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Controlling cell morphology on ion beam textured polymeric surfaces

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Abstract

Objectives: Nano and micro sized textures on surfaces are generally useful biomaterials. Although the importance of roughness degree has been reported for cell morphology, texture morphology can be more determining for cell adhesion and proliferation. Biodegradable polymers are extensively used as scaffolds as well as implant materials in the human body. The aim of this study was to investigate the effect of ion beam bombardment on cell morphology.

Methods: Gold and carbon ion implantation was made on biodegradable polymers polyglycolic acid, polycaprolactone and polylactic-co-glycolic-acid. Roughness data were obtained from atomic force microscopy. After surface modification with ion beam, B35 neural cells were evaluated on surfaces for cell compatibility. Morphology and cell surface interaction were analyzed with scanning electron microscopy.

Results: We observed improved cell adhesion after ion beam surface modification. Cell adhesion was greater on gold implanted surfaces compared to the carbon implanted. Neural cells attached to lamellar wrinkles, spread by taking the shape of the pattern and exhibited high aspect ratios and axon extension. In contrast, cells that attached on the untreated surfaces remained rounded with low spreading.

Conclusion: The findings of this study are important for development of ion beam modified cell cultures and scaffold systems to understand texture-based cell adhesion mechanisms.

Keywords: cell culture; ion implantation; polylactic-co-glycolic acid; surface treatment; topography; wettability

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Introduction

Biomaterials have a broad application in biomedical researches due to their biocompatibility, biodegradability characteristics and ease of processing. Specifically, biopolymers have a great potential on cell related applications. A number of different synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL) and polylactic-co-glycolic-acid (PLGA) have been used for biomedical applications. The availability of these polymers and the approval of FDA make these materials even desired for the biomedical research.[1]

Biomedical researches are strongly dependent on the success of cell attachment on material surfaces. Due to this dependence, most material research focus on improving surface characteristics, thus, increasing cell attachment. There are plenty of techniques to improve surface characteristics of materials including ion implantation and plasma deposition.

Surface modification with ion implantation and plasma deposition has been shown as a viable method to enhance the adhesion properties without affecting the bulk characteristics and modify polymeric surfaces. Despite many complicated effects, the implantation process may generally be envisaged as two major com-

peting processes: chain scission and crosslinking. In this process, ions are penetrating into a polymer surface by interacting with substrate atoms *via* electronic (ionization) and nuclear (recoil) interactions. Ionization is the dominant phenomenon and generally leads to crosslinking in adjacent polymer chains, whereas recoils generally lead to chain scission.[1–3] By selecting the appropriate implantation parameters, one can create a three-dimensional cross-linked surface layer with hardness exceeding that of steel in addition to improved wear resistance.[1] Implantation sometimes also results in selective enhancement or reduction of functional chemical groups that can, by modifying the chemical interactions on the polymer surface, change the surface wettability and critical surface tension.

A simple way to evaluate the modified polymeric material surface is by the contact angle with deionized water. The dependence on the circumstance of contact angle is reserved. After implantation, the relaxation or stabilization of the polymer surface gradually occurs as time passes by rearrangement and adsorption of oxygen atoms to induce defects. This results in a gradual change of the contact angle of ion-implanted polymers with time. The contact angle depends on the ion-implanted conditions, the measuring period after implantation, and the reserve circumstance. According to preliminary results and our previous experience, polymers with higher hydrophilicity tend to have good biocompatibility.[2,4–6]

In this study, we investigated the effect of ion beam irradiation on B35 cell morphology attached to biodegradable polymers.

Materials and Methods

Polymer film preperation

Samples of poly-L-lactide (PLA; PURASORB PL18 with an inherent viscosity of 1.8 dL/g), poly-D, L-lactide-co-glycolide 50/50 (PDLG; PURASORB PDLG 5010 with an inherent viscosity of 1.03 dL/g), and poly-L-lactide/caprolactone 70/30 (PLC; PURASORB PLC7015 with an inherent viscosity of 1.63 dL/g) were purchased from PURAC (Netherlands) in granule form. As a solvent, chloroform (Merck) was used. PLA, PDLG, and PLC film samples were prepared by a solvent casting method using a 5 wt% polymer–chloroform solution. Granules were dissolved in chloroform and prepared by casting of the polymer solution on clean glass slides. The solvent was allowed to evaporate slowly at room temperature for 24 h, and the samples were then dried under vacuum at 50 °C.

