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Cell patterning on a glass surface by a mask-assisted ion implantation

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ABSTRACT

A simple patterning method of cells on a glass has been developed by using ion implantation. The glass was implanted through a pattern mask with 150 keV Ar ions in the absence or presence of oxygen. Surface properties of the ion-implanted glass were investigated by means of X-ray photoelectron spectroscopy, contact angle measurement and cell culture test. The results showed that more hydrophilic groups were formed on the glass surface implanted in the presence of oxygen. Thus, the glass surface implanted in the presence of oxygen showed lower contact angle compared with the glass surface implanted in the absence of oxygen. The cells were strongly adhered to and proliferated on the ion-implanted regions of the glass. The cell population was found to be the highest on the glass implanted at a fluence of 1×10^{16} ions/cm^2 in the presence of oxygen.

1. Introduction

Patterning of cells on various substrates is essential for tissue engineering, bioelectronic devices and fundamental biological studies [1–3]. A variety of techniques including photolithography, soft lithography, ion implantation, biological laser printing and dip-pen lithography, have been used to place cells on selected areas of substrates [4–10]. However, surface modifications of those substrates are necessary for those applications using different techniques including physical and chemical processes. Among them, surface modification by ion implantation has attracted much attention due to the following merits: (a) it is a surface-specific modification without detrimentally affecting the bulk properties and (b) the projected range, kind of ion and the ion fluence of the implanted region can be selected accurately [11,12]. Thus, this technique has been widely used for surface modification of various organic and inorganic materials for improvement of their surface properties such as their surface hardness, wear resistance, chemical erosion, biocompatibility, and so on [13–17]. Thus, cell patterning on various polymeric substrates has been studied by using ion implantation [7,18,19]. However, research on a cell patterning on a glass surface by ion implantation has not been studied extensively.

In this study, we report on a simple patterning method of cells on a glass surface via ion implantation. The major advantage of this process is that it does not require any additional reaction step to introduce cell-adhesive materials such as gelatin and RGD-peptides that are normally used for the immobilization of cells. The glass was implanted through a pattern mask with 150 keV Ar ions in the absence or presence of oxygen. The changes in the surface properties were investigated by using X-ray photoelectron spectroscopy (XPS), contact angle measurement and cell culture test.

2. Materials and methods

The glass (76×26 mm^2, Superior, Germany) used in this study was ultrasonically cleaned in water and acetone, and then dried in an oven at 100 °C for 3 h. The cleaned glass were then implanted through a pattern mask at an energy level of 150 keV with fluences ranging from 1×10^{15} to 1×10^{17} ions/cm^2 by using a 300 keV ion implanter in the Advanced Radiation Technology Institute (ARTI), Korea. The pressure in the implanter's target chamber was 10^{-5}–10^{-6} Torr and the ion beam current density was less than 30 μA/cm^2. The ion implantation was also executed in the presence of oxygen (O_2, 99.99%) as an atmosphere gas in the target chamber. The flow rate of O_2 was 8.0 ml/min.

The wettability of the glass surface after ion implantation was evaluated by contact angle measurements for the water droplets.

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The measurements were performed by using a contact angle analyzer (Phoenix 300, Surface Electro Optics Co.) as follows: redistilled water (10 μl) was gently placed on the glass surface. Each value of the contact angles was taken as an average value measured from five different samples fabricated under the same experimental conditions.

The chemical composition of the glass surface before and after ion implantation was compared by using X-ray photoelectron spectrometer (MultiLab 2000, Thermo electron Corporation, England) by employing Mg Kα radiation. The applied power was 14.5 keV and 20 mA.

In order to investigate cell proliferation, HaCaT cells (human keratinocyte) were in vitro cultured on the glasses implanted in the absence and presence of O_2. Cell number was counted by a trypan blue exclusion assay after the designated culture time. The 3×10^3 cells/ml of the cells were seeded onto the implanted glasses. The medium was Dulbecco's modified eagle's medium (DMEM) supplemented with heat inactivated 10% fetal bovine serum. The cell-cultured glasses were incubated at 37 °C in the presence of 5% CO_2. After treating the glasses with a trypsin/EDTA solution for 10 min, followed by washing with a phosphate buffered saline (PBS) solution and drying thoroughly in a hood. For staining the cells with hematoxylin and eosin (H&E), the glasses were immersed in a hematoxylin solution for 3 min and then an eosin solution was applied on the glasses for 30 s. After washing and mounting the cells with a mounting medium, the formation of red-stained cell patterns on the glasses was observed with an optical microscope.

