Adhesion of L929 mouse fibroblast cells on poly(styrene)/poly(methyl methacrylate) films modified by cold oxygen plasma process

A. M. G. Borges and V. Soldi

Departamento de Química – UFSC, Florianópolis/SC

Introduction

Biomaterials are one of the most productive research areas in materials science. As a result, a great diversity of new classes of materials has been created. Researchers are currently using synthetic and/or natural macromolecule biomaterials for the fabrication of devices designated to replace a biological tissue or organ with the ultimate goal of promoting life. In this sense, the synthesis of new polymeric materials that interact favorably with biological organisms may favor the replacement of damaged organs, hence promoting the quality of life of patients[1]. Thus, it is of interest to produce polymers that have physical-chemical properties as similar as possible to the tissues in which they are employed. A number of important properties of polymeric materials, including their interaction with biological systems, are controlled by their surface chemistry and morphology[2,3]. Polymer films have a wide field of application, due to some properties that are inherent to this type of material, such as good mechanical strength, chemical resistance to acids and bases and low production cost. However, polymer films have limitations such as low surface tension, low roughness and poor adhesion, which prevent their use in some instances, especially when such use requires that the material has a good wettability, hence requiring the use of techniques capable of modifying their surfaces[4]. The cold plasma generated in a dielectric barrier discharge (DBD) device operating close to atmospheric pressure was used for the purpose of surface treatment. The advantage of this process is that the surface properties and biocompatibility can be enhanced selectively, whereas the attributes of the material remain unchanged[5]. In the present study, polymeric films of PS, PMMA and 1:1 PS/PMMA blends were prepared and characterized. The biocompatibility of the polymeric films was assessed by studying cell adhesion and proliferation of L929 mouse fibroblasts.

Experimental

Poly(styrene) (PS), Mw = 300500 g mol^{-1}, and poly(methyl methacrylate) (PMMA), Mw =139595 g mol^{-1}, were obtained from Aldrich Chemical Co. (St. Louis, USA) and chloroform ($CHCl_3$) was obtained from Nuclear (São Paulo, Brazil). All materials were used without further purification.

Films were prepared by dissolving the polymers in 2% (m/v) $CHCl_3$ in a closed flask under magnetic stirring for 24 h at room temperature followed by solvent evaporation (casting method).

The plasma system used in this work is schematically shown in Figure 1. The films were wrapped up in order to cover the inner surface of the quartz tube in the plasma discharge zone. Afterwards, the system was purged with O_2 for a few minutes (1 L min^{-1}) in the DBD reactor using the input gas positioned at the bottom cap of the reactor, in order to eliminate possible gaseous contaminants present in the system. The plasma system was turned on during the treatment time (1-4 hours) and triggered the gas phase chemical reactions, which promoted the modification of the polymeric surface. Finally, the films were taken from the DBD reactor and maintained in an argon atmosphere inside desiccators, until further use. Subsequent tests were performed 24 hours after plasma treatment.

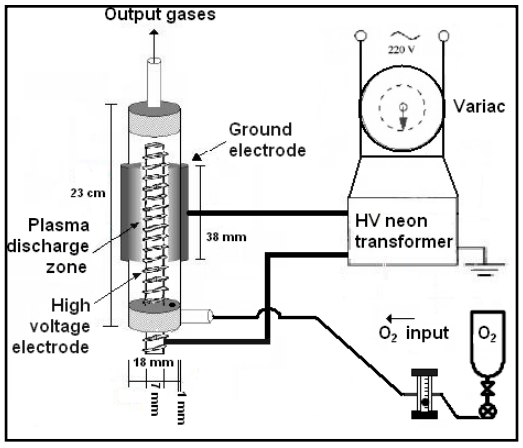

Figure 1 - Experimental setup used in this work.

The contact angles were measured after deposition of drops of distilled and deionized water on the films under study. A DATA PHYSICS goniometer with Image Tool software especially designed for this type of analysis was used. A high-resolution camera was used for image capture. Five measurements were carried out for each sample, and the mean value was considered.

Each of the polymeric films was cut into circular disks, soaked in 70% (v/v) ethanol, placed under UV light for 30 min for sterilization, and thoroughly washed with sterile phosphate-buffered saline (PBS) solution. For the experiments, cells were detached with trypsin, counted in a hemacytometer and seeded at a density of 50000 cells/well. Two types of control were used: cells seeded directly onto the well of the plastic plate or on top of a glass coverslip lying on the bottom of the well. The cells were stained with acridine orange and, after a 24 h incubation period, cell morphology was assessed by optical microscopy.

Results and Discussion

Figure 2 shows the variation of the interaction of the drop of deionized water with the material surface before and after plasma treatment. It can be observed that after treatment there was a significant decrease in the contact angle, as a result of an increase in the hydrophilicity of the material. This phenomenon is related to the concentration of oxygen in the polymeric surface, which resulted in formation of polar groups. It can be observed that the contact angle varies with the time used for plasma treatment, stabilizing at approximately 38° after 3 and 4 hours of treatment, as shown in Figure 3. The hydrophilicity of all sample surfaces was significantly improved after plasma treatment. Differences exceeding 60% in the values of contact angles of treated samples when compared to the same polymeric films that were not modified by the DBD plasma were observed. This means that the plasma treatment in any of the conditions used in this work increased substantially the wettability of the films. Figure 3 also evidences that the variation in treatment time of the polymeric films did not promote significant changes after 3 hours, as the contact angle observed following a 3 h-treatment was similar to that from a 4 h-treatment. This aspect is particularly relevant, as it represents significant time-saving, which can be especially critical for large-scale production of the material.