Surface modification

The polymer samples were ion implanted using a vacuum arc ion-source-based ion implantation system at the Ege University Surface Modification Laboratory. This facility has been described in detail elsewhere.[5,6] The broad-beam ion source can be repetitively pulsed at rates up to ~50 pulses/s, and the extracted ion beam current can be up to ~1 A peak or ~10 mA time averaged. The ion source extraction voltage can be as high as near 100 kV. Mixed metal/gas ion beams can be generated by adding gas to the arc discharge region. In this paper, carbon (C) and (separately) gold (Au) were implanted into polymer-on-glass samples at fluences that varied over the range of 10^{14}–10^{17} ions/cm^2 and at ion energies spanning the range of 20–80 keV.

Surface characterization

In order to determine the changes in surface properties, SEM and AFM has been used. Roughness data before and after implantation for each polymer also collected via AFM. Contact angle measurements have been done for the examination of hydrophilicity.

FEI Quanta 200 FEG scanning electron microscopy (SEM) was employed to examine the surfaces of unimplanted, Au and C implanted specimens. The results are compared with respect to the reference - untreated PDLG samples.

PSIA XE-100E model atomic force microscopy (AFM) was used to investigate the surface morphology of untreated and Au and C implanted PLA, PDLG and PLC. Scan size 5.000 μm, scan rate 1.001 Hz. and data scale 300.0 nm were applied through silicon nitride of tip for scanning the sample surfaces. The roughness of untreated and Au and C implanted PLA, PDLG and PLC determined with the AFM. XEI software was used for image processing and roughness calculation, in terms of average roughness, R_a (the average deviation of the measured z-values from the mean plane), root-mean-square roughness, R_q (standard deviation of an entire distribution of z-values for a large sample size), and maximum roughness, R_{max} (the difference between the largest positive and negative z-values).

Cell culture

B35 cells (ATTC, CRL2754) were seeded at a cell density of 4×10^5 cells / ml and incubated in a humid, 5% CO_2 cell culture incubator at 37 °C for 48 h. The cell culture medium was DMEM, Gibco supplemented with 10%FBS, 1% L-glutamine, 100 U/ml penicillin, and 100

mg/ml streptomycin. If otherwise specified, all cell culture reagents were purchased from Biochrom, Germany. After 2-days in culture, the attachment of neural cells on the modified surfaces was observed using SEM (Phillips XL 30S-FEG). The cell viability has been assessed using cell contrast ratio. Morphology and the adhesion points investigated using SEM. Cell contrast ratio was calculated as the ratio of number of cells attached on the implanted sample to that on an untreated sample.

$$\text{Cell contrast ratio} = \frac{\#\text{of cells attached on ion implanted surface } (^{cell}/_{area})}{\#\text{of cells attached on pristine surface } (^{cell}/_{area})}$$

Results

In this study, surface topography of untreated, Au and C implanted biodegradable polymers including PLA, PLC and PDLG were examined with SEM. SEM micrographs were obtained randomly with several measurements (**Figure 1**). Results represent the surface morphology before and after implantation. We observed the formation of a wide range of surface wrinkling patterns, lamellar and a highly ordered pattern, in swollen poly(L-lactide/caprolactone) (PLC) samples.

Wrinkle morphology and size were controlled by the ion beam dose and energy and initial film thickness, respectively. Wrinkle geometry remained stable for gels with equilibrium Young's modulus >100 kPa (results not shown).

Roughness measurements were performed on PDLG and PLC films for both unimplanted and implanted samples. The treatment of ion bombardment into surface significantly increased the material roughness. The roughness of Au implanted PDLG films increased to 249 nm and C implanted ones increased to 213 nm, whereas the roughness of unimplanted films was 79 nm. For PLC films, measurements showed that Au implanted films had the roughness of 254 nm and C implanted films had the roughness of 236 nm, whereas the roughness of unimplanted films was 32 nm. Both Au and C ion bombardment increased roughness more than 10 times (**Figure 2**). Roughness is a desired characteristic for cell attachment and it is known that the rougher surface stimulates the higher cell attachment. Hence, this treatment makes the materials more applicable in the biomedical field due to their improved surface topology.

