

Plasma polymerization of 3-aminopropyl-trimethoxysilane inside closed plastic bags at atmospheric pressure

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Abstract: Surface modification of the inner surfaces of closed plastic bags enables cultivation of stem cells under GMP (Good Manufacturing Practice) conditions. The surface coating is performed in an atmospheric PECVD process based on dielectric barrier discharges using specially designed automated equipment. In former investigations coatings made from 3-aminopropyl-trimethoxysilane (APTMS) were found to be particularly suitable for adherent cell cultivation. This contribution presents investigations of the coating process within the bag with a focus on film composition and stability of the deposited film. Effects of treatment parameters, diffusion processes through the permeable polymer bag and the influence of the relative humidity were studied.

Keywords: plasma polymerization, DBD, cell culture, plastic bag

1. Introduction

Plasma treatment to enhance cell cultivation is well described in the literature [1-2]. Usually two dimensional substrates, like polymer foils or glass slides were coated. As the cultivation of mesenchymal stem cells is challenging, the use of open systems may be involved with high risk of contamination. The use of closed plastic bags as reaction vessels for cell cultivation enables cell growth under GMP conditions. However, for cultivation of adherent cell lines a modification of the inner surface of the plastic bag is required.

Surface functionalization as well as coating of the closed plastic bag can be achieved by a plasma process at atmospheric pressure which was developed at Fraunhofer IST [3]. Thin coatings of plasma polymerized 3-aminopropyl-trimethoxysilane (APTMS) were identified as a suitable surface modification for adherent cell cultivation [4]. This contribution focuses on the film composition as a function of the process parameters and also on the stability of plasma-polymerized APTMS (pp-APTMS) films.

2. Experimental

Plasma treatment was performed in an automated system designed and built at Fraunhofer IST (see Figure 1). The process is based on a DBD arrangement where the plastic bag is pressed between two parallel electrodes, a high voltage electrode (150 x 300 mm²) covered with a 3.4 mm thick glass plate, and a ground electrode with a 5 mm thick polyethylene dielectric [5]. Helium is used as a carrier gas for the precursor, because its breakdown voltage is lower than that of air. This makes a plasma formation exclusively in the inside of the bag at a properly adjusted electrode voltage feasible. The film forming agent, 3-aminopropyl-trimethoxysilane (APTMS, > 97 % Sigma-Aldrich, Steinheim, Germany) was added by passing the carrier gas through a bubbler system containing the liquid monomer.

Gas permeable and transparent bags made of several laminated polymers including EVA (ethylene vinyl acetate copolymer) as the main part and polypropylene on the inner side (Cell Differentiation Bag – 100, Miltenyi Biotec, Bergisch Gladbach, Germany) were used as substrates.

Parameters used for plasma treatment are summarized in Table 1. A HV 2000 generator from Tantec SE (Lunderskov, Denmark) was used as a power supply.

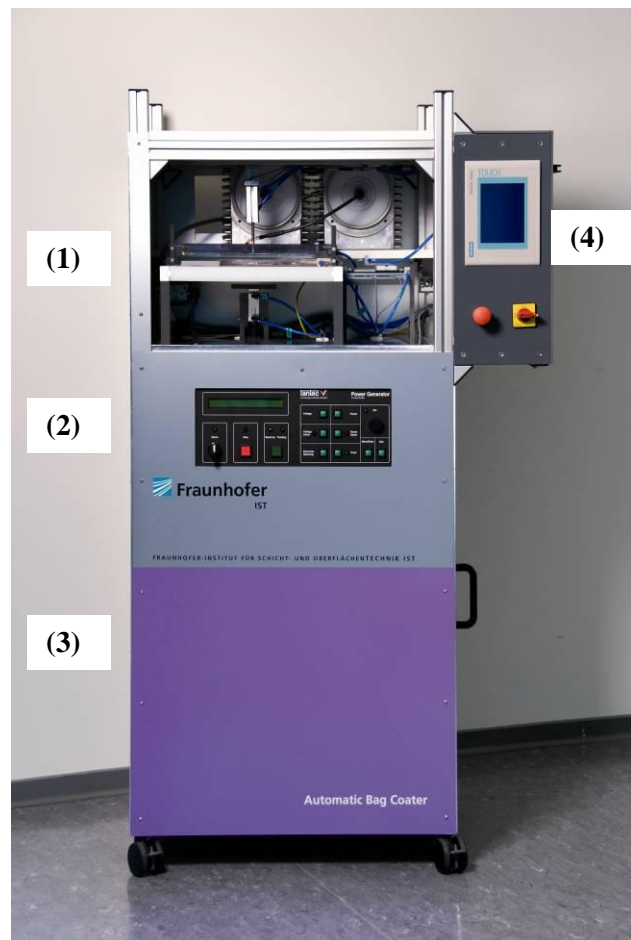


Figure 1: Automated bag coater for the internal coating of plastic bags; (1) electrode system; (2) generator HV 2000, Tantec SE; (3) not seen: bubbler system; (4) touch panel to adjust the process parameters.

