Influence of carrier gas on the behavior of plasma polymerized polystyrene films in aqueous media

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The purpose of this research is the characterization of plasma polymerized polystyrene (pPS) films synthesized by atmospheric pressure plasma technique, using helium and argon, as carrier gas and styrene vapors, as monomer. A special attention was focused on the pPS coatings stability during aqueous media exposure. In addition, the coatings cytotoxicity was studied by Cell Count Kit-8 (CCK-8) assay using HEK (Human Embryonic Kidney) cells.

1. Introduction

Plasma polymerization is a versatile technique for the preparation of functional films. It has many advantages: it is a solvent-free technology, fast and one step process, numerous monomers can be polymerized on a wide range and complex shaped substrates. The properties of plasma polymers such as thickness, chemical composition, morphology and stability can be tailored by controlling plasma process parameters.

Plasma polymerized styrene films can be used as protective coatings and insulating layers for applications in floating gate type organic memory devices [1], optical biosensors or biomaterials [2]. Although for biomedical applications stable surfaces are requested, only few studies deal with the stability of plasma polymers exposed to biological media.

Our study is focused on the influence of biological environment on pPS characteristics, like chemical composition, homogeneity and morphology. The pPS films were prepared by atmospheric pressure plasma obtained in a mixture of helium or argon gas and styrene vapors. To evaluate the aqueous media effects (soluble components leach out), the pPS samples were analyzed before and after soaking for 1 h up to 72 h in 10 mL of water solutions, followed by 1-2 h drying at 37 °C. The pPS films composition was analyzed by means of Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The surface morphology of the pPS films was examined by atomic force microscopy (AFM) and optical microscopy. Wettability properties were evaluated by contact angle measurements. In addition, a systematic ageing study was performed. UV-Vis spectroscopy was used to monitor mass loss as a function of soaking time.

2. Experimental

Plasma deposition of pPS films

The common method to synthesize pPS films is radio frequency plasma-enhanced chemical vapor deposition (RF-PECVD). In our study, plasma polymerization of styrene (S, C_8H_8, Fluka, 99% purity) was carried out using a dielectric barrier discharge (DBD), which operates in helium (pPS_{He}) and argon (pPS_{Ar}). Compared with He, Ar is cheaper and requires low ionization energies, but He plasmas are more efficient to obtain a favorable plasma regime which allows uniform film deposition.

A schematic representation of the plasma reactor is presented in Fig. 1. Because our DBD system operates at atmospheric pressure, it does not require an expensive vacuum chamber.

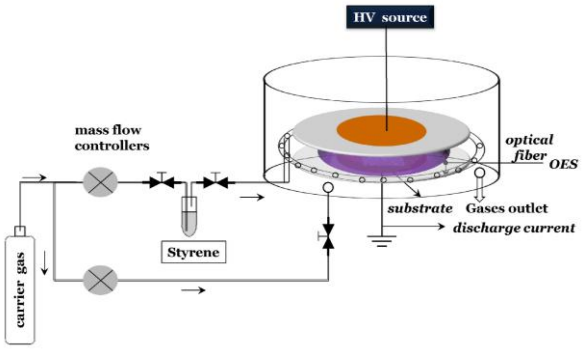

Fig. 1. Schematic representation of the plasma polymerization system.

Plasma was produced between two cylindrical parallel copper electrodes, deposited as thin films on glass substrate. The gap width between the electrodes was fixed to 5 mm to provide stable plasma operation. Positive high voltage pulses with 6 kV amplitude, 2 kHz frequency and 250 μs width were

applied on the high voltage electrode. Plasma reactor is equipped with two gas lines, one for carrier gas (He or Ar) used to sustain the discharge and other line to transport the styrene vapors into the plasma volume. The S monomer was introduced into the discharge region by He bubbling with a rate of 17 µl/min and by Ar bubbling with a rate of 5 µl/min. The polymerization process was performed for 10 min. In order to clean and activate the glass slide used as substrate, the surface was exposed 1 min to Ar plasma (pPS_{Ar*}).

Characterization of the pPS films

Wettability was evaluated by measuring the contact angle between water drops (2 µl) and the plasma polystyrene surface using a horizontal optical microscope with a digital camera (OPTIKAM 4083.B5) connected to a computer and with a Digidrop instrument (GBX, France). All measurements were made under ambient conditions.

X-ray photoelectron spectroscopy (XPS) spectra were recorded using PHI 5000 VersaProbe (Physical Electronick) spectrometer with a monocromated Al Kα radiation source (1486.7 eV), under a vacuum of about $2x10^{-6}$ Pa and 45° photoelectron take – off angle.