Different ion doses applied on the material surfaces to examine changes on the surface topography. AFM images in **Figure 2** show how surface texture changes in nanoscale with respect to ion dosage. When the dose value of ions increased, the cavities on the surface became larger and decreased in number. On the other hand, when we decreased the dose value, the cavities on the surface got smaller but they increased in number. Thus, it is shown that surface texture can easily be controlled by changing the dose value of ions. Ion bombardment significantly changed the surface texture at nanoscale. While there was no surface wrinkles on unimplanted samples, ion bombardment gave surfaces a wrinkled, rough texture which is supposed to stimulate cells to change their morphology (**Figure 2**).

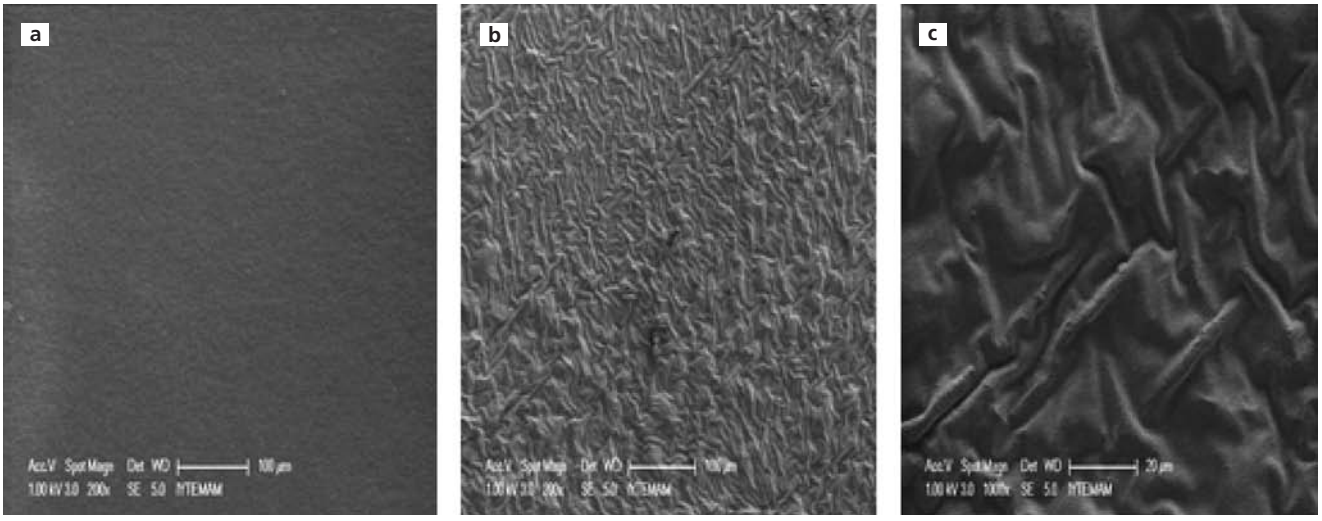


Figure 1. Surface morphology of polymeric films unimplanted PLC surface (**a**) and ion bombarded surfaces (**b**, **c**). Growth of deposited Au layer, island growth and coalescence by ion bombardment are observed.

