Extreme ultraviolet (EUV) surface modification of polytetrafluoroethylene (PTFE) for control of biocompatibility

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abstract

Extreme ultraviolet (EUV) surface modification of polytetrafluoroethylene (PTFE) was performed in order to enhance the degree of biocompatibility. Polymer samples were irradiated by different number of EUV shots using a laser–plasma based EUV source in the presence of nitrogen gas. The physical and chemical properties of EUV modified PTFE samples were studied using Atomic Force Microscopy, X-ray photoelectron spectroscopy and water contact angle (WCA) methods. Pronounced wall type micro and nano-structures appeared on the EUV treated polymer surfaces resulting in increased surface roughness and hydrophobicity. Stronger cell adhesion and good cell morphology were observed on EUV modified surfaces by in-vitro cell culture studies performed using L929 fibroblasts.

Keywords:
Extreme ultraviolet, PTFE surface modification, Surface patterning

1. Introduction

Polymers are widely used biomaterial in applications ranging from cardiovascular implants to drug delivery systems. This wide usage is due to their ease of fabrication, flexibility, resistance to biochemical attack, lightweight and their ability to be made biocompatible. In the case of medical implants, the material has to remain in contact with host tissues for a prolonged period of time. Therefore, it is crucial to investigate the degree of biocompatibility of a material and tune its surface properties in order to control the interaction between the material and host extracellular environment [1,2].

Polytetrafluoroethylene (PTFE) is a fluoropolymer (fluorocarbon-based) with long chain of $(CF_2–CF_2)_n$. PTFE is hydrophobic in nature, non-biodegradable and has low friction characteristics [2]. PTFE can be fabricated in numerous forms, including porous mesh like structures, tubes, strands and sheets. Therefore, in the healthcare industry, PTFE has been employed in the fabrication of vascular prostheses, tubes for nerve regeneration, subcutaneous augmentation materials, and in the maxillofacial surgeries [1–4]. PTFE is chemically stable and due to its low surface energy, protein adsorption on its surface within the biological environment is low. This property is quite advantageous making it suitable for vascular prostheses and tubes for nerve regeneration, as the cells and proteins from blood plasma do not attach on its surface, the risk of thrombus generation is low. However a PTFE implant will be encapsulated by connective tissues that will not adhere to its surface due its bio-inertness. This increases the risk of vascular occlusion, which would be counter integrative. PTFE has low wear resistance which results in the production of wear particles under compressive or abrasive loading which can lead to chronic inflammation [1]. Due to this abrasion the wall roughness is promoted which results in an increased risk of platelet aggregation and blood clotting. An endothelial layer is required to form on the vascular implant surface to inhibit the blood clot formation. However due to lack of cell attachment, endothelialization fails to occur [5]. Therefore for vascular prosthesis, improved cell adhesion of PTFE would be advantageous.

PTFE is semicrystalline in morphology and has a low glass transition temperature (70 C) that enables exceptional chemical resilience [6]. Therefore it is quite difficult to tune its surface properties [5–8]. Since tuning of surface properties would be difficult at the fabrication stage of polymers, various surface modification techniques are currently being employed or developed [4,9–17]. Various extracellular matrix (ECM) peptide sequences, which have been determined to influence the cell behavior, have been isolated and grafted on biomaterials to enhance biological properties. RGD cross-linked fibrin gel, WQPPRARI, P15 peptide, cyclic CRRETAWAC and many other peptides have been derived from ECM proteins or other moieties [18]. These have been used as coatings on PTFE surfaces to mimic the features of the ECM and assist the specific cell type adhesion [18]. However as described above, due to low surface free energy, protein adhesion to PTFE surfaces is quite limited. Plasma treatments have been used to control the wettability of PTFE [19,20]. However plasma treatments are quite restricted due to non-uniformity, formation of by-products and lack of sustainability [21–23].

