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Polymer films with surfaces unmodified and modified by non-thermal plasma as new substrates for cell adhesion

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ABSTRACT

The surface properties of biomaterials, such as wettability, polar group distribution, and topography, play important roles in the behavior of cell adhesion and proliferation. Gaseous plasma discharges are among the most common means to modify the surface of a polymer without affecting its properties. Herein, we describe the surface modification of poly(styrene) (PS) and poly(methyl methacrylate) (PMMA) films using atmospheric pressure plasma processing through exposure to a dielectric barrier discharge (DBD). After treatment the film surface showed significant changes from hydrophobic to hydrophilic as the water contact angle decreasing from 95° to 37°. All plasma-treated films developed more hydrophilic surfaces compared to untreated films, although the reasons for the change in the surface properties of PS and PMMA differed, that is, the PS showed chemical changes and in the case of PMMA they were topographical. Excellent adhesion and cell proliferation were observed in all films. In vitro studies employing flow cytometry showed that the proliferation of L929 cells was higher in the film formed by a 1:1 mixture of PS/PMMA, which is consistent with the results of a previous study. These findings suggest better adhesion of L929 onto the 1:1 PS/PMMA modified film, indicating that this system is a new candidate biomaterial for tissue engineering.

1. Introduction

The use of biomaterials for tissue repair has increased considerably in recent decades, due to both the development of new materials and a better understanding of the mechanisms of interaction with living organisms. The synthesis of new polymers that interact favorably with organisms, allowing cell adhesion may, for example, encourage the replacement of damaged organs or tissues [1–5].

In order to have a good cell-biomaterial interaction it is necessary to promote adhesion of the cells to the substrate. Although the substrate does not necessarily need to have characteristics similar to the extracellular matrix (ECM) for cell adhesion to occur, physico-chemical similarity is desirable. The topography, chemistry and surface energy play an essential role in cell adhesion to and proliferation on the biomaterial. In addition, biomaterials that have rougher surfaces may have a better response in terms of cell adhesion [6–9].

When a biomaterial is exposed to a biological environment, the first molecules to reach the surface are water molecules, so there is a relationship between the hydrophilicity of materials and cell adhesion and in the case of some parameters more interaction takes place. Hydrophobic polymers have chemically inert surfaces with low surface free energy, which makes them non-receptive to the adherence of other substances [10,11].

It is generally known that cell adhesion to artificial materials is mediated by molecules off extracellular, e.g. proteins, which are spontaneously adsorbed to the materials from the culture media and other body fluids, or are deposited by the cells themselves. If the materials are too hydrophobic, these molecules are adsorbed in a denaturated and rigid state. Their geometrical conformation is inappropriate for binding to cells, because specific sites on these molecules are less accessible to cell adhesion receptors [12].

Due to the great difficulty in obtaining materials that allow particularly rapid adhesion, proliferation and differentiation, several studies have sought to modify the surface properties of compounds by means of special treatments. Consequently, the modification of surfaces is becoming a widely used strategy because the new surface properties may be more suitable for biomedical applications. The most commonly used techniques include chemical wear, plasma treatment and exposure to gamma radiation. Of these techniques, plasma treatment is particularly versatile because the modification is restricted to the surface without compromising the material properties as a whole [13–16].

Several authors have presented and discussed results related to the effect of plasma treatment on polymer substrates. However, almost all

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of these treatments are focused on increasing the wettability. Zhu and collaborators [17] modified chitosan membranes with plasma to improve the hydrophilicity of the surface and promote the proliferation of fibroblast cells. The results showed that treatment increased the proliferation of fibroblast cells derived from human skin. Esposito and collaborators [18] studied the interactions between Vero cells and poly (lactic-co-glycolic acid) (PLGA) previously treated by oxygen plasma in order to increase the hydrophilicity of the studied samples, observing, in plasma-treated membranes, a decrease in contact angles and better cell adhesion.

Non-thermal plasma generated in a dielectric barrier discharge (DBD) operating at close to atmospheric pressure has been used for the purpose of polymeric surface treatment. The advantage of this technique is that it can operate at close to atmospheric pressure in vacuum-free systems. In addition, surface properties and cellular response can be improved, while the attributes of the material remain unchanged [19–21].

