Amine coatings are useful for numerous applications including tissue engineering and biosensing. The bio-applications of amine thin films, such as biomolecule immobilization, biosensors or cell adhesion enhancement require good layer stability in water. However, typically for allylamine or ammonia/ethylene plasma polymerization, increased plasma polymer cross-linking, leading to improved film stability, is achieved at the expense of the amine-group density. Cyclopropylamine (CPA) is a promising non-toxic monomer recently used for the deposition of amine-rich thin films. The stability of the CPA plasma polymers depends on power, discharge mode, monomer flow rate and the discharge configuration. In this paper it is demonstrated that the deposition of CPA plasma polymers can be processed on various substrates including sensor electrodes (for biosensing application) and on the nanofibrous meshes (for a tissue engineering). The thickness loss below 1% combined with high amine concentration (>7at.%) can be achieved at the optimized plasma conditions and therefore these coating have promising future for bio-applications.

Introduction

The preparation of amine-rich surfaces has attracted attention of numerous researchers due to its great potential applications, such as biomolecule immobilization [1,2], microfiltration membranes [3], enzyme electrodes [4], adhesion enhancement [5] or biosensor development [6]. The simplest technique of amine grafting relies on surface plasma treatment in nitrogen or ammonia discharges [2, 7]. However, these techniques lead to unstable functionalization of a thin near surface layer with rather short duration. It has been observed that the grafted groups almost disappeared after several days of aging in air, and the composition of the surface became comparable with the untreated layer [8].

An alternative efficient way to create amine surfaces is a plasma polymerization of amine-based monomers. In this case the stability of amine groups can be enhanced and, thus, aging effect reduced [1]. Due to a high reactivity of primary amines the plasma polymerization should be performed at mild conditions, i.e., low power input and pulsing the discharge, avoiding oxygen species. The presence of oxygen contamination can easily oxidize and “de-activate” the amine groups. Thus, the preparation of amine-rich surfaces is more often performed in low pressure discharges.

For many years, allylamine has been the monomer of choice [3,9,10,11] thanks to the presence of vinyl group (Figure 1) that enable free radical polymerization of this amine compound. However, allylamine is a highly toxic flammable chemical compound with a lethal dose LD₅₀ equal to 35 mg/kg. Additionally, the stability of amine-rich allylamine plasma polymers is not sufficient for bioapplications. A successful preparation of amine-rich films was achieved for example using diaminocyclohexane [3] and ethylenediamine [6,4]. Another promising approach for the amine-rich coating deposition is the plasma polymerization of the isomer of allylamine, cyclopropylamine (CPA). R. Snyders' group showed that pulsed plasma polymerization of CPA using inductively coupled RF discharge lead to more efficient incorporation of amine groups than in the case of allylamine [12].
(t_{OFF}) in which the reactions are governed by existing radicals. It leads, in general, to a higher retention of the monomer structure. Therefore, the plasma conditions are for simplicity described by a mean RF power $P_{\text{mean}}$ calculated as the on-time power $P_{\text{ON}}$ multiplied by the duty cycle D.C.

$$P_{\text{mean}} = P_{\text{ON}} \times \frac{t_{\text{ON}}}{t_{\text{ON}} + t_{\text{OFF}}} = P_{\text{ON}} \times D.C.$$

The mean power is used in the composite parameter $W/F$ that expresses an average power input per unit of monomer flow. Although the description of the complex plasma polymerization process by this parameter is crucial simplification it proved to be applicable as scaling parameter in some case [17].