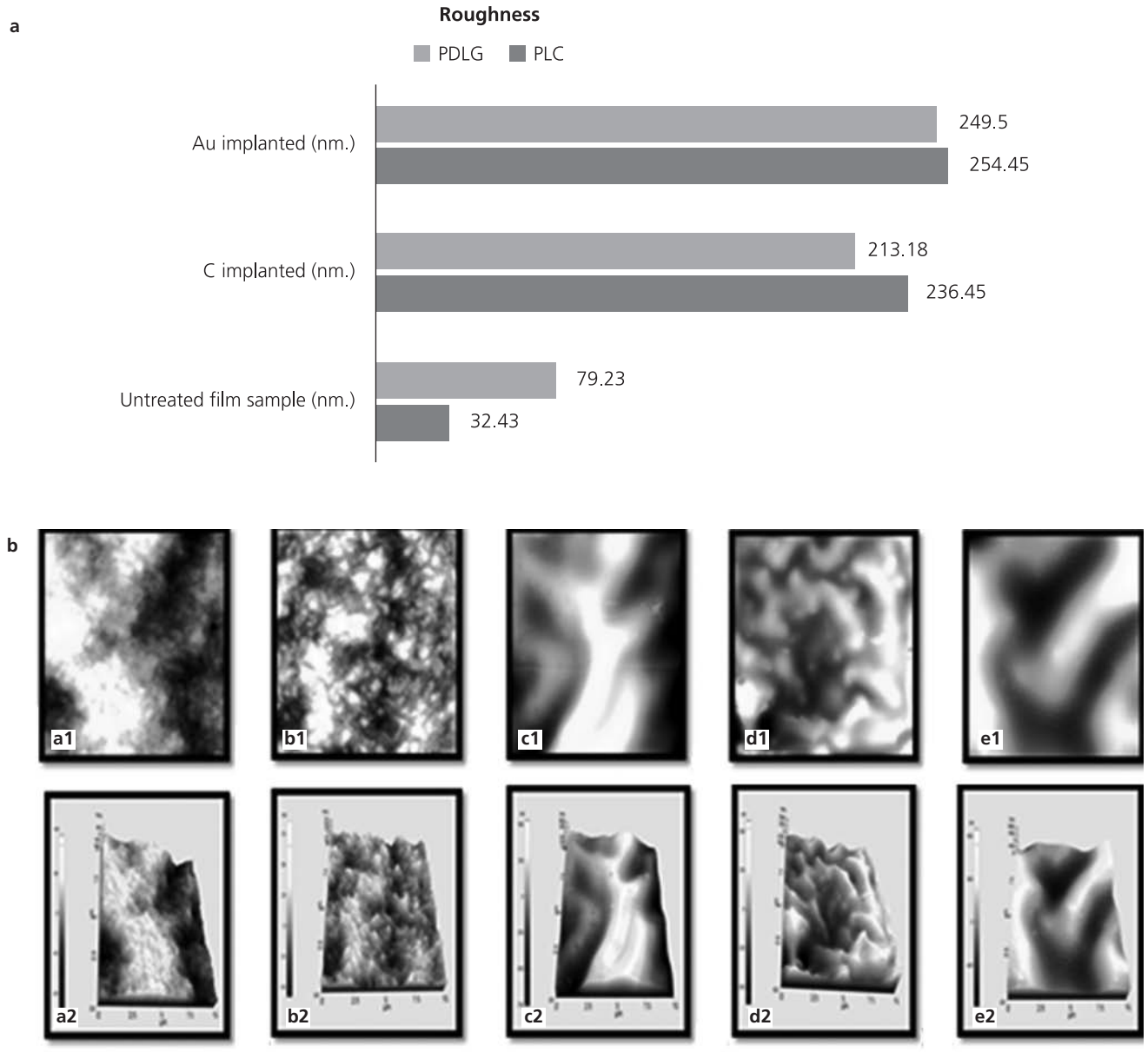
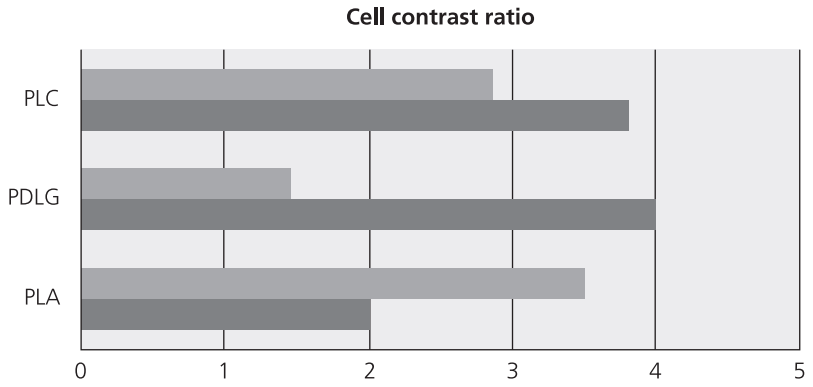