Liu and collaborators [22] studied the influence of the DBD plasma treatment on the adsorption of bovine serum albumin on the surface of poly (methyl methacrylate) (PMMA) films. The authors found that the DBD process significantly influences the surface wettability of the film and this fact can be attributed to the change in surface composition and topography. Lee and collaborators [23] used a DBD plasma treatment under atmospheric pressure to modify the surface of poly(caprolactone) films in order to improve adhesion and proliferation of human prostate epithelial cells. The results showed a significant improvement in cell proliferation when compared to the unmodified film, and this difference may be related to the oxidation of the surface, through the emergence of more hydrophilic groups. In addition, the authors conclude that the increased hydrophilicity on the surface of the films is directly related to the increase of the applied power, providing an improvement in adhesion properties. D'Sa and collaborators [21] modified the surface of poly(styrene) (PS) and poly (methyl methacrylate) (PMMA) using an atmospheric pressure DBD reactor. The authors observed changes in both surface chemistry and topography, and these changes were characterized by contact angle measurements in water, photoelectron spectroscopy and X-ray atomic force microscopy. Through this study, the authors concluded that processing by DBD plasma is able to modify the surface of PS and PMMA and that a change in the surface can induce an improved response against the albumin and human epithelial cells, being classified as a suitable biomaterial in tissue engineering.

Polymeric blends, which have been widely studied, show surface properties that are often a combination of those of the pure polymers, and their surface composition is often different from that of the bulk. Furthermore, because of the immiscibility of most polymers, the surface of polymeric blends usually presents segregated structures with domains predominantly composed of each individual polymer [24,25]. Recently, polymeric films of PS, PMMA and a 1:1 PS/PMMA blend were evaluated for cell adhesion using L929 mouse fibroblasts. Although all films showed chemical properties able to support cell adherence and proliferation, these processes were slightly favored in the blend. The authors concluded that the use of the 1:1 blend in future experiments would be advantageous since this material has an additional benefit which is its cost-effectiveness [26].

Within this context, the aim of this study was to prepare polymeric films of PS and PMMA and modify the film surface employing a DBD reactor in order to increase the hydrophilicity of the surface and improve its ability to promote cell adhesion and proliferation.

2. Experimental section

2.1. Sample preparation

Poly(styrene) (PS), Mw = 300 500 g mol^{-1}, and poly(methyl methacrylate) (PMMA), Mw = 139 595 g mol^{-1}, were obtained from Sigma 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 $CHCl_3$ (2% m/v) in a closed flask under magnetic stirring for 24 h at room temperature followed by solvent evaporation (*casting* method).

2.2. Plasma modification

2.2.1. Experimental setup

The experimental setup used for plasma generation is shown in the Fig. 1A and also a similar plasma device has been described elsewhere [27].

2.2.2. Sample modification procedures

In order to modify the PS, 1:1 PS/PMMA blend and PMMA polymer surfaces, the films were folded to cover the inner surface of the quartz tube in the plasma discharge zone (Fig. 1B). Purging with the O_2 feeding gas (2 L min^{-1}) was carried out for a few minutes in the DBD reactor through the input gas positioned at the bottom cap of the reactor in order to eliminate possible gaseous contaminants present in the reactor. The DBD reactor was turned on during the treatment time (60 min) and the gas phase chemical reactions that promoted surface modification were triggered. Finally, the films were removed from the DBD reactor and kept in an argon atmosphere inside a desiccator until use.

2.2.3. Experimental design

A 2^2 standard design with one central point was used in this study. The ultimate goal was to investigate the effect of the O_2 gas flow rate (fo_2) and the DBD treatment time (t) on the measured contact angle (θ) for PS, 1:1 PS/PMMA blend and PMMA films, in order to select the most appropriate combination of these variables for further experiments. For the experimental design, the two variables were confined to two levels: 0.1 (low level) to 2.0 L min^{-1} (high level) for fo_2 and 1 min (low level) to 60 min (high level) for t. A central point (1.05 L min^{-1} O_2; 30.5 min) with three replicates was also included for statistical evaluation (at the 95% confidence level), resulting in seven experiments being performed for the factorial design. The STATISTICA 6.0 software was used to estimate the equation model as well as to draw the response surface based on θ measurements.

2.3. Determination of contact angles and calculation of surface energy

The effect of DBD plasma processing on the wettability of the polymer films was evaluated in terms of water contact angle. The equilibrium contact angles (θ_e) were measured after 24 h of treatment by 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. Images were captured with a high-resolution camera. Five measurements were carried out for each sample, and the mean value was considered. The advancing and receding angles (θ_a and θ_r respectively) were measured using the same goniometer and hysteresis ($\Delta\theta$) was calculated as the difference between θ_a and θ_r.

The surface energy of unmodified and modified films was calculated following contact angle (θ) measurements using the Owens–Wendt geometric model [28]. Distilled and deionized water and diiodomethane were used for the measurements. The surface parameters of these liquids were taken from the literature [29]. Other theories were similarly investigated (Wu — harmonic model) [30], but the results showed the same trend in terms of surface energy as those obtained with the Owens–Wendt model.

2.4. Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS)

Micrographs of the films were obtained using a XL 50 microscope (Philips, The Netherlands), equipped with a tungsten filament as the