![Figure 2](image-url)

According to the fitting of X-ray photoelectron spectroscopy (XPS) N1s signal the films deposited in the PW mode of the tubular reactor at optimum $W/F$ contained about 12 at.% of NH$_x$ groups (Figure 3b). A high concentration of amine groups was accompanied by a presence of nitriles (C≡N), isonitriles (C≡N-) and/or conjugated imines (-N=C=N-) identified in the infrared spectra as a double peak at 2245 and 2190 cm$^{-1}$ (Figure 3a). The deposition in vertical reactor confirmed that the PW process can produce films with a higher amount of amine groups (Figure 3c) but the comparison of FT-IR spectra of both amine-rich films revealed less amount of nitriles and conjugated imines (Figure 3a). The difference between the chemistry of the films deposited in tubular and vertical reactor at different potentials requires further clarification. A high amount of nitrile groups can affect the antibody immobilization chemistry due to possible nucleophilic addition reaction with the participation of nitriles [18]. Moreover, the conjugated imines can overcomplicate the analysis of the layer chemistry during the immobilization process. Anyway, both types of the films are suitable for bioapplications because they possess surfaces rich of amine groups and they are still sufficiently stable in water as discussed in the next section.

![Figure 3](image-url)

**Water Stability of Amine-Rich Plasma Polymers**

The application of amine-rich thin films for cell adhesion enhancement or immobilization of biomolecules requires high stability of the layers in aqueous media and prevention of trapped toxic molecules. The amine-rich plasma polymerized allylamine thin films showed a significant decrease in nitrogen concentration (N/C decreasing from 0.22 to 0.06) [9, 11] and film thickness loss up to 90% after the immersion [10]. The stability can be improved by an increase of plasma power at the expense of amine concentration in the films [19].

Recently, it was found that copolymerization of acetylene and NH$_3$ or ethylene and NH$_3$ leads to the deposition of stable amine coatings (thickness loss 15% in water) bearing around 5 at.% of amine groups, if a high flow rate of C$_2$H$_4$ or C$_2$H$_2$ is used [7,19,20]. In order to obtain even higher concentration of the amine groups, a high flow rate of ammonia is necessary and the coatings deposited in these conditions suffer from thickness loss (up to 70%) after immersion in water. By plasma polymerization of n-heptylamine it is possible

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to deposit sufficiently stable (thickness loss 15 %) amine coatings but the concentration of amine groups and nitrogen in such films is significantly lower compared to the allylamine plasma polymers (N/C ~ 0.12) [21].

The CPA plasma polymerization in the tubular reactor (Figure 2a) yielded films with various degrees of water stability. However, optimized conditions employing PW mode of the discharge resulted in sufficiently water-stable polymers rich of amine groups [13]. The film R3, whose chemistry is reported in Figure 3c, exhibited thickness loss about 20 % after immersion in water for 48 hours. XPS analyses revealed that immersion in water induced an oxygen increase by 4-5 at.% at the expense of nitrogen. The XPS N1s curve fitting showed a decrease of the NH3 contribution by 4.5 at.% accompanied with an increase of amide (N-C=O) environment by 2.0 at.%. Although, the water stability these films can be considered as promising it is desired to obtain amine-rich films that do not show any changes induced by interaction with water.

Recently, the CPA pulsed plasma polymerization was carried out in the reactor depicted in Figure 2b. The film R2, whose chemistry is reported in Figure 3b (NH3 environment of 9.7 at.%), exhibited a thickness loss below 1% after water immersion for 216 hours. This is an excellent result compared to previously published behavior of amine-rich films. The NH3 environment decrease only by 2.8 at.% while the amide concentration was not affected by the immersion in water. Figure 4 shows the SEM micrographs of amine-rich films after 48 hours in water.

![Figure 4. SEM micrographs of amine-rich films after 48 hours immersion in water.](image)

Biosensors Prepared with Help of Plasma Polymerization

The biomolecule immobilization step is critical for the development of any sort of a biosensor. It provides the core of the biosensor and gives it its identity. Moreover, the immobilized biomolecule needs to keep its original functionality as required for the function of the biosensor. Therefore, care has to be taken to avoid a steric hindering of the recognition sites [22]. Antibodies remain by far the most frequently used biorecognition elements. They offer high affinity and specificity against the target analyte [23]. In order to immobilize biomolecule on the surface a covalent bond between the surface functional group and biomolecule (e.g. protein) must be developed.