More recently, micro and nano-patterned structures are induced on to the polymer surfaces using ultraviolet radiation. The surface modification of PTFE surfaces using ultraviolet lasers have been performed to induce microstructures in order to improve cell adhesion [4,7,16,17]. Human umbilical-vein endothelial cells (HUVEC), human aortic smooth-muscle cells (HASMC), 3T3 mouse fibroblasts and rat aortic smooth muscle cells (SMC) cell culture studies have been performed on PTFE samples irradiated with 172 nm excimer lamp in an ammonia atmosphere, depicting improved biocompatibility [16,17]. Improved adhesion of 3T3 mouse fibroblasts was demonstrated on PTFE surfaces irradiated at by F_2 laser at 157 nm wavelength [7]. A comparative study of ultraviolet and extreme ultraviolet surface modification of polyethylene terephthalate (PET) demonstrated that Chinese hamster ovary

(CHO) cells seeded on UV-laser-induced structures showed less pronounced alignment comparative to those cells seeded on extreme ultraviolet (EUV) induced structures [24]. This motives the further investigation of EUV surface modification for biocompatibility control.

A crucial requirement of any surface modification technique is to leave bulk properties intact as alteration in mechanical properties of the bulk material of the bioimplant may result in hostinduced biodegradation. EUV radiation is high-energy ultraviolet radiation, having photons with energies from 30 eV up to 250 eV (corresponding to wavelengths in vacuum from 40 nm to 5 nm respectively) [25]. Such photon energies are able to break molecular bonds more efficiently and effectively as compared to excimer lasers or excimer lamps [26]. In addition to that, EUV photons are highly absorbable even in the quite low-density medium. The penetration power of EUV photons in upper surface layers of polymers is limited to 100 nm. Therefore the EUV surface modification technique can be used for smooth ablation of polymers without producing undesirable impacts on bulk material [21].

The three regular adhesive sites between cells and solid substrata are focal adhesion at cell boundaries (10–20 nm gap), close contact surrounding focal adhesion (30–50 nm gap), and extracellular matrix contacts (more than 100 nm gap) [2]. Focal adhesion points represent strong cell adhesion sites responsible for cell attachment to the surface [16]. In addition, crossing the interfacial free energy barrier of adhesion which is a function of surface free energy of the substrate is needed for cell adhesion [2]. Therefore the wettability being a function of the surface energy of a material is an important factor responsible for cell adhesion. The wettability can be tuned by the surface roughness of the material as well as surface chemistry. EUV surface modification has been successfully demonstrated to control the surface topology and chemistry [26– 28]. These wall type nano-topographic structures could provide focal adhesion sites to control cell morphology, alignment and adhesion [29–31]. There are various physical parameters of biomaterial surfaces that correlate with bio-reactivity. Bio-reactions like protein adsorption, platelet adhesion and bacterial adhesion are often associated with surface properties like surface roughness, wettability, specific surface groups and surface chemistry etc. Unfortunately powerful mathematical models which fully explain the multivariate correlation are not yet available [2]. The degree of biocompatibility can have many manifestations, therefore it is difficult to approximate all the trends to allow mathematical modeling. For example, it has been demonstrated that positive influence on relative cell spreading can be modeled as a function of increasing substrate surface free energy [32]. However, reduced cell spreading and adhesion has also been reported with high surface free energy substrates [33]. Nevertheless generalized relationships dependent upon repeated observations provide good indications of positive or negative correlations between surface physical characteristics and bioreactivity. Fibroblasts are the most common cells of connective tissues in animals. Fibroblast cell culture studies are performed by various researchers as a starting point of the biocompatibility evaluation of a material intended for medical use [7,23,34–36]. The surface topography of biomaterials can be characterized according to surface roughness, porosity and texture. It has been reported that the microand nanotextured polymer surfaces provides good cell adhesion and overall enhanced biocompatibility for various cell types as compared to smooth surfaces [4,16,24]. Contact angle measurements are normally taken to estimate the surface free energy as first line characterization of materials. The surface free energy of a material surface is highly significant as it provides good correlation approximations for various biological interactions. The energy barrier to cross adhesion threshold is higher for low surface energy substrates as compared to hydrophic surfaces. Generalization of thermodynamical model indicates the direct proportional relationship between interfacial free energy of adhesion and substrate wettability under certain limits [2].

