Michael Teske*, Katharina Wulf, Joschka Fink, Andreas Brietzke, Thomas Eickner, Daniela Arbeiter, Volkmar Senz, Niels Grabow and Sabine Illner

Controlled biodegradation of metallic biomaterials by plasma polymer coatings using hexamethyldisiloxane and allylamine monomers

Abstract: Plasma enhanced chemical vapor deposition is a promising process for the generation of tailor-made polymer coatings on medical devices in order to improve their implant/ host interaction. The ultra-thin coatings can fulfil a variety of purposes, depending on the monomers used, the process conditions and the location of the coated implants in the human body. In addition, even complex geometries can be coated easily and without the application of solvents. Particularly hydrophilic and hydrophobic plasma polymer coatings can improve biocompatibility, especially in blood contact. Furthermore, the selection of the monomers used enables the generation of specific functional groups for further surface immobilization of drugs, such as proteins, by chemical crosslinking. The release of toxic residues from polymeric implants, such as monomers, additives or degraded components, can also be avoided. The aim of our investigation was the generation of plasma polymer films, their characterization and application as coatings for biodegradable metallic biomaterials in order to retard the degradation process. Metallic biomaterials, in various forms are frequently used in orthopaedics, dentistry, cardiovascular and neurosurgical equipment, because of their tensile strength, fracture toughness, fatigue strength and electrical conductivity.

Plasma polymerization was performed using the monomers hexamethyldisiloxane and allylamine. The resulting plasma polymers were analysed in an accelerated degradation test. Both plasma polymers appear to be promising, while polyHMDSO appears to degrade over time and polyallylamine indicates to be stable.

Keywords: PECVD, plasma polymers, allylamine, hexamethyldisiloxane, HMDSO, biomaterials

<https://doi.org/10.1515/cdbme-2019-0079>

1 Introduction

Plasma Enhanced Chemical Vapor Deposition (PECVD) is used to apply thin layers of e.g. polymers in the range of a few nanometres of 1-2 micrometers to surfaces [1]. The properties of the layers depend on the choice of the used monomers or monomer mixtures. PECVD coatings are characterized by a high degree of crosslinking, a short chain length and a random distribution of the monomers [2].

They allow to influence the permeation properties, e.g. as a top layer of spray coatings to delay the release of the pharmaceutical active ingredient or as a diffusion barrier of water to delay the degradation of degradable scaffolds. Moreover, suitable functional groups can also be generated on the surface by the selection of the monomer, which can be used for further surface modifications [1,2].

In this study we apply the common term “plasma polymerisation” for the PECVD of allylamine and hexamethyldisiloxane (HMDSO). The potential of the plasma polymers as protective layers to delay the degradation of metallic biodegradable biomaterials, which are increasingly used in medical technology [3], will be considered. Aluminium was used as model metal substrate for the plasma polymers. The plasma coated samples were examined morphologically and infrared spectroscopic. In addition, accelerated degradation of the samples and cell colonization tests of the surfaces were performed.

***Corresponding author: Michael Teske** Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany, michael.teske@uni-rostock.de

Katharina Wulf, Joschka Fink, Andreas Brietzke, Daniela Arbeiter, Thomas Eickner, Volkmar Senz, Niels Grabow, Sabine Illner: Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany

2 Materials and Methods

2.1 Materials

HMDSO, allylamin and aluminium foil were purchased by Sigma Aldrich (Taufkirchen, Germany). Calcein AM staining solution has been obtained from Thermo Fisher Scientific (Bremen, Germany).

2.2 Methods

Plasma polymers were generated using a plasma chamber with a radio frequency generator (13.56 MHz, Diener electronic GmbH & Co. KG, Ebhausen, Germany) [4].

Morphological changes were examined in a QUANTA FEG 250 (FEI Company, Frankfurt/Main, Germany) scanning electron microscope (SEM) according to Teske *et al.* [5].

Fourier transform infrared spectroscopy-attenuated total reflection (FTIR-ATR) measurements were performed using a Bruker Vertex 70 IR-spectrometer (Bruker, Ettlingen, Germany) equipped with a DLaTGS-detector. Each spectrum was recorded in the range of $4000\text{--}500\text{ cm}^{-1}$ at a spectral resolution of 4 cm^{-1} and with 32 scans on the average using a Graseby Golden Gate Diamond ATR-unit. All spectra were analysed using OPUS software (Bruker, Ettlingen, Germany) and were subsequently atmosphere and baseline corrected.

