XXXI International Conference on
Surface Modification Technologies (SMT31)

JULY 5-7, 2017, MONS, BELGIUM

INVESTIGATING THE STABILITY OF PLASMA POLYMERIZED ACRYLIC ACID COATINGS

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Abstract
In this study, a dielectric barrier discharge set-up operating at medium pressure was employed for the thin film deposition of acrylic acid based coatings. The plasma discharge power (21-27 W) was systematically varied to achieve a series of carboxylic acid-rich films ranging from unstable to stable when stored in human body like conditions (PBS, 37°, 24 h). The coating chemical composition was analyzed both before and after incubation using FT-IR and XPS to establish a correlation between functional group preservation and coating stability. The entire range of samples was also analyzed *in-vitro* to test the cell-surface interactions with human foreskin fibroblasts. Only for the highest discharge power (27 W), no coating dissolution/delamination could be observed during the *in vitro* analysis. As a result, only for these coatings, the human foreskin fibroblasts maintained their normal cell morphology.

Keywords: Acrylic acid, plasma polymerization, stability study, human foreskin fibroblasts, DBD

1. Introduction

The plasma-assisted deposition of acrylic acid-based coatings, has received considerable attention from the research community, as it has shown high potential for biomedical applications (O'Toole et al. (1996), Jafari et al. (2006)). The biggest challenge for these carboxylic acid rich coatings is their stability when incubated in aqueous-based media. When suboptimal plasma parameters are chosen, the thin film will start dissolving over time. This results both in a loss of function as well as a considerable acidification of its immediate environment (Finke et al. (2009), Morent et al. (2010)). Several attempts have been made to overcome this stability issue by using a pulsed plasma system or adding an additional cross-linker (Alexander et al. (1998), Jafari et al. (2006), Carton et al. (2012)). The stability of the carboxylic acid-rich films in this study is tested in 2 different ways: firstly, incubation in a phosphate buffered saline (PBS) solution (pH 7.4) at human body temp (37°C) in a shaking bath configuration (70 rpm) to renew the surface-liquid interface continuously for up to 1 day, as this is the most critical time step for the stability process (Lerouge et al. (2015)). Secondly, an *in vitro* static incubation is performed for 48 and 72 hours in growth medium while seeded with fibroblasts.

2. Materials and methods

2.1. Materials

Polystyrene (PS) film substrates were purchased from Goodfellow (UK) and press cut into circular samples with a diameter of 1.25 cm. Acrylic acid (99%), NaCl, KCl and all phosphate salts required to prepare a PBS

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solution were bought from Sigma-Aldrich (Belgium) and used as such. Helium (Alphagaz 1) was purchased from Air Liquide (Belgium).

2.2. Plasma set-up

A parallel-plate DBD set-up was used as described in previous experimental work (Cools et al. (2017)) to deposit plasma polymerized acrylic acid coatings on PS substrates. The used experimental parameters can be found in Table 1.

Table 1: Experimental plasma parameters

Plasma parameter	
Discharge gas	Helium
Gas flow rate	3 standard liters per minute
Acrylic acid monomer concentration	0.25 g/h
Discharge power	21-23-25-27 W
Treatment time	3 min
Discharge pressure	500 mbar
Electrode distance	7.8 mm

2.3. XPS

XPS measurements were performed on coated PS substrates in a PHI 5000 Versaprobe 2, using a monochromatic Al K_α ($h\nu$ = 1486.6 eV) X-ray source operating at 50 W. For each condition, 4 random points were measured on a single sample. Vacuum within the XPS analysis chamber was kept below 10^{-6} Pa for all conditions and the emitted photoelectrons were collected at an angle of 45° with respect to the normal of the sample. Survey spectra were recorded at a pass energy of 187.85 eV (0.8 eV step) and were analyzed using Multipak software (version 9.6.1).