Atomic force microscopy (AFM) investigations were performed using NT-MDT Solver Pro-M type apparatus. The images were recorded in the tapping mode using standard silicon nitride tips (NT-MDT, NSG03). The topography, magnitude and phase were recorded simultaneously for each sample on various scanned areas. The root mean square roughness (Sq) of each surface was calculated from the images obtained.

Fourier transform infrared (FTIR) spectra of pPS films were performed using a BOMEM MB-Series 104 spectrometer. The spectrum of each sample, deposited on NaCl plates, was acquired in the transmission mode and averaged over 5 scans in the range of 4000 – 500 cm^{-1} with a resolution of 2 cm^{-1}. The assignment of the most important S and pPS peaks was done according to the literature data.

The coatings thickness was quantified using an interferometric-based technique (Linnik MII type). For 10 min deposition time, the pPS_{He} thickness is around 550 nm and for pPS_{Ar} is around 950 nm.

UV-Vis spectroscopic measurements were carried out on a dual beam spectrometer (Thermo Scientific Evolution 300) operating at a resolution of 1 nm in a 1 cm spectrometric cuvette.

3. Results and discussion

Behavior of pPS films in aqueous media

As shown in Fig 2, the pPS_{He} and pPS_{Ar} spectra are very similar. FTIR studies indicate a partial retention of the monomer characteristic groups in the polymer films. In addition, for S monomer spectrum in the 1665 cm^{-1} and 2000 cm^{-1} region, the presence of the aromatic CH vibrations, named the five aromatic fingers, can be observed. For plasma polymerized coatings the intensity of these five peaks decreases, meaning that the phenyl rings can be destroyed by the plasma polymerization process [3, 4]. The presence of OH and C-O groups in the FTIR spectra indicates that the films are oxygenated. This result is confirmed by the XPS analysis. The oxygen incorporation in the plasma polymers may become from two sources, the presence of oxygen and water vapors in the plasma reactor during plasma polymerization and from post oxidation of the coatings exposed to air after plasma deposition.

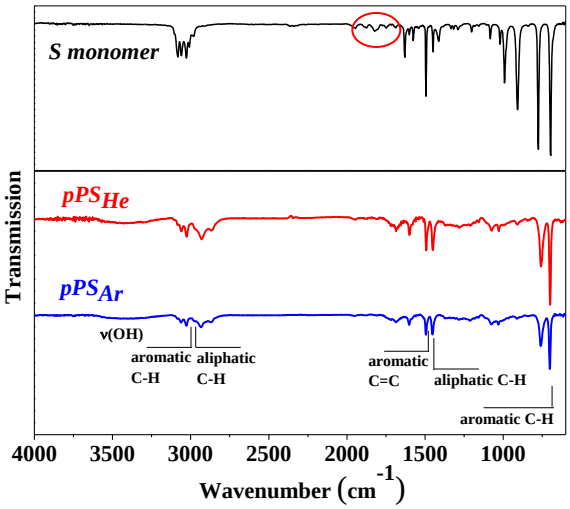

Fig. 2. FTIR spectra of monomer S, pPS_{He} and pPS_{Ar} deposited onto NaCl plates.

The XPS results are summarize in Table 1 and Fig. 3. Oxygen and carbon are the elements detected in the pPS_{He} and pPS_{Ar} spectra. The spectra show peaks assignable to C-C and/or C-H (at 284.6 eV), C-O-C and/or C-OH (at 286.3 eV), C=O and/or H-C=O (at 288 eV) groups and π-π* shake-up satellite (at 294.2 eV). The characteristic π-π* shake-up satellite is due to resonance of the PS aromatic ring, emphasizing that the films retained the monomer structure. After immersion of pPS_{He} and pPS_{Ar} films in distilled water for 72 h, an increase of the oxygen content was observed.

Table 1. Relative atomic concentrations and the functional groups contents (%) of pPS films before and after immersion in distilled water for 72 h.

pPS	He		Ar	
	before	after	before	after
C	92.3	84.8	92.7	87.7
O	7.7	15.2	7.3	12.3
O/C	**8**	**17**	**8**	**14**
C-C, C=C, C-H	85.9	73.9	86.4	78.8
C-O	9.3	19.8	10.4	17.7
C=O	-	3.3	-	2.4
π-π*	4.8	3	3.2	1.1

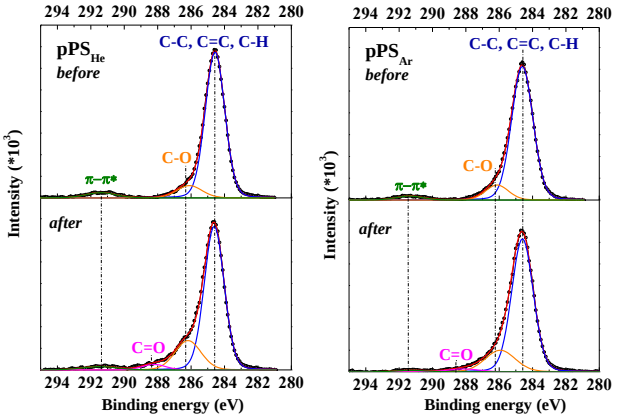

Fig. 3: High-resolution XPS C1s spectra of pPS_{He} and pPS_{Ar} before and after 72 h of immersion in water.