The accelerated degradation study of the samples ($\varnothing = 12\text{ mm}$) was carried out in 1 M hydrochloric acid at $25\text{ }^{\circ}\text{C}$ and was monitored using a digital camera Canon 80 A (Canon, Krefeld, Germany) for about 2 h.

Biological evaluation was carried out with L929 mouse fibroblasts (CCL-1, ATCC) generally according to cell culture protocols as described previously [6] with $n = 6$. Cell staining was conducted with calcein AM staining solution and imaging was realised with Olympus IX81 confocal laser scanning microscope (CLSM, 488 nm excitation and 505 - 605 nm fluorescence imaging filters).

3 Results

In order to achieve an adequate plasma polymer coating on aluminium foils, several process parameters such as generator power, chamber pressure and plasma process time regarding layer thickness and surface hydrophilicity were

examined (data not shown). The applied process parameter for HMDSO and allylamine plasma coating for this investigation are shown in Table 1.

Table 1: Applied process parameter chamber pressure, generator power and process time for plasma polymerisation of aluminium foils with HMDSO or allylamine.

	chamber pressure [mbar]	generator power [W]	process time [min]
HMDSO	0.6	10	20
allylamine	1.3	100	20

Figure 1 shows an exemplary SEM image of a partly polyHMDSO coated aluminium foil. Here the successful plasma coating with HMDSO can be plainly seen. Furthermore, a relatively homogeneous and uniform coating was achieved.

Figure 1: Representative SEM images of polyHMDSO coated site (grey, right) and uncoated aluminium foil (white, left).

Chemical changes by plasma polymerisation were also visible by FTIR-ATR. For polyHMDSO (Fig. 2A green) coated samples, differences of the IR bands in the range of $1300 - 700\text{ cm}^{-1}$ and 3000 cm^{-1} can be observed compared to the IR spectra of the uncoated aluminium foils (Fig. 2A blue). These changes are also reflected in the HMDSO spectra (Fig. 2A red). The same differences are also observable for plasma polyallylamine coatings of aluminium foils in the range of $4200 - 2800\text{ cm}^{-1}$ and $1750 - 1300\text{ cm}^{-1}$ in accordance to the allylamine spectrum.

Figure 3 shows the accelerated degradation of the samples at several time points. The polyallylamine coating (Fig. 3 column C) detaches from the aluminium foil after only 1 hour. After 2 h the uncoated (Fig. 3 column A) and the primal polyallylamine coated aluminium foils degraded completely. However, the polyallylamine layers are still completely preserved. Samples coated with polyHMDSO (Fig. 4 column B) degraded slower compared to untreated

Figure 2: FTIR-ATR spectra of plasma PolyHMDSO (A) and polyallylamine (B) coated aluminium foils in the range of 4000 – 500 cm^{-1} .

aluminium foils. Even after 2 hours, two of the four polyHMDSO coated samples are partially preserved. There was no polyHMDSO coating observed to be in the degradation media. Therefore, we assume that the plasma coating degrades and does not detach from the aluminium foil.

Figure 3: Representative macro images of untreated aluminium foils (A), polyHMDSO coated aluminium foils (B) and polyallylamine coated aluminium foils (C) at different time points during the accelerated degradation in 1 M hydrochloric acid ($n = 4$).

Figure 4 shows representative images of vital L929 mouse cells in direct contact with the untreated and plasma coated aluminium foils. Microscopic imaging revealed a slightly lower but remarkable cell growth on polyallylamine coated aluminium foil compared to the uncoated aluminium foil control. Coating with polyHMDSO resulted in poor cell growth.

Figure 4: Confocal micrographs of viable L929 mouse fibroblast (Calcein AM stained, green) cultivated on uncoated aluminium foil (A), polyHMDSO (B) and polyallylamine (C) coated aluminium samples.

4 Discussion

The morphological and infrared spectroscopic analyses show successful coating of the aluminium foil with the plasma polymers. The broadening of the bands of the plasma polymers in the IR spectra compared to their monomers also indicates polymerization [7, 8]. The coatings appear to be very thin, since the original surface structure of the aluminium foil is still visible after plasma coating. However, they are very homogeneous and smooth without recognizable structures. These results agree with different other studies [7-10].