2.4. FT-IR

A Bruker Tensor 27 spectrometer equipped with a single reflection ATR accessory (MIRacle^{TM}, Pike technology) was used to perform FT-IR analysis on coated PS substrates, using a germanium crystal as internal reflection element. 3 points distributed evenly on a single sample were analyzed for each condition. All spectra were recorded using an MCT-detector (liquid N_2 cooled) with a resolution of 4 cm^{-1} and 64 scans were made for each sample. OPUS 6 software was used to analyze the obtained spectra and to correct for the presence of CO_2 peaks within the spectra.

2.5. In-vitro cell testing

The cellular interactions on the plasma polymerized coatings were also examined in detail. HFF-1 cells (human foreskin fibroblasts, ATCC) were cultured in DMEM glutamax medium (Gibco Invitrogen) supplemented with 10% fetal bovine serum (FBS, Gibco Invitrogen), 0.5% P/S (10 U/ml penicillin, 10 mg/ml streptomycin, Gibco Invitrogen) and 100 mM sodium pyruvate (Gibco Invitrogen). The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. Afterwards, cells were seeded on the coated substrates at a density of 40 000 cells/ml medium per material in 24-well suspension culture plates and evaluated after 48 and 72 hours with fluorescence microscopy.

3. Results and discussion

3.1. Chemical analysis

Figure 1 depicts the O/C ratio as a function of discharge power as determined via XPS analysis. As can be seen, there is an almost linear decrease (R^2 = 0.92) in O/C ratio as the discharge power is increased, indicating a progressive fragmentation of the acrylic acid precursor as energy is added to the system. When comparing the obtained O/C ratios to the theoretically expected value (0.67), even for the lower discharge powers, the fragmentation is extensive. Both the absolute values and the downwards trend suggest that the plasma system is operating in the monomer deficient regime, which is considered a requirement for obtaining stable coatings as determined by Yasuda et al. (1984).

To analyze the effects of the precursor fragmentation on the coating stability, the acrylic acid plasma coated samples were analyzed before and after incubation in PBS solution (see Figure 2). After 24 hours, the coatings deposited at the lower discharge powers (21-23 W) were dissolved/delaminated, as the corresponding peaks in the FT-IR spectra were no longer detected. For the coatings deposited at 25-27 W, the expected peaks at 1710 cm^{-1} (C=O stretch) and 3500 cm^{-1} (OH stretch) were still there, confirming the preservation of the coating. Alongside the expected peaks, additional peaks were found at 1650 cm^{-1}, 1550 cm^{-1} and 1410 cm^{-1}. The first was linked to the presence of residual water in the deposited coating while the other two could be linked to the formation of carboxylic anions, thus further confirming the presence of carboxylic acid groups in the coating. High discharge powers are thus required to generate stable plasma polymerized acrylic acid coatings.

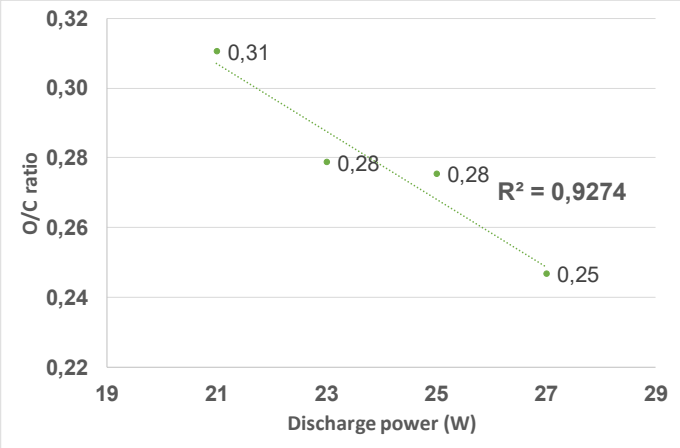

Figure 1: O/C ratio of acrylic acid based plasma coatings as a function of the applied discharge power