Our results suggest the pPS WCAs are independent of the carrier gas nature. The pPS WCAs after 14 days of storage under ambient condition remain constant at about 82° ± 2°. As can be seen in Fig. 4, in the case of the surfaces pre – treated with Ar plasma for 1 min prior to plasma film deposition, there was no detectable difference in the WCAs after immersion in water for 72 h.

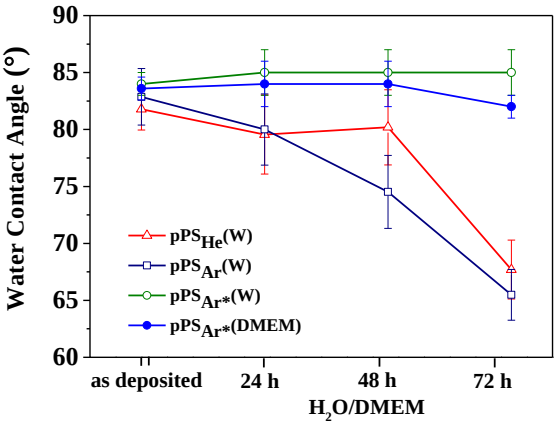

Fig. 4: Water contact angles measured as a function of immersion time in water (W) and cell culture medium (DMEM). Error bar shows a standard deviation of 10 drops for one sample.

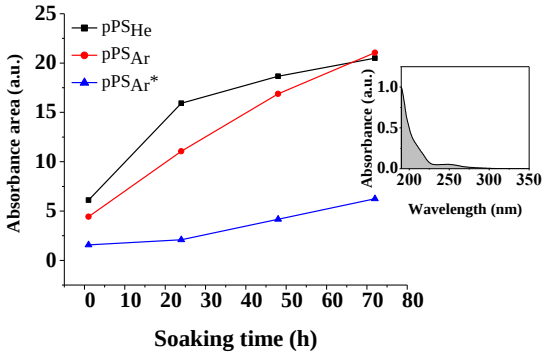

Fig. 5: Water stability of pPS_{He} and pPS_{Ar} films. Insert: UV spectrum of water in which the pPS_{He} was soaked for 72 h.

Compared with untreated substrate, the substrate pre–treated with Ar plasma for 1 min manifests less extraction of water soluble material (Fig 5). The coatings are dissolved due to the presence of oligomers in the polymer structure, which are released upon contact with water. Generally, the coatings that contain a high amount of polar groups are expected to be soluble in water.

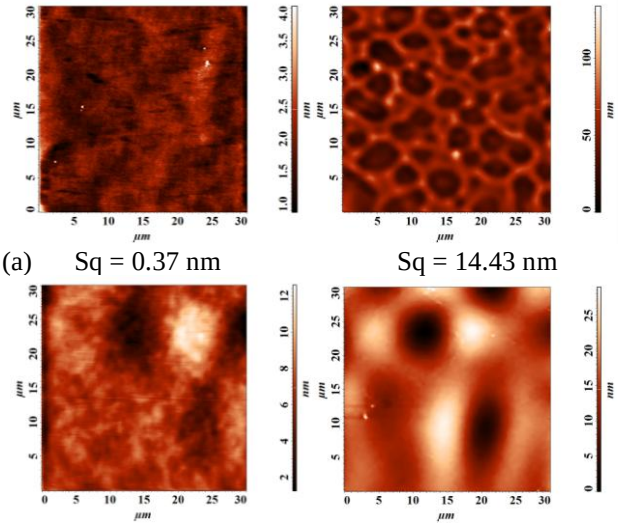

Fig. 6: AFM topographic images 30 x 30 μm^2 of the (a) pPS_{He} and (b) pPS_{Ar} films before and after immersion in distilled water for 24 h.

