JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 15 (2004) 447–449

Cell interactions with laser-modified polymer surfaces

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The performance of a polymeric biomaterial depends on the bulk and surface properties. Often, however, the suitability of the surface properties is compromised in favour of the bulk properties. Altering the surface properties of these materials will have a profound effect on how cells and proteins interact with them. Here, we have used an excimer laser to modify the surface wettability of nylon 12. The surface treatment is rapid, cost-effective and can cause reproducible changes in the surface structure of the polymers. Polymers were treated with short wavelength (< 200 nm) UV light. These wavelengths have sufficient photon energy (6.4 eV) to cause bond scission at the material surface. This results in a surface reorganisation with incorporation of oxygen. Surface wettability changes were confirmed using contact angle measurements. Cell interactions with the surfaces were examined using 3T3 fibroblast and HUVEC cells. Cells morphology was examined using a confocal laser scanning microscope (CLSM). Cell activity and cell number on the treated nylon were assessed using biochemical assays for up to seven days. Both fibroblasts and endothelial cells initially proliferated better on treated compared with untreated samples. However, over seven days activity decreased for both cell types on the control samples and endothelial cell activity and cell number also decreased on the treated polymer.

Introduction

Laser-induced modification of polymers provides a useful method for modifying the surface of materials whilst minimally changing the bulk properties [1]. This is particularly useful in that some current materials have acceptable bulk or mechanical properties for biomedical use but poor surface properties [2]. Incident photons can be used to break and form bonds in the surface of the material and hence change the surface wettability of the material [3]. These changes will have a strong influence on the capacity of a material to encourage or resist protein adsorption [4], as appropriate to the application. Methods such as plasma treatment have successfully produced these types of changes in the past [5, 6], but laser treatment offers the possibility of ''tuned'' laser pulses to break specific surface bonds.

This study involved the treatment of commercially available nylon. Polymers were treated with short wavelength (< 200 nm) UV light. These wavelengths have sufficient photon energy (6.4 eV) to cause bond scission at the material surface [7]. This results in a surface reorganisation with incorporation of oxygen [7]. Surface wettability changes were confirmed using contact angle measurements. Cell interactions with the surfaces were examined using 3T3 fibroblast and HUVEC cells. Cell morphology was examined using a confocal laser scanning microscope (CLSM). Cell activity and cell number on the treated nylon were assessed using biochemical assays for up to seven days.

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Materials and methods

Polymer treatment

Samples of Nylon 12 were prepared by sectioning, then treating using ultrasound for 8 min in methanol. The ethanol was rinsed using double distilled water (DDW) and then the polymers were treated using an excimer lamp for 30 min operating at 172 nm wavelength and 50 mW cm^{-2}.

Surface measurement

Contact angle measurements were performed using the sessile drop technique. Drops of deionised water were placed onto the surface of the polymer. Images of the

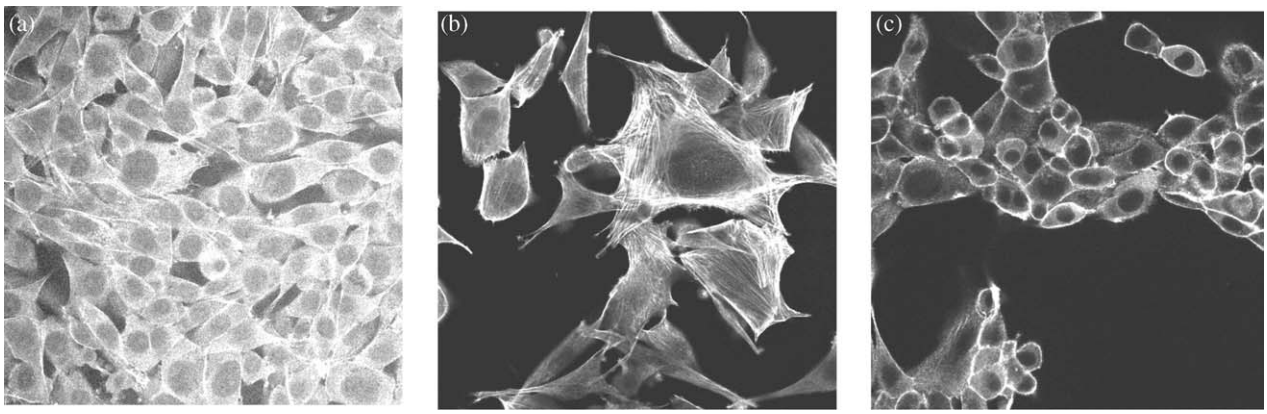

Figure 1 Fibroblast adhesion on nylon 12 after 48 h. (a) control (Thermanox) (b) laser-treated nylon; (c) untreated nylon. Cells have coated the control (a) after 48 h, and remain well spread on the treated sample. In contrast, cells on the untreated sample remain small and rounded.

