Plasma polymerized ethylenediamine (PPEDA) thin films for cell-adhesive implant coatings

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Plasma polymerized ethylenediamine (PPEDA) thin films (20 nm - 70 nm) were deposited on the powered electrode of the low pressure capacitively coupled 13.56 MHz plasma. The chemical composition of the thin PPEDA films was characterized by FTIR spectroscopy. During storage of the PPEDA films in air, characteristic changes in the FTIR spectrum were observed immediately after deposition and over long-time period of 360 days. Additionally, the adhesion of human osteoblastic cells MG-63 (ATCC) on PPEDA coated samples was investigated. Corundum blasted titanium alloys (Ti6Al4V) were used as specimens. The cell adhesion was characterized by flow cytometry and the cell shape was analysed by scanning electron microscopy. A significant increase of the initial cell adhesion was observed compared with the uncoated reference sample. The enhanced initial cell adhesion is remaining after long-time storage in air.

1. General

Plasma Enhanced Chemical Vapour Deposition (PECVD) is a useful tool for synthesis of thin films with specific physical and chemical properties [1]. In particular, the plasma chemical conversion of organic precursor molecules in a low temperature plasma and the interaction with material surfaces results in deposition of thin organic films with high content of functional groups. An interesting field of application is the coating of implant materials for endoprosthesis to achieve bioactive surfaces, e.g. the nitrogen-rich coatings with enhanced initial adhesion of human osteoblastic cells. Here, thin plasma polymer films with N/C ratio up to 50% from ethylenediamine (EDA) as precursor [2] are of great interest. This paper concerns with investigations of the stability of nitrogen-rich thin PPEDA films under ambient conditions to have knowledge about the physical and chemical film modification during long time storage and its influence on the initial adhesion and proliferation of osteoblastic cells.

2. Experimental set-up

In figure 1 it is shown the used experimental set-up for synthesis of thin nitrogen-rich organic films by plasma polymerization. The stainless steel vacuum chamber with diameter and height of 400 mm was pumped by a turbo pump to a base pressure of $10^{-5}$ Pa. To ensure a process gas pressure between 20 and 200 Pa a stage rotary pump is applied. The process gas was a mixture of the carrier gas argon and the precursor ethylenediamine (EDA). The liquid precursor EDA in the storage vessel was held on constant temperature at 30°C to ensure a constant vapour pressure. The gas flow rates could be varied between 4 and 60 sccm for argon and between 4 and 20 sccm for EDA. The EDA gas flow rate is adjusted by a needle valve. The gas supply including the needle valve was also heated to avoid condensation.

The discharge arrangement consists of a planar electrode powered by the rf-generator at 13.56 MHz via matching network with rf power between 20 and 100 W. The electrode shielding and the chamber wall provide the grounded electrode. To ensure a defined temperature of the samples on the powered rf-electrode its temperature (10-20°C) was controlled by a cooler. The discharge can operate in continuous (cw) or pulsed mode. Therefore, a pulse-delay generator was connected with the rf-generator which allows pulse frequencies between 2 and 10
Hz. The typically used plasma processing parameters are the mixture of argon and EDA 5:1 at total pressure of 60 Pa and gas flow rate of 24 sccm, the rf power of 60 W pulsed with frequency of 10 Hz at duty cycle of 50%.

Additionally, a floating cover plate is mounted above the powered electrode to allow a temporary covering of planar samples on the rf electrode. This cover plate enables the investigation of thin film deposition after defined state of plasma chemical conversion of the precursor molecules without interruption of the rf plasma. The plasma chemical conversion of the precursor gas was studied by mass spectrometry and FTIR transmission spectroscopy of the gas phase as well as the thin film deposition on the powered electrode and the wall of the plasma reactor by ellipsometry and quartz crystal microbalance, respectively. The thickness of the thin PPEDA films was determined by spectroscopic ellipsometry using a single layer Cauchy dispersion model for PPEDA on silicon substrate.

The chemical composition of the PPEDA thin films and the molecular changes due to storage at ambient conditions was studied by FTIR reflection absorption spectroscopy (FT-IRRAS). For the IRRAS measurements the thin film is deposited on highly reflective aluminium layer.

Corundum blasted titanium alloys (Ti6Al4V) were used as sample material in cell adhesion tests.