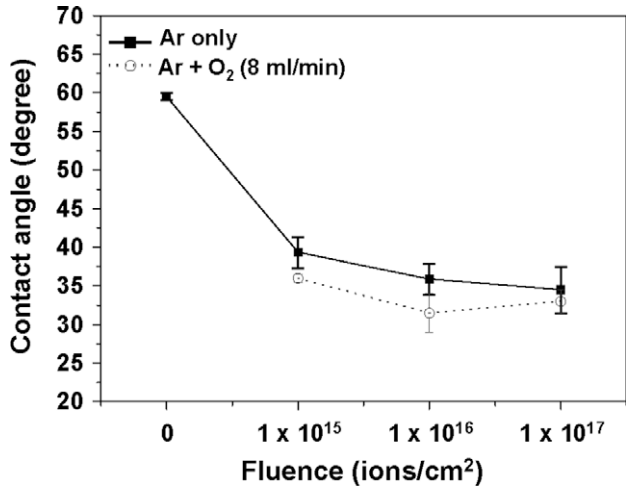

Fig. 1. Variations of contact angles as a function of the fluence in the absence and presence of O_2.

3. Results and discussion

The changes in the wettability of the glass surface as a function of fluence were investigated by contact angle measurements as shown in Fig. 1. The contact angle of the control glass was around 58°. After ion implantation in the absence of O_2, the contact angle gradually decreased up to 36° with an increasing fluence. In the case of the glasses implanted in the presence of O_2, the changes in the contact angle showed a similar tendency to those implanted in the absence of O_2, but the contact angles were lower at all the fluences. These results mean that the glass surface was converted into a more hydrophilic one via ion implantation in the presence of O_2.

To investigate the chemical changes of the glass surface after ion implantation, the glass surface was analyzed with XPS. Figs. 2 and 3 show the C1s region of the XPS spectra of the control glass and the glasses implanted in the absence and presence of O_2. As shown in Figs. 2 and 3, the C–H, C–O, and C=O peaks of the control glass appeared at 285, 284.6 and 288.5 eV, respectively. The intensities of the three peaks existing on the control glass surface were changed slightly with an increasing fluence. However, there was no significant difference among them.

As shown in Table 1, in the case of the glasses implanted in the absence of O_2, the atomic concentration of carbon increased from 20.7 to 39.8 at.% with an increasing fluence above which it de-

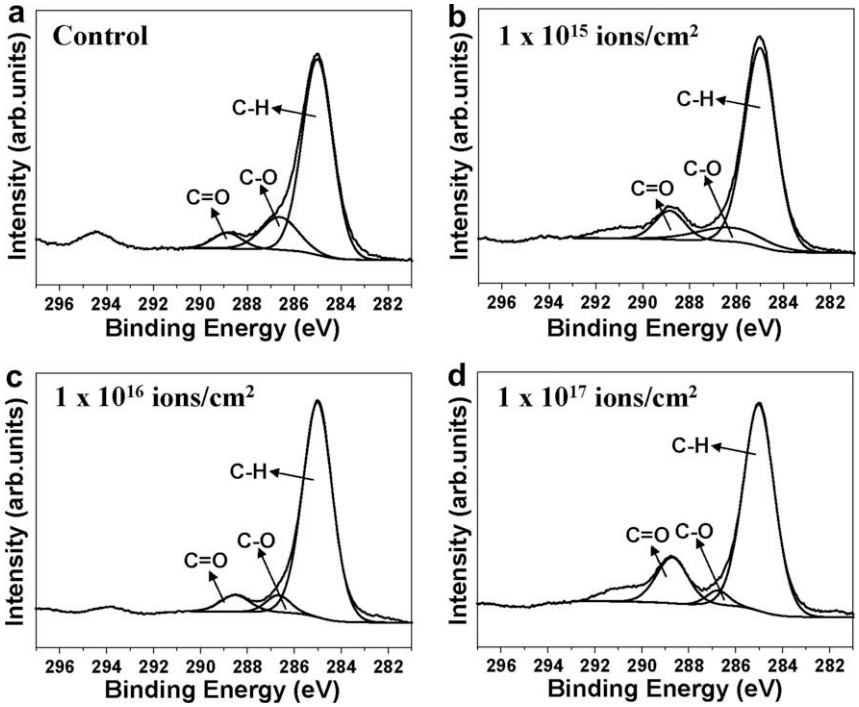

Fig. 2. Cls core-level spectra of the control (a) and the glasses implanted with fluences of 1×10^{15} (b), 1×10^{16} (c) and 1×10^{17} (d) ions/cm^2.