Following the promising results on the plasma polymerization of CPA in CCP discharges shown in previous sections the films were applied to the development of a quartz crystal microbalance (QCM) immunosensor. The pulsed CPA plasma polymer was deposited onto the QCM gold electrode and then the antibody specific to human serum albumin (HSA) was attached to the QCM surface via cross-linkage obtained by intermediate reaction with glutaraldehyde (Figure 5a). The XPS N1s curve fitting confirmed the immobilization of the antibody, as high concentrations of amide environment (N-C=O) as well as the protonated amine groups (NH3+) attributed to aminoacids were detected (Figure 5b).

The sensitivity of the sensor with a CPA plasma polymer thickness of 20 nm, was evaluated as the change of the frequency due to the antigen binding, i.e. approximately 100 Hz for 50 μg/ml HSA, is a very promising achievement (Figure 5c). It significantly exceeds responses obtained using QCMs with the antibody immobilized via a thiol-based self-assembled monolayer because their signal to HSA was typically around 35 Hz [24].

![Figure 5. Development of QCM biosensor: a) scheme of the biosensor functionalization, b) XPS N1s curve fitting of the surface after antibody immobilization (PP-GA-Ab), c) the response of the QCM biosensor in the form of frequency change Δf.](image)

Modification of Polymer Nanofibers by CPA Plasma Polymers

Electrospun nanofibers with a high surface area to volume ratio are gaining increasing interest because of their potential applications in biomedical devices, tissue engineering scaffolds and drug delivery carriers [25]. However, an exploitation of nanofiber meshes for these purposes requires activation of their surface by reactive groups, such as carboxylic acid, hydroxyl or amine groups. Therefore, the plasma polymerization of CPA was tested also on the surface of biodegradable polycaprolactone (PCL) micro/nanofibers. Fibrous meshes were prepared by NanospiderTM technology using the laboratory machine NS LAB 500S from Elmarco s.r.o. The nanofibers were formed from 50 cm length wire rotating electrode (or roller) and collected on nonwoven...
polypropylene foil. As shown by SEM (Figure 6), the fibers were 1.2 ± 0.4 μm in thickness and mesh pore size was 8 ± 2 μm. As shown in Figure 6, the uncoated PCL fibers exhibited a high water contact angle (above 120°) whereas the deposition of the CPA polymers using R2 process led to its significant decrease to 30°. The XPS analysis revealed that the entire top surface of the nanofibers was coated by the CPA plasma polymer as the elemental and the functional compositions of the surface were very similar to the reported CPA plasma polymerization results (Figure 3b). The concentration of the NHx environment determined by the XPS N1s curve fitting exceeded 7 at.%. It is well known that surfaces exhibiting low water contact angle and high amine concentration are more favorable for a cell adhesion and proliferation [1] and, therefore, a perspective application of the CPA plasma coated nanofibers is foreseen.

Figure 6. Water contact angles (on left) of uncoated (120°) and plasma coated (30°) PCL nanofibers. SEM micrograph (on right) of plasma coated PCL nanofibers.

Conclusion

The CPA plasma polymerization is a versatile technique allowing for the deposition of amine coatings exhibiting high NHx concentration combined with the essential film stability. The concentration of amine groups depends on the plasma power discharge mode and monomer flow, while the increased film stability can be achieved by optimization of the configuration of the discharge. Here, it was shown that layers deposited on the D.C. self-biased substrate exhibited the decrease of thickness loss by a factor of 20 compared to the coating grown on the substrates at a floating potential. At the same time the NHx environment was decreased very slightly, by 3 at.% and moreover, layers exhibiting more NHx >7at.% can be considered as the amine-rich ones.

The combined water stability and amine density is a great advantage of the CPA plasma polymerization compared to the commonly used ammonia/ethylene or allylamine plasmas, where the increased amine concentration was always achieved at the expense of the film stability. In perspective, the CPA plasma layers with even higher chemical stability can be synthesized by the optimization of the plasma parameters.

By depositing CPA plasma polymers onto biodegradable PCL nanofibers, the amine-rich films (NHx >7 at.%) Furthermore, the obtained sensitivity of the sensor with a CPA plasma polymer is very promising because it significantly exceeds responses obtained using QCMs with the antibody immobilized via a thiol-based self-assembled monolayers.

References

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