Table 1: Process parameters for the internal plasma treatment of the plastic bags (cw = continuous wave excitation)

| Parameter | Value |
|-------------------|---------------------------|
| Total gas flow* | 5 L min ⁻¹ STP |
| Pulse-pause ratio | cw, 1:1 (25 ms : 25 ms) |
| Power | cw: 50 W pulsed 2-10 W |
| U _{eff} | 7.5 kV |
| Treatment time** | 4 x 5 s (in total 20 s) |

*to ensure a saturated APTMS vapor in the process gas, all gas was flushed through the bubbler system.

**Plasma treatment was performed step-wise, as one precursor filling is consumed within about 5 s [5].

Plasma-polymerized APTMS coatings were analyzed by FTIR-ATR spectroscopy, XPS and SIMS. For the analyses the bags were opened.

Film homogeneity in dependence on the plasma parameters (pulse-pause ratio, electrode gap) was examined by FTIR-ATR spectroscopy (FTIR Nicolet 5700, Thermo Fisher Scientific Inc., Waltham MA, USA). FTIR-ATR spectroscopy was also used to analyze film stability after incubating the samples in different media (ethanol, cell culture media, trypsin) and for the determination of the film composition after storing the closed bags under different external conditions (air with ~ 40 % relative humidity (r. h.), air with ~100 % r. h., dry N₂ atmosphere).

XPS measurements were performed on a PHI 5500 Multi-Technique system from Perkin Elmer equipped with a MgK α X-ray source (1253.6 eV) without monochromator.

3. Results and Discussion

FTIR-ATR spectroscopy was used to analyze film composition and homogeneity. For this purpose, difference spectra were generated by subtracting a spectrum of an untreated polymer bag from a spectrum of a coated sample. To achieve coatings with sufficient thickness the treatment process was performed four times, with a process time of 5 s for each run. In former investigations it was seen that the precursor in the gas phase is already consumed within this time period [5].

Over the sample width film homogeneity with a thickness variation of $\pm 5 - 7 \%$ was achieved by a pulsed plasma treatment ($t_{on} = 25$ ms, $t_{off} = 25$ ms) and in a continuous wave mode plasma process performing the experiments with an electrode gap of ~ 1 cm. At higher distances (electrode gap ~ 2 cm, corresponding to a higher gas volume and precursor

content in the bag) the thickness variation rose to $\pm 10\%$. From the intensity of the extinction band between 1200 and 1000 cm^{-1} it was seen that the film thickness is smaller on the upper inner side of the bag than on the lower inner side of the bag (see results of the area of the extinction band in Figure 2, bottom). This effect is observed independently of the electrode gap and is due to the electrical asymmetry of the arrangement with two different dielectrics on the electrodes. Moreover, film thicknesses as estimated from the absorption bands in the spectra are roughly the same for 1 and 2 cm electrode distance, resp. (see Figure 2, bottom).

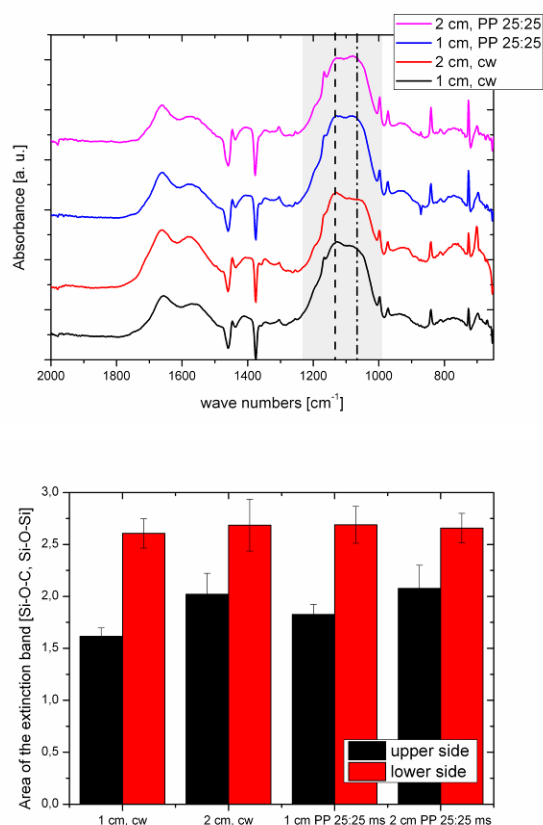


Figure 2: (top) FTIR-ATR difference spectra of pp-APTMS coated surfaces at different treatment conditions; 1 cm/2 cm: electrode gap, cw: continuous mode, PP 25:25 ms: 25 ms plasma on/25 ms plasma off. (bottom) Area of the absorption bands due to Si-O-C and Si-O-Si vibrations between 1000 and 1260 cm^{-1} (highlighted in grey).