In this study, surface modification of PTFE foils has been performed using a 10 Hz laser–plasma EUV source based on a double gas puff target [37]. The polymer samples were irradiated in the presence of nitrogen gas. Incorporation of nitrogen onto the polymer surface promotes cell attachment [38]. The generated surface roughness and patterning were investigated by Atomic Force Microscopy (AFM). Chemical modifications and incorporation of nitrogen atoms in treated polymer samples were analyzed by Xray photoelectron spectroscopy (XPS). The water contact angle measurements were taken to study the impact of EUV irradiation on the wettability of the treated samples. Cell interaction with the EUV modified PTFE surfaces, including morphology and adhesion test results are also presented.

2. Materials and methods

2.1. EUV surface modification

PTFE foils 0.1 mm thick from Goodfellow Cambridge Limited, UK were irradiated using a 10 Hz laser–plasma EUV source based on a double stream gas puff target. The gas puff (Kr/He) target was irradiated with a 3-ns/0.8 J Nd:YAG laser pulses. Injecting pulsed krypton gas into a hollow stream of helium gas created the gas target. The gas target upon irradiation by the laser generates EUV radiation without producing debris. The EUV photons were focused using a gold-platted ellipsoidal grazing incidence mirror in order to obtain maximum intensity. This innovative setup allows controlling the spectral range of radiation spanning the wavelengths from 9 to 70 nm. The maximum intensity attained was at a wavelength of 10 ± 1 nm. The EUV fluence at the center of focal spot was more than 60 mJ/cm^2. This laboratory scale compact EUV source was equipped with an auxiliary gas nozzle within the EUV-sample interaction chamber. Injection of an additional gas (such as nitrogen or helium) was possible through this nozzle during EUV exposure on to the sample. Further detailed description of source construction and parameters can be found in previous studies by our group [26–28,37,39–44].

The PTFE foils of about 12 mm by 12 mm size were mounted on an XYZ translation stage and kept 2 mm away from the EUV collector focal point. The spot size of EUV beam on the polymer sample was about 1.5 mm in diameter. The translationary movements along *X* and *Y* planes allow irradiation of 10 mm by 10 mm area of the polymer samples. The polymer samples were irradiated with 50, 200 and 300 EUV shots per millimeter at 10 Hz repetition rate in the presence of nitrogen gas at the pressure of 3 bars. This was achieved by controlling the motorized movement of sample stage along *x*and *y*-axes. The stage was moved with speed of 0.2 mm/s, 0.05 mm/s and 0.003 mm/s for 10 mm along *x*-axis in order to irradiate 1 mm of polymer sample with 50, 200 and 300 EUV shots. After the movement of 10 mm along *x*-axis, the laser was stopped and the stage was moved along *y*-axis by 1.5 mm. The sample was

then moved back in the negative *x*-direction at the set speed by 10 mm with continuous EUV exposure. By repeating this procedure six times, the area of about 10 mm by 10 mm was raster scanned and irradiated with the specified number of EUV shots. There is small chance of overlapping of EUV irradiation over the edges. However it is expected that this overlapping does not influence the surface structuring. The details of experimental design are shown in Table 1. Three identical sets of polymer samples for each set of experimental conditions, as described in Table 1, were prepared for AFM, water contact angle (WCA) and cell culture studies. For XPS measurements, one pristine sample and one EUV irradiated sample for each condition as described in Table 1 were examined.

2.2. Morphological characterization

The morphology of the pristine and EUV treated polymers was studied by Atomic Force Microscopy (AFM). The commercially available AFM supplied by NT-MDT, Russia was used with standard tip NSG03. The tip curvature radius was 10 nm. The spring constant of the cantilevers ranged from 0.35 to 6.06 N/m for noncontact (semi-contact) measurements with resonance frequency of 47–150 kHz. The samples surface roughness was investigated in semi-contact mode at ambient temperature by acquiring the images at two resolutions, 25 lm 25 lm and 50 lm 50 lm.

The cross section analysis has been performed and histograms of the AFM scans were obtained using software provided by NTMDT. This allowed evaluation of the surface structure and its relation with the number of EUV shots. Average surface roughness of pristine and EUV modified surfaces were calculated. The changes in average surface roughness could provide meaningful insight to morphological characteristics of pristine and EUV modified surfaces [45].