Figure 2. (**a**) Surface roughness (nm.) of untreated and Au and C ion implanted PDLG and PLC samples. Surface roughness values increased by ion bombardment. (**b**) Topographical changes by ion bombardement on PLC samples. Images taken by AFM in order to show nanoscaled textures on the surfaces. PLC film samples were ion implanted with the dose value of (**a1**, **2**), $1x10^{14}$ (**b1**, **2**), $1x10^{15}$ (**c1**, **2**), $1x10^{16}$ (**d1**, **2**), $5x10^{16}$ (**e1**, **2**) $1x10^{17}$ ion/cm^2.

To investigate the effects of pattern geometry and size on neural cell morphology and spreading, we focused on lamellar patterns on PLC sample. After sterilization, polymeric films were incubated with cells without any further surface modification. Cell contrast ratio is calculated for all PLC, PDLG and PLA for both Au and C bombarded samples (**Figure 3**). We took SEM images of PLC, PDLG and PLA (PDLG and PLA images are not shown) samples in order to observe morphological changes on the cells with respect to different ions and as well as the untreated samples. There were almost no morphological changes on the unimplanted surfaces compared to implanted samples where neuron-like cell morphology was observed. Neurites formed bridges between the surface wrinkles and aligned themselves with these bridges. Besides, neurite bridges lengthened to other cells and spread as neurons did (**Figure 4**).



	PLA	PDLG	PLC
Au implanted sample	3.5	1.45	2.85
C implanted sample	2	4	3.8

Figure 3. Cell contrast ratio of B35 cells on Au and C ion implanted different polymeric surfaces (PLA, PDLG and PLC).

Discussion

In this study, we used different polymers and modified the polymer surfaces with different ions to change surface topology to examine it effects on neural cell morphology. Au and C bombardment significantly increased surface roughness and this stimulated a higher cell attachment to the material surface. On the other hand, we observed that PLC is the best polymer for cell growth and proliferation. We clearly observed the extracellular matrix formation, cell spread and neurite formation on implanted samples.

Although we obtained reproducible pattern structures and sizes, the structural properties of the polymeric films differ significantly for each condition.[7] Importantly, this technique is applicable to a variety of photo-cross-linkable materials. For instance, for methacrylated hyaluronic acid gels.[8,9]

Increased surface roughness of materials will provide an increase on the cell attachment. Regardless of cell type, all adherent cells first, need to attach surface to proliferate and show their morphology. It has been shown that cell spreading elongated facilitates calcium deposition,[10,11] whereas rounded cells shape in circular with low spreading allows for maximum lipid storage.[12,13] Here, we observed that control of surface structure morphologically using by ion beam bombardement technics could directly effect neural cell attachment and neurite extension.

Recent studies on ion implantation also questioned the use of this technique in nerve regeneration.[7,14,15] Ishikawa et al.[14] studied negative ion implantation on polymer surfaces and showed that PC12 cells were able to extend neurites. Improved and selective attachment properties of nerve cells, cultured *in vitro*, as well as their neurites were obtained.

In this study, the best cell attachment and neurite formation were observed on PLC samples. Ion bombardment increased the cell attachment. Cells attached and spread better on ion implanted surfaces. On C bombarded surfaces, we were able to observe a better neurite formation. When we compare the length of neuritis, best results were found in the C implanted samples. On the other hand, a better cell spread was observed on Au bombarded samples. We observed an extracellular matrix-like structure, which is a significant signal for cell viability, on implanted samples. This structure is more prominent on t Au implanted samples where cell spread is also more apparent. When there is no extracellular matrix-like structure on unimplanted samples, this can be evaluated as a signal for a fitting environment created with ion implantation for cell growth and proliferation.