Within biological evaluation, direct contact test with L929 showed distinct differences between the plasma polymer coatings polyallylamine and polyHMDSO in cell growth. Remarkable cell growth on polyallylamine confirms findings from similar experiments in literature [9]. Poor cell growth on polyHMDSO might be effected by the already described hydrophobic surface [10]. Due to the fact, that eluate tests (data not shown) revealed no differences in cell viability between plasma coated aluminium foils and the uncoated aluminum control, differences in cell growth should not originate from toxic substances and might be caused by properties of the surfaces.

Clear differences between the two plasma polymers can be observed in the accelerated degradation. The polyHMDSO coating stronger adheres to the aluminium surface than the polyallylamine. As a result, only this plasma polymer was able to protect aluminium foils until the end of the experiment. While the polyHMDSO layer degraded over time, the polyallylamine coatings were still intact, but detached from the aluminium foil, probably due to poor adhesion. Consequently, the unprotected aluminium foils degraded.

In summary, a degradation delaying effect could be observed for the polyHMDSO coating. The potentially biodegradable coating [11] appears to be suitable for biodegradable medical devices that require low cell adhesion. For the polyallylamine layer further investigations are planned to clarify whether the degradation protection can be improved by a stronger adhesion to the aluminium surface.

Acknowledgment: The authors would like to thank Andrea Rohde, Caroline Dudda, Gabriele Karsten, Martina Nerger and Babette Hummel for the technical assistance.

Author's Statement

Research funding: Partial financial support by the European Regional Development Fund (ERDF) and the European

Social Fund (ESF) within the collaborative research between economy and science of the state Mecklenburg-Vorpommern and by the Federal Ministry of Education and Research (BMBF) within RESPONSE “Partnership for Innovation in Implant Technology” is gratefully acknowledged.

Conflict of interest: Authors state no conflict of interest.
Informed consent: Informed consent has been obtained from all individuals included in this study.

References

- [1] Kawai K, Ikegami H, Sato N, Matsuda A, Uchino K, Kuzuya M, Mizuno A. Industrial Plasma Technology: Applications from Environmental to Energy Technologies. Wiley-VCH Verlag GmbH & Co. KGaA; 2010.
- [2] Cvelbar U, Canal, Hori M. Plasma-inspired biomaterials. *J. Phys. D: Appl. Phys.* 2017; 50(4):040201.
- [3] Prakasam M, Locs J, Salma-Ancane K, Loca D, Largeteau A, Berzina-Cimdina L. Biodegradable Materials and Metallic Implants-A Review. *J Funct Biomater.* 2017; 8(4):44.
- [4] Teske M, Sternberg K. Surface functionalization of poly(ϵ -caprolactone) and poly(3-hydroxybutyrate) with VEGF. *BioNanoMaterials* 2017; 18(1-2):20170001.
- [5] Teske M, Arbeiter D, Schober K, Eickner T, Grabow N. Systematic analysis about residual chloroform removal from PCL films. *Current Directions in Biomedical Engineering* 2018; 4(1):567-569.
- [6] Brietzke A, Siewert S, Schmidt W, Stahnke T, Kreiner C, Grabow N, Guthoff R, Schmitz K-P. Hydrophilic silicone elastomer with excellent cell adhesion capability - A promising material for ophthalmic micro-implants. *CDBME* 2018; 4(1):571-4.
- [7] Radeva E, Tsankov D, Bobev K, Spassov L. Fourier transform infrared analysis of hexamethyldisiloxane layers obtained in low-frequency glow discharge. *J. Appl. Polym. Sci.* 1993; 50:165-171.
- [8] Fally F, Doneux C, Riga J, Verbist JJ. Quantification of the functional groups present at the surface of plasma polymers deposited from propylamine, allylamine, and propargylamine. *J. Appl. Polym. Sci.* 1995; 56:597-614.
- [9] Hamerli P, Weigel Th, Growth Th, Paul D. Surface properties of and cell adhesion onto allylamine-plasma-coated polyethyleneterephthalat membranes. *Biomaterials* 2003; 24(22):3989-3999
- [10] Kuhn S, Kroth J, Ritz U, Hofmann A, Brendel C, Müller LP, Förch R, Rommens PM. Reduced fibroblast adhesion and proliferation on plasma-modified titanium surfaces. *J. Mater. Sci. Mater. Med.* 2014; 25(11):2549-60.
- [11] Behnisch J, Tyczkowski J, Gazicki M, Pela I, Holländer A, Ledzion R. Formation of hydrophobic layers on biologically degradable polymeric foils by plasma polymerization. *Surface and Coatings Technology* 1998; 98(1-3):872-874.