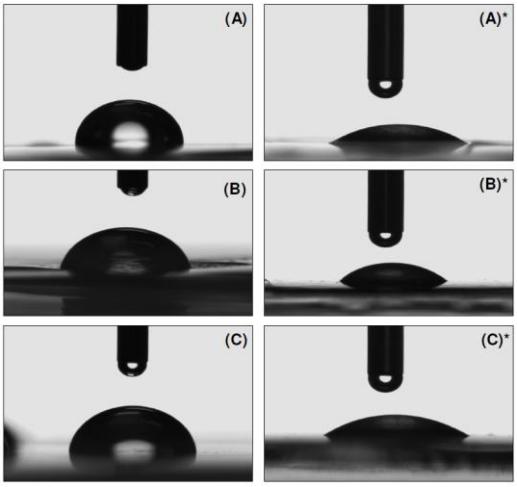

Figure 2 – Images of water droplets on the surface of films (A) PS, (B) 1:1 PS/PMMA blend and (C) PMMA films unmodified and * films modified t= 3 hours of plasma.

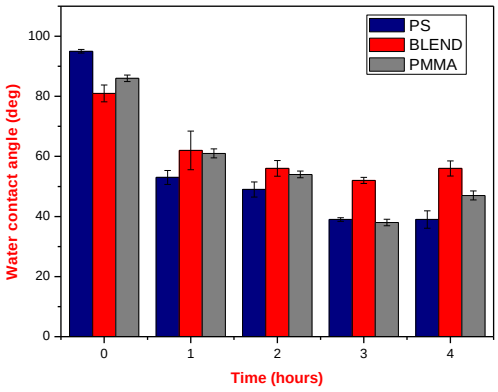

Figure 3 - Contact angle to the surface of the films before and after modification by DBD plasma.

As can be seen in Figure 4, L929 cells attached to and spread over the surface of all polymeric films and the results for cell adhesion after treatment of plasma did not differ from those of the control wells. Several adhesion processes can be seen in all images, assuring that the L929 cells adhered strongly to the polymeric films. The results of optical microscopy analysis revealed that there were no significant differences regarding the number of cells adhered to the polymeric films after treatment when compared to the untreated films. The pure PS film showed the highest number of adhered cells after treatment, which can be explained by the contact angle value. According to Yang *et al.*,[6] hydrophilicity is a relevant factor in defining cell adhesion. Polymeric films with moderate hydrophilicity are more efficient at promoting cell adhesion.

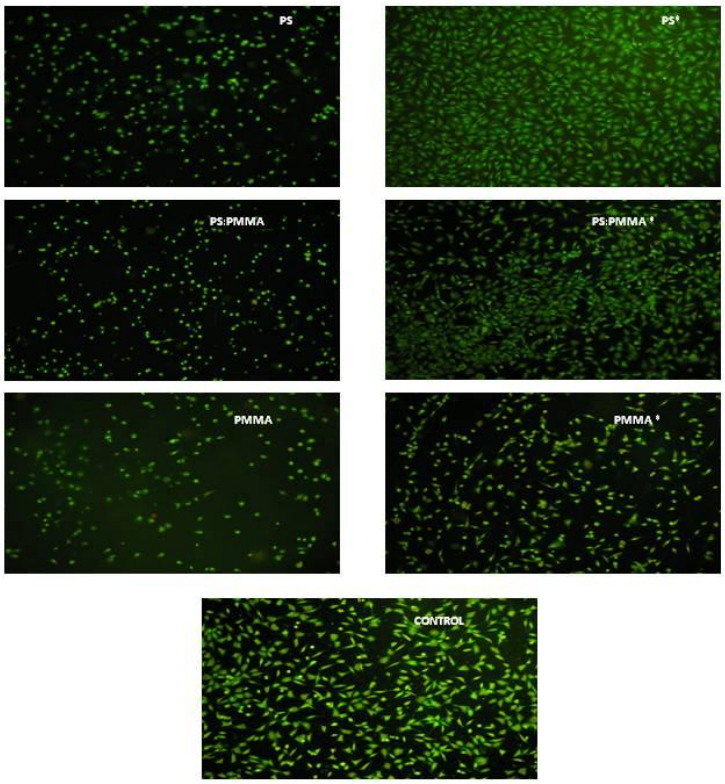

Figure 4 – Pictures of L929 fibroblasts on the surface of (A) PS, (B) 1:1 PS/PMMA blend and (C) PMMA films unmodified and * films modified t = 1 hour of plasma.

Conclusion

The oxygen plasma treatment using a dielectric barrier discharge (DBD) promoted an increase in the hydrophilicity of the studied samples, observed by a decrease in the contact angles of the plasma-treated film. This study suggests that plasma-induced surface changes increase the effectiveness of cell adhesion, since the cells adhere and spread more easily to surfaces with higher wettability. All films showed good cell adhesion after treatment, although analysis using optical microscopy showed that the pure PS film had the highest number of adhered cells after treatment, which can be explained by the increased contact angle observed in this film.

References

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