reference: 100106122. Conflict of interest: Author states no conflict of interest. Informed consent: Informed consent is not applicable. Ethical approval: The conducted research is not related to either human or animals use

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