3. Results and discussion

3.1. Thin film molecular structure and stability

In figure 2 the absorption spectrum of a 45 nm thin plasma polymerized ethylenediamine (PPEDA) film, taken immediately after the deposition (black), is compared with its absorption spectrum (red) after storage over 360 days in air. The IR absorption spectrum of PPEDA after the deposition (day 0) is characterized by absorption bands of the N-H stretching and deformation overtone vibrations broadened due to hydrogen bridge bonds (3500-3000 cm⁻¹), the C-H symm./asymm. stretching vibrations (2980-2880 cm⁻¹), the stretching vibrations of nitrile group C≡N and carbon-carbon triple bond C≡C (2200-2150 cm⁻¹), the imine group C≡N and carbon-carbon double bond C≡C vibrations (1690-1650 cm⁻¹), and the deformation vibrations of the amine group N-H (1650-1510 cm⁻¹) [3]. The comparison of the thin film absorption spectrum directly after preparation with that after 360 days storage under ambient conditions shows significant changes in the absorption spectrum. The difference of these absorption spectra provides information about the formation or the degradation of molecular groups as well as the broadening and shift of absorption bands, see figure 3.

![Fig. 2 FT-IRRAS spectrum of thin PPEDA film directly after preparation and after 360 days storage in air.](image)

For example, characteristic changes due to ageing of the thin PPEDA films are the formation of O-H groups (stretching vibrations at 3500-3000 cm⁻¹), carbonyl groups C=O (stretching vibrations at 1700-1680 cm⁻¹) which may be assigned to acid amide. In the fingerprint region changes are found which are assigned to the deformation vibrations of C-H and O-H groups at 1465-1375 cm⁻¹ as well as to the stretching vibrations C-N and C-O at about 1250 cm⁻¹ and 1100 cm⁻¹, respectively. Furthermore, the loss of the C≡N and C≡C molecular structures is observed.

![Fig. 3 The difference of the spectra from Fig. 2 shows the formation and degradation of molecular structures/groups after storage over 360 days under ambient condition.](image)

Beside the reactions with oxygen or water from ambient, the absorption spectrum after storage, shown in figure 2, reveals more clear separated absorption bands, e.g. in the fingerprint region. This might be also interpreted by more defined molecular structure due to chemical reactions and relaxation...
processes inside the cross-linked amorphous plasma polymer film.

In figures 4 and 5 the temporal development of the absorbance at different wavenumbers is shown which indicates the formation of new molecular groups or the modification of PPEDA characteristic molecular groups during storage in air.

The considerable increase of the absorbance at the wavenumber range between 1700 and 1680 cm\(^{-1}\), assigned to the carbonyl group in acid amine [2], is observed over the first 30 days (logarithmic time scale in figure 4), but is continued over the complete storage time of 360 days. A similar behaviour is observed for the stretching vibrations assigned to C-N and C-O in the fingerprint region. On the other hand, the content of the nitrile groups C≡N is significantly reduced over the first 30 days and decreases further during the storage time, whereas the carbon-carbon triple bond C≡C in the long-time storage is already strongly reduced after 2 days and remains on low level.

Therefore, the PPEDA film ageing was additionally investigated on a short-time scale over 3 hours immediately after the film deposition. Both, the IRRAS thin film spectra were analysed during storage of the PPEDA film under vacuum conditions and in contact with ambient air. The storage under vacuum shows no significant ageing effects, whereas the storage in air results in strong decreasing absorbance of the triple bonded carbon (C≡C), see figure 6. The absorbance of other molecular structures in PPEDA is slightly changed under vacuum conditions, only.

3.2. Cell adhesion and morphology

The short time cell adhesion experiments were carried out by flow cytometry (FACSCalibur, BD Biosciences) after 10 min. On PPEDA functionalized corundum blasted (cb) Ti6Al4V surfaces we could find out a significant increase of attached cells within the time frame of 360 days of storage (see figure 7), although the PPEDA thin film is already altered significantly within 0-30 days after deposition as seen in figures 4-6. Furthermore, the sterilization process of the PPEDA-treated samples with \(\gamma\)-irradiation, which is necessary for medical applications, did not influence the adhesion behavior of our osteoblasts (human MG-63 osteoblastic cells, ATCC).

The same long term stability of the cell attracting capacity of PPEDA could be observed using scanning electron microscopy (SEM). The cells melt into the surface structure and the cell morphology is flattened and well spread, see figure 8.
Fig. 7 Adherent cells on sterile and unsterile PPEDA thin films on cb-Ti6Al4V within 360 days of storage. Both, significant enhanced initial cell adhesion is observed on sterile and unsterile films compared with the reference sample without PPEDA deposition.

3. Outlook
Further investigations will focus on the improvement of the mechanical as well as water stability of the PPEDA thin layers and their impact on cell functions.

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5. References

Fig. 8 Morphology of adherent cells on PPEDA thin films measured by SEM (scaling factor: x1000)
a) untreated, b) day 4 and c) day 360.
Note that cells on PPEDA seem to melt into the rough samples topography of the corundum blasted Ti6Al4V also on day 360 (arrow).