2.3. Elemental analysis

Smooth laser ablation results in chemical modification of the upper layer surface of EUV treated polymers. Incorporation of functional groups often desirable for enhanced proliferation and cell adhesion of particular cell types. X-ray photoelectron spectroscopy

(XPS) scans were obtained from XPS with analyzer R3000 from VG Scienta Sweden and X-ray lamp with the anode Mg Ka (205 W X-ray power) from PREVAC sp. z o.o., Poland to investigate the incorporation of nitrogen gas injected through an electromechanical valve during EUV irradiation of polymers. The analyzer pass energy was set at 100 eV. The acquisition time for each scan was 15.5 min with point spacing of 200 meV. Measurements were made without the neutralizer, binding scale was referenced so that the F1s peak of the unmodified PTFE was in the position of 689.0 eV [46]. In this case, the C1s peak is shifted by 7.3 eV to higher binding energy as is apparent from its chemical environment. Detailed elemental analysis to visualize the changes in bonding energy of carbon and fluorine were performed using CasaXPS Version 2.3.15 from Casa Software Ltd.

Table 1
Experimental design of EUV irradiation of PTFE polymer samples.

Sample number	EUV treatment (EUV shots/mm)	Additional gas	Sample stage speed during irradiation (mm/s)
1	Pristine	–	–
2	50	Nitrogen	0.2
3	200	Nitrogen	0.05
4	300	Nitrogen	0.0033

2.4. Water contact angle

For the measurement of the static water contact angle of pristine and EUV treated polymers, KSV Instruments CAM 100 was used. The equipment was calibrated according to the user manual from KSV Instruments. The water contact angles (WCA) were measured using the static sessile drop method. The distilled and deionized 10 ll water droplets were used for all measurements. Each polymer sample was measured in triplicate and standard deviation was measured to assess the reproducibility of the results. The percentage change in WCA with respect to increase in EUV pulses irradiated on PTFE samples was calculated.

2.5. Cell culture

L929 fibroblasts (ECACC, UK) with concentration of 2 10^4 cells/ml, were seeded in 24 well plates and cultured with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of FBS, 1% of glutamine and 1% of antibiotic at 37 C in 10% CO_2. After 24 h medium was changed. The tested samples were cut into disks with a diameter of 16 mm, sterilized with 70% ethanol, placed in each well (the modified surface was in direct contact with cell layer) and fixed with well inserts (Sigma–Aldrich) and incubated in 37 C for 24 h. After that time, samples were removed, cells were washed with Dulbecco's Phosphate-Buffered Saline (DPBS) and Live/Dead Staining Kit working solution was added (200 ll/well, working solution prepared according to manufacturer's protocol). Cells were incubated with the solution (37 C, 15 min) and analyzed using fluorescence microscopy (Nikon, Eclipse Ti-U). Live/Dead Double Staining Kit enables to distinguish live and dead cells by using two fluorescence stains: calcein (stains live cells green) and propidium iodium (PI) (stains dead cells red). In live cells the nonfluorescent calcein AM is converted to a greenfluorescent calcein after acetoxymethyl ester hydrolysis by intracellular esterases. PI binds to DNA by

intercalating between the bases and once the dye is bound, its fluorescence is enhanced 20to 30-fold. PI is not permeant to living cells. Hence dead cells could be detected. Additionally, in order to visualize cells adhered to the modified surfaces, material samples removed from the culture was also stained and analyzed.

MTT assay was performed in order to assess the cell viability after contact with pure and EUV modified samples. The assay was conducted according to ISO 10993-5 standards, using indirect method (incubation with materials' extracts). L929 fibroblasts were seeded in 24 well plates and cultured with DMEM supplemented with 10% of FBS, 1% of glutamine and 1% of antibiotic at 37 C in 10% CO_2 for 24 h. The tested samples were cut into disks with a diameter of 12 mm, sterilized with 70% ethanol, placed in 24-well plate, fixed with well inserts (Sigma–Aldrich) and incubated with DMEM (500 ll/well) at