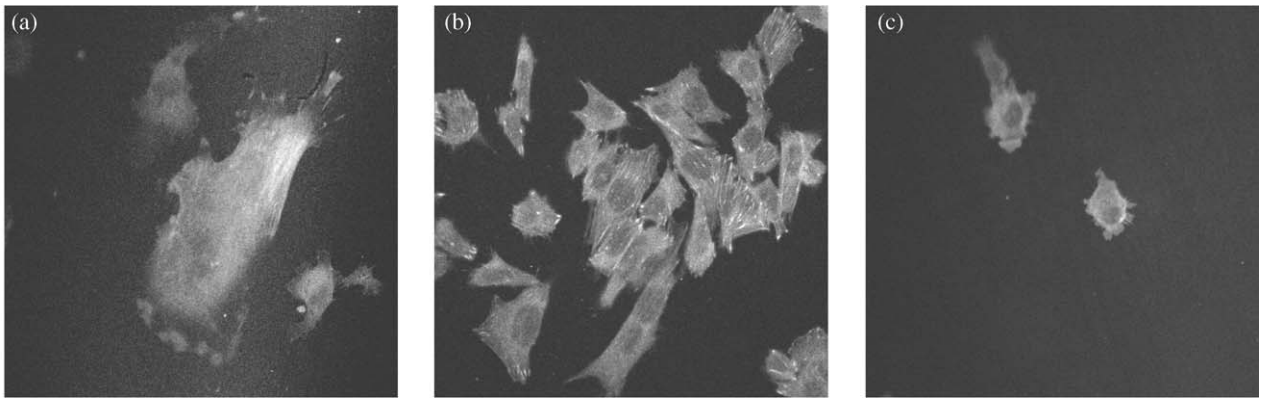

Figure 2 Adhesion of HUVEC on nylon 12 after 48 h. (a) control (Thermanox); (b) laser-treated nylon; (c) untreated nylon. Cells on the control sample are large and well spread, with some focal contacts visible. HUVECs on the treated sample are also well spread with clear cortical actin staining. In contrast, the cells cultured on the untreated sample are small and rounded.

droplets were captured and processed using an image-processing package.

Cell culture

Polymers were used in triplicate, untreated nylon samples were negative controls and Thermanox® discs were the positive controls. Polymers were sterilised by washing with 70% ethanol, rinsed with water and washed with Hanks' balanced salt solution (HBSS) prior to seeding with 3T3 fibroblasts or human umbilical vein endothelial cell (HUVEC) cells. The 3T3 cells were maintained in DMEM medium supplemented with 1% penicillin/streptomycin and 10% serum, HUVECs in EGM-MV BulletKit media (Cambrex, UK). Cells seeded at 1×10^4 cells cm^{-2}.

Cell adhesion

After 24 h culture at 37 °C, 5% CO_2, cells were fixed with 4% paraformaldehyde/2% sucrose, permeabilised with buffered Triton X100 and stained using tritc-conjugated phalloidin. Cells were examined using a Zeiss CLSM.

Cell activity

To examine metabolic activity of cells on the surfaces, triplicate repeat samples were prepared as previously for

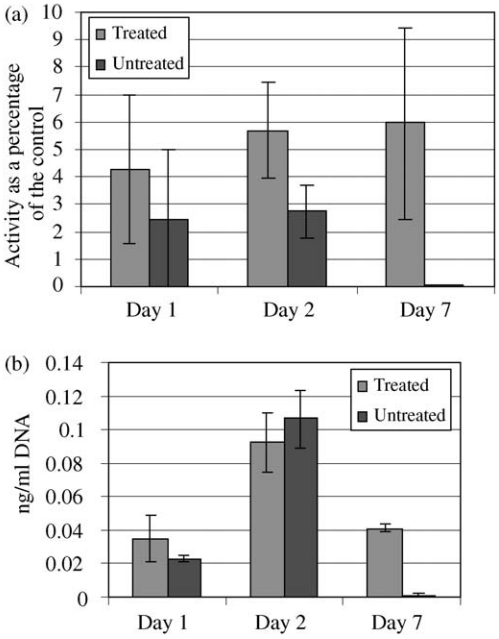

Figure 3 The 3T3 fibroblasts after 24 h culture on treated and untreated nylon 12. Bars show means and standard deviation of triplicate samples. (a) cell metabolic activity as measured by the alamar blue assay. Activity on treated samples is significantly higher than that on untreated samples as measured by two-way ANOVA ($p < 0.05$). (b) cell number, as measured by using an assay of total DNA. The cell number changes over time, but is not dependent on the sample (two-way ANOVA, $p < 0.05$).