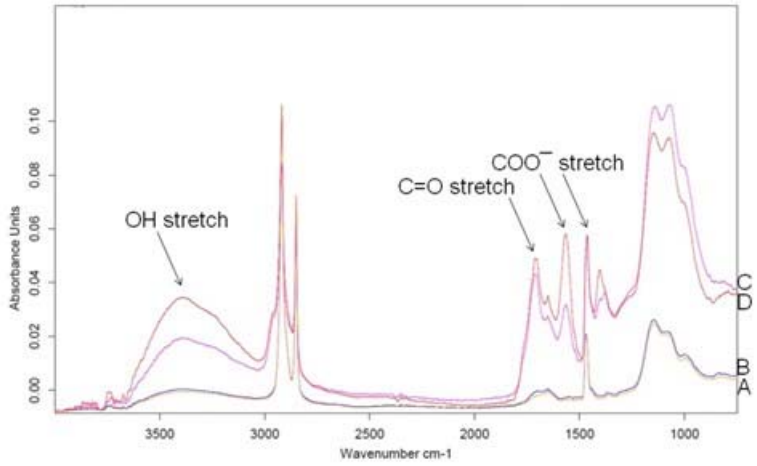

Figure 2: FT-IR spectra of acrylic acid plasma coated samples at 21 W (A), 23 W (B), 25 W (C) and 27 W (D)

3.2. In-vitro analysis

Figure 3.A-D presents the fluorescent micrographs 48 hours after cell seeding. For the lowest discharge power (21 W), it is evident that most of the coating has delaminated/dissolved (as shown by the red staining of the coating) within a period of 2 days. Furthermore, it is clear that the dissolution process is sufficiently slow to be non-toxic, as the cells start growing on the underlying plasma activated substrate. For the 25 W case, slight signs of coating dissolution can be observed across the coating as marked by the white arrows. Cells seem to take a slightly irregular morphology, which most likely can be linked to the strong acidification of its immediate environment. The 2 highest discharge powers are characterized by a uniform coating, showing no signs of coating instability. In these cases, the fibroblasts exhibit a normal spindle shaped cell morphology. In addition, for the highest discharge powers, some signs of supracellular parallel orientation could also be observed.

Figure 3.E-H gives an overview of the live/dead stained samples 72 hours after cell seeding. For the 21-23 W samples (E, F), the coating is completely gone as proven by the lack of a red background, thus confirming the FT-IR results of potential instability. A lot of viable cells can however still be seen in these images, but in this case cells just continue to grow on the underlying plasma activated PS substrate. For the 25 W sample, extensive coating delamination/dissolution could be observed, similar as to what was observed after 48 hours for the lower discharge powers. This indicates that for this specific condition, the coating only exhibits a short-term stability. For the 27 W condition, there are still no signs of coating dissolution after 72 hours, confirming that at this discharge power, medium-to-long term coating stability can be achieved. In this case, cells on top of the coating also show a normal spindle shaped morphology, indicating an enhanced cell surface compatibility.

