Plasma Surface Modification of Chitosan Membranes and Its Effect on Cell Adhesion and Proliferation

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Statement of Purpose: Every year, thousands of people suffer cutaneous lesions such as burns, abrasions or wounds caused by traumatisms that need cicatrization. In cases of severe and large amounts of skin loss, or in the presence of difficult wounds, immediate coverage of the wound surface with an adequate dressing is needed. In this context, a variety of materials (hydrogels, membranes, patches) obtained from different biodegradable polymers has been developed in an attempt to supply the high demand of new materials for skin repair as wound cover or dressings in the treatment of different wounds. Chitosan-based materials have been evaluated as a potential wound dressing solution due to its characteristics including biodegradability, biocompatibility, adhesiveness, non-toxicity, and fungistatic activities. Nevertheless, previous studies [1-2] showed that chitosan-derived membranes are not cytotoxic towards fibroblasts but tend to inhibit cell proliferation. Plasma surface modification is a versatile method to modify surfaces without altering bulk properties which might be an interesting approach to increase cell proliferation on the polymer. The aim of this study was to promote changes on the chitosan membrane surface, such as introduction of specific surface functionalities, etching and/or cross linking by means of a plasma treatment using nitrogen and argon gas in different operational conditions. In addition, the effect of plasma treated-chitosan membranes on the cell adhesion/proliferation of fibroblasts like cells was evaluated.

Methods: Chitosan with a deacetylation degree of about 91% (Vanson, USA) was dissolved in 0.2M acetic acid at concentration of 1 wt%. Membranes were obtained by solvent casting technique, followed by neutralisation in a NaOH solution. The plasma treatment was carried out on a plasma reaction (PlasmaPrep5, Germany), using a power of 20 W. The duration of the glow discharge treatment was varied from 10 min to 40 min, using nitrogen and argon gas. The studied surface properties on dry samples were: roughness (SEM - scanning electron microscopy, AFM - atomic force microscopy), surface chemistry (XPS - X-ray photoelectron spectroscopy) and wettability (contact angle). In order to evaluate the effect of plasma treatments on the cells attachment and proliferation onto membranes, a cell suspension (L929 mouse fibroblast cell line) was seeded at a concentration of $8x10^4$ cells/cm^2 and cultured for 7 and 14 days. The cell viability was evaluated by a MTT assay. The membranes were stained with blue methylene for optical observation and fixed, dehydrated and coated with gold for SEM observations.

Results / Discussion: The SEM micrographs and AFM analysis showed that all treated materials have different surface morphology (rougher) compared to the original chitosan membrane. In addition, an increase in the water contact angle was detected for all samples in the used conditions. Regarding surface chemistry, an increase of the surface energy was observed for nitrogen plasma-treated materials as the treatment time increased. However, in the argon plasma- treated materials this effect was not clearly detected. The nitrogen and argon gas plasma in the used conditions might have promoted a modification and reorganization of chemical groups that are already present at the polymer surface. This involves the breaking of covalent bonds and forming new ones, which can explain the changes in the surface chemistry of the treated materials. These statements are consistent with the XPS studies performed on the plasma treated membranes. MTT assay showed that none of the treatments (under all studied conditions) induced any cytotoxic effect. SEM and optical microscope observations showed that cells clearly attach and proliferation in argon and nitrogen plasma modified membranes improved in comparison with chitosan untreated membranes. In general, after 7 days of culture, the argon treatment seems to produce better results (in terms of cells attachment and proliferation) than the nitrogen plasma treatment.

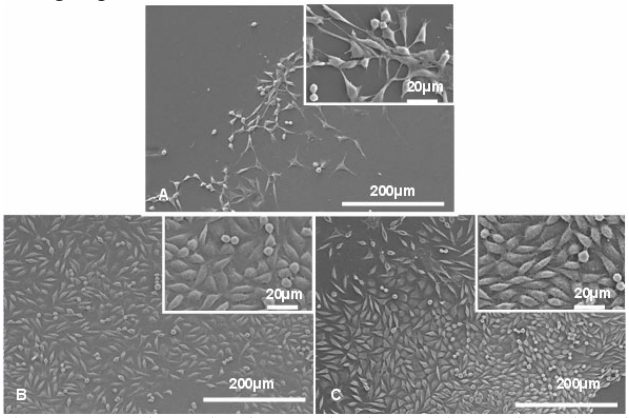

Figure 1: SEM micrographs of L929 cultured on membranes after 7 days of culture: (a) chitosan membrane; (b) nitrogen plasma treated chitosan membrane (20W, 40min); (c) argon plasma treated chitosan membrane (20W, 40 min).

Conclusions: Nitrogen and argon plasma treatment performed under optimized conditions seems to be a suitable technique for modifying the surface properties enhancing the biological response to chitosan-based membranes.

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