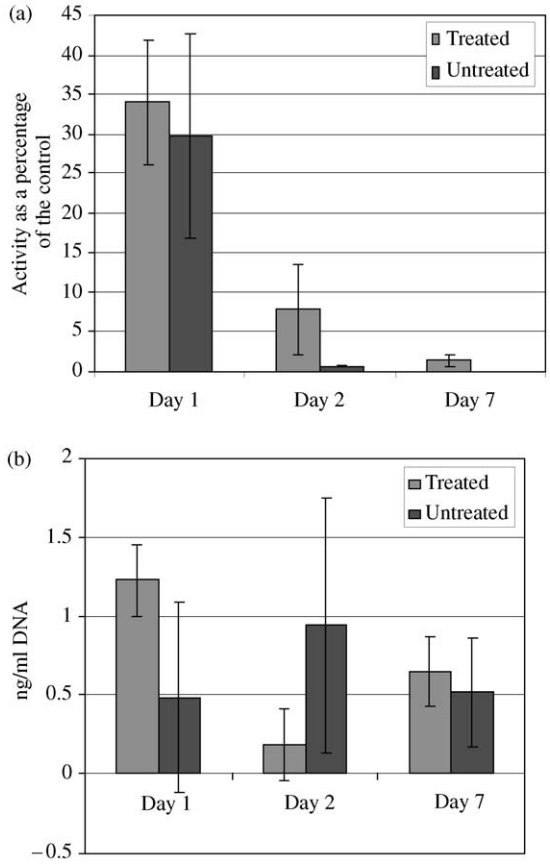

Figure 4 HUVECs after 24 h culture on treated and untreated nylon 12. Bars show means and standard deviation of triplicate samples. (a) cell metabolic activity as measured by the alamar blue assay. Activity on treated samples is significantly higher than that on untreated samples as measured by two-way ANOVA ($p < 0.05$). (b) cell number, as measured by using an assay of total DNA. The cell number does not significantly change over time, but is highly variable.

assay at one, two and seven days. Cells were seeded onto the polymer samples at a density of 1×10^4 cells cm^{-2} and placed in the tissue culture incubator as described. After 24 h, samples were removed, rinsed with HBSS and a 1 in 10 dilution of alamar blue dye in HBSS added to each well. The samples were incubated for 1 h, and then the dye was removed, placed into fresh wells and read on a microfluorimeter at 520 nm in excitation, 590 nm in emission. Cells were then lysed by repeated freezing and thawing in DDW and the DNA content of the suspensions measured by adding buffered Hoechst 33258. Fluorescence was measured against a calf thymus DNA standard at 360 nm excitation and 460 nm emission wavelengths. This was repeated at two and seven days. All experiments were repeated three times. Significance values were measured statistically by ANOVA.

Discussion

Cells are highly dependent on proteins adsorbed onto material surfaces to mediate their adhesion. The process of modifying the surfaces of the polymer is essentially to change the nature of the extracellular matrix protein interaction with the surface to mediate cell adhesion. The data shows that fibroblasts have responded to the surface alterations by increasing cell adhesion and spreading and maintaining cell activity over seven days. In contrast, fibroblasts on untreated polymers are small, rounded and have decreased activity after seven days. However, whilst endothelial cells are more spread on the treated polymer than the untreated polymer after two days, cell numbers are highly variable and activity decreases steadily for both samples over time. This highlights that different cells may respond differently to the same surfaces. This may be a consequence of cellular expression of adhesion molecules. Endothelial cells express both $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrins [8] and restriction of either of these can induce apoptosis [9]. $\alpha 5\beta 1$ is normally associated with fibronectin binding, while $\alpha v\beta 3$ is considered as the classic vitronectin receptor [10]. Restricted expression of one or other of these integrins could possibly allow the spreading seen on the confocal micrographs (Fig. 2) but still cause progressive cell death due to apoptosis.

Therefore, although this technique has proved successful in encouraging fibroblast adhesion to nylon, more study into the nature of the adsorbed protein and the expression of cell adhesion molecules will be required to allow the design of a substrate that can encourage adhesion of other cell types.

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Received 4 October
and accepted 10 October 2003