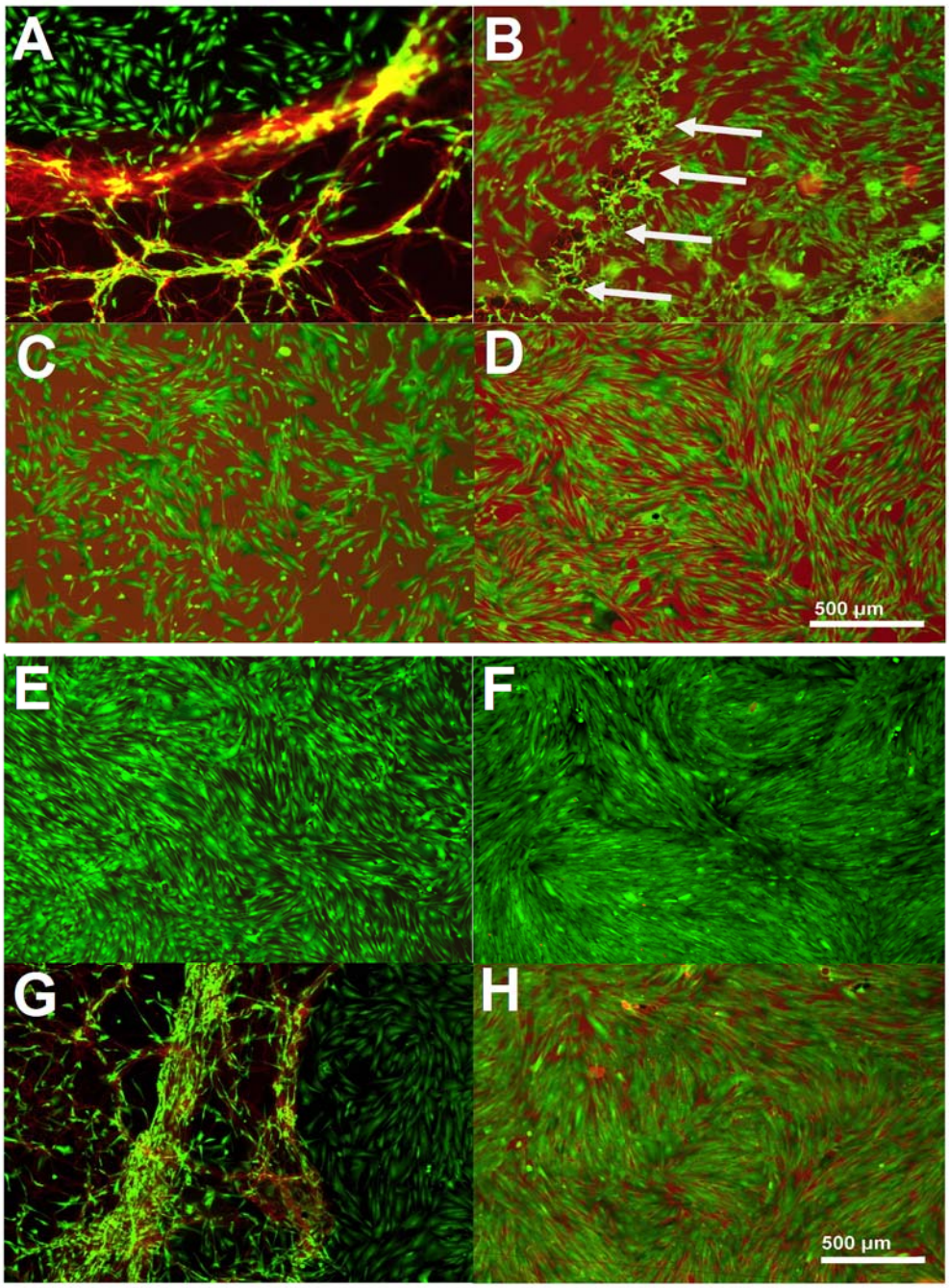

Figure 3: Live/dead stained fluorescent images 48 h (A-D) and 72 h (E-H) after incubation for 21 W (A, E), 23 W (B, F), 25 W (C, G) and 27 W (D, H).

4. Conclusions

In this study, a parallel-plate DBD plasma reactor was used for the deposition of a series of acrylic acid-based plasma coatings ranging from very unstable to completely stable. XPS analysis showed a progressive fragmentation of the carboxylic acid group as a function of discharge power, indicating a strong loss of functional group density. Incubation analysis showed that a minimum discharge power of 25 W was required to maintain a 24-hour coating stability. *In vitro* analysis using human foreskin fibroblasts indicated that coating instability occurred in the form of both dissolution and delamination. Local acidification of the cell environment at lower discharge powers resulted in significant changes in cell morphology but did not induce cell death. Only for the 27 W condition, a completely stable coating could be observed 72 hours after cell seeding. For these samples, cells maintained their normal spindle shaped morphology, indicating a good cell surface compatibility.

Acknowledgments

This research was supported by a grant from the Research Foundation – Flanders (G.0516.13N) and has also received funding from the European Research Council under the European Union's Seventh Framework Program (FP/2007-2013) / ERC Grant Agreement n. 279022.

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