Conclusion

Our results strongly indicate the influence of surface modification by ion treatment on cell attachment, cell growth, cell proliferation and cellular morphology com-

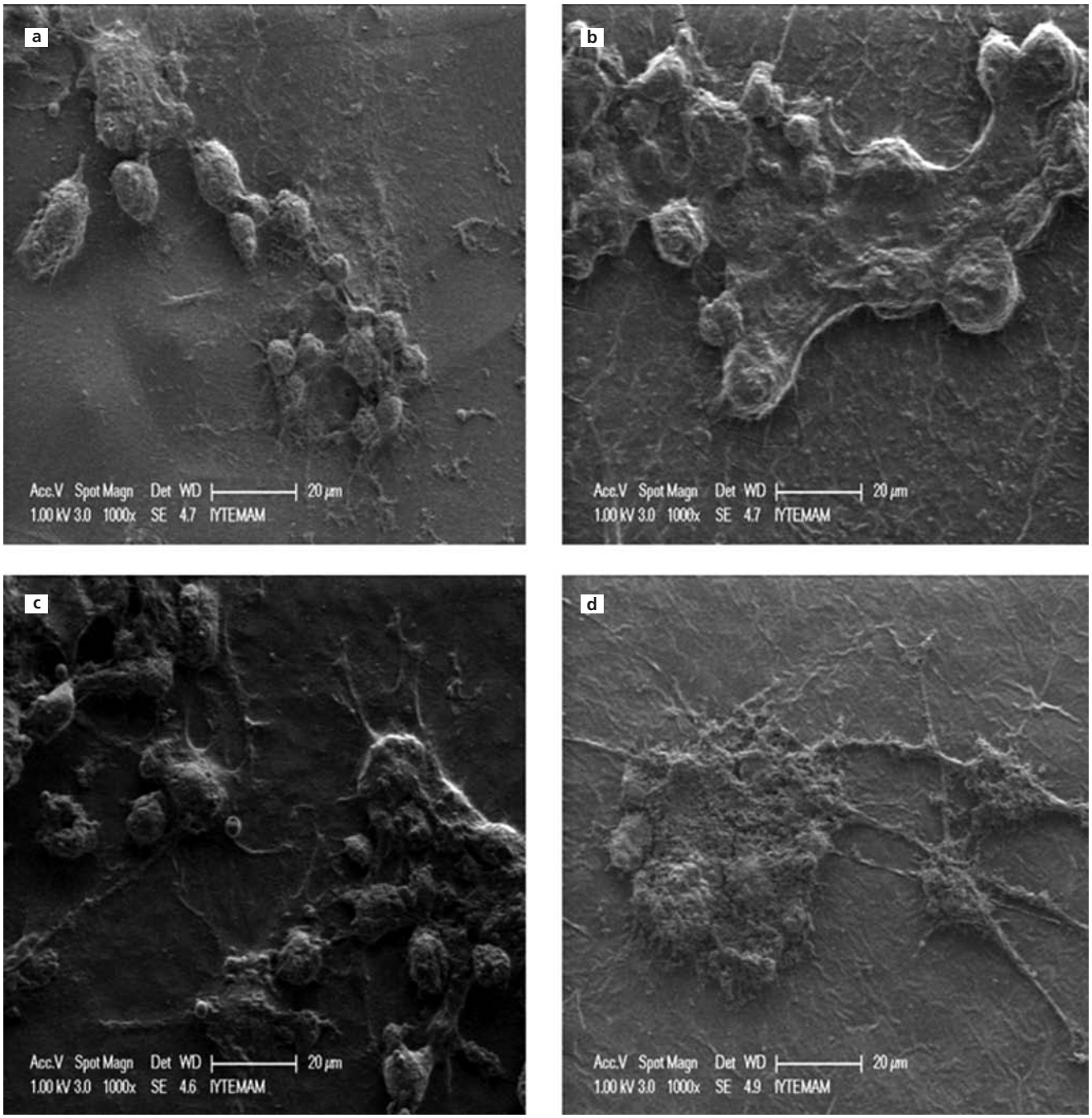


Figure 4. Cell morphology on differently textured ion beam bombarded surfaces. Polymeric film samples are compared as untreated (**a**), treated by Au (**b**), and C-ion bombarded (**c** and **d**) (SEM-1000X).

pared to unimplanted samples. These results are important to show that we can create a viable environment for cells where they secrete their own extracellular matrix and show their own phenotype and morphology without getting stressed. We have a great control over the surface topology just by changing the ion dose value and energy which makes it possible to create desired surface structures for different applications.

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