This effect indicates that only a small portion of the monomer introduced in the bag is used for film deposition, independently of the electrode gap.

In the broad absorption band in the spectra between 1200 and 1000 cm^{-1} two peak maxima could be identified, probably due to Si-O-C and Si-O-Si vibrations, resp. Comparing different excitation modes (continuous wave mode vs. pulsed plasma operation), slight differences were obtained in the shapes of the resulting adsorption bands, indicating different film compositions, e. g. differences in cross-linking of the film by hydrolysis and subsequent condensation reactions between intact $\equiv\text{Si-O-CH}_3$ moieties left after the plasma deposition.

The effect of condensation was also investigated by XPS measurements of the elemental film compositions obtained after gas-phase derivatizing primary amino groups with trifluoromethylbenzaldehyde (TFBA) (see Table 2). In the samples which were deposited under pulsed conditions, a lower amount of nitrogen was detected, showing that the monomer was decomposed differently. Regarding the elemental composition of the other elements (except F) virtually no differences were observed on the film surface as detected by XPS.

As expected the amount of carbon within the film decreased by a condensation reaction of the Si-OCH₃ groups, forming a Si-O-Si-network. After one week the sample stored under dry nitrogen shows the same trend in film composition as the sample stored in humid air. An explanation could be the water content of the polymer laminate used for the bags [6]. Future investigation will focus on shorter time periods between the measurements to get a better understanding of the chemical hydrolysis, condensation and crosslinking processes in the plasma polymer films within the bag.

Table 2: Elemental composition of pp-APTMS coated samples. Fluor is introduced by the reaction of $-\text{NH}_2$ with TFBA.

*These bags were stored in air ($\sim 40\%$ r. h.) at room temperature and under nitrogen at room temperature, resp. for one week before the measurement.

| Sample | C[%] | N[%] | O[%] | F[%] | Si[%] |
|----------------------------|-------|------|-------|------|-------|
| Cw | 72.28 | 0.82 | 21.16 | 1.51 | 4.23 |
| PP 25:25 | 73.22 | 0.16 | 21.23 | 0.95 | 4.45 |
| PP 25:25, Air* | 52.44 | 4.92 | 26.64 | 5.82 | 10.17 |
| PP 25:25, N ₂ * | 58.74 | 2.03 | 26.17 | 3.44 | 9.6 |

For freshly prepared films the $[\text{NH}_2]/[\text{C}]$ ratio was calculated from equations given in the literature [7]. The amount of primary amino groups is higher in coatings deposited in the continuous wave plasma mode (cw: $[\text{NH}_2]/[\text{C}] = 0.73$; PP 25:25 $[\text{NH}_2]/[\text{C}] = 0.44$).

After storage in air of 100 % relative humidity an increase of water and possible silanol content in the films is indicated by an increase of the absorption band at wave numbers beyond 3300 cm^{-1} which are attributed to the vibrational absorption of O-H groups (see Figure 3). Moreover, the shape of the Si-O-C/Si-O-Si absorption band changed under these condition which leads to the conclusion that the film composition also changed. This implication will be investigated in more details in future experiments.

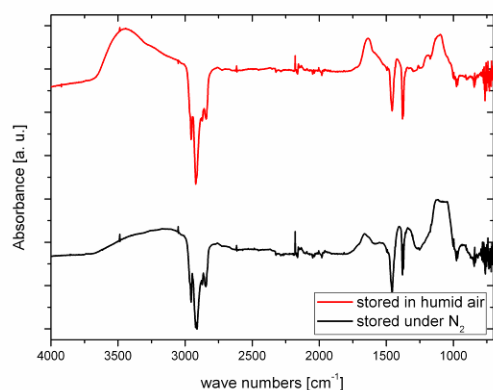


Figure 3: FTIR-ATR difference spectra after storage of the closed bags in different atmospheres; in humid air (red) and under nitrogen (black).

To use the coated plastic bags for cell cultivation, film stability in different media has to be guaranteed. The films were analyzed with regard to their stability in ethanol, cell culture medium (EMEM: Eagle's minimum essential medium) and trypsin (which is used to detach the cells from the surface). For all media sufficient stability was proved.

Attempts to determine the film thickness quantitatively were done by SIMS. Unfortunately, as the inner surface of the polymer bag seems to be comparably rough no reliable values for the film thickness could be obtained.

4. Conclusions

In this contribution it was demonstrated, that stable coatings of pp-APTMS could be achieved in closed plastic bags. On first sight the process does not seem to be very sensitive to variations of the electrode gap. Slight differences were obtained by changing the plasma mode (from continuous mode to pulsed operation). Further experiments to analyze the film composition in more detail have to be carried out, especially the determination of the film thickness in these bags seems to be challenging.

5. Acknowledgements

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