The as-deposited films were smooth without any visible features. Immersion of pPS in water induced significant changes in the surface morphology, as shown in Fig. 6. After immersion of plasma polymerized coatings in water or DMEM, the surfaces become microporous, resembling a honeycomb pattern. The structures dimensions and densities depend on the plasma polymerization conditions. The structure diameter of pPS_{He} films are 2 to 5 μm and of pPS_{Ar} films 12 to 15 μm. A main

cause of the formation of these voids is the extraction of soluble low molecular weight polymeric material from the polymer matrix. As can be shown in Fig 7.c, these voids appear to be uniformly distributed on the entire film surface. A similar result was found by Vasilev *et al.* (2008). They observed that the solvent induced formation of pores in n-heptylamine plasma polymers (HApp) [5].

FTIR and WCAs results showed no detectable difference from both He and Ar pPS films after 14 days of storage under ambient conditions.

Investigation of pPS films cytotoxicity

HEK (Human Embryonic Kidney) cells were used to evaluate the pPS_{Ar^*} cytotoxicity. The HEK cells were maintained in DMEM supplemented with 10 % fetal-calf serum (FCS) and penicillin/streptomycin in a 5% CO_2 atmosphere at 37 °C. We investigated cell adhesion and spreading by means of a Nikon Eclipse TS 100 optical microscope with a 4x, 10x and 20x objective lens for 24 h, 48 h and 72 h. After 72 h at 37 C, cell viability was colorimetrically measured using a Cell Counting Kit-8 (CCK-8). The absorbance intensity was acquired by a microplate reader (PHERAstar FS, BMG LAB-TECH GmbH, Germany). The sterile round glass coverslips and the PS culture well (cell grown without any sample in the culture plate) were used as control. The cells grown on the bottom of the PS wells represented the 100 % viability value.

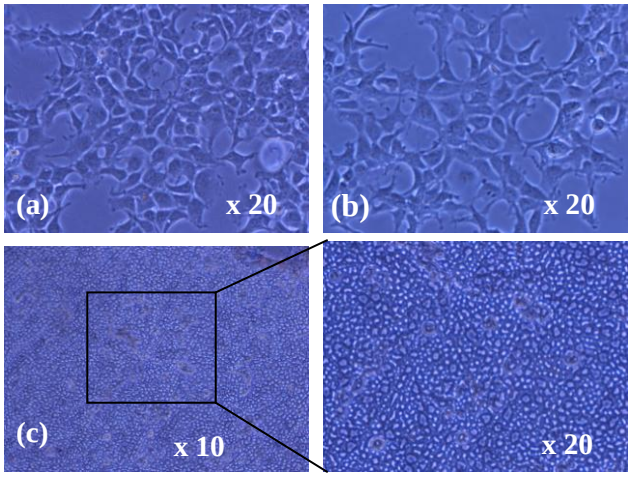

Fig. 7. Optical images of HEK cells on (a) PS, (b) glass and (c) pPS_{Ar^*} film after 48 h at 37 °C.

As can be seen in Fig 7 and Fig. 8, the PS culture, as control polymer, showed significantly better performance than the pPS_{Ar^*} and 5 min UV/ozone treatment of the pPS_{Ar^*} coating (θ = 35°) enhances their biocompatibility.

These results suggest that the surface morphology, chemistry and wettability may have a strong influence on cells behavior. Owen *et al.* (2005) reported that the rough poly(lactic-co-glycolic acid) surfaces inhibited epithelial cell attachment, migration and proliferation compared to a smooth surface [6]. In addition, it is reported that the coatings containing EO-groups reduce biological molecule adsorption and cell adhesion [7].

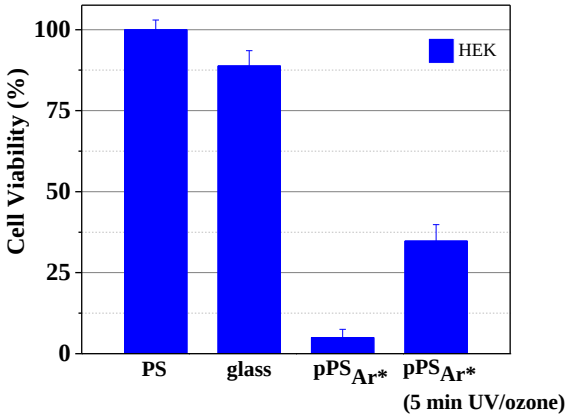

Fig. 8: *In vitro* cytotoxicity of pPS_{Ar^*}.

4. Conclusion

Plasma polymerized polystyrene (pPS) films were successfully synthesized by atmospheric pressure plasma technique, using helium and argon, as carrier gas and styrene vapors, as monomer. Furthermore, the films prepared using argon/styrene gas mixture has a higher deposition rate. Our results show that 1 min substrate pre-treatment with Ar plasma is effective in improving the coatings aqueous media stability and the pPS_{Ar^*} surface properties are unsuitable to attach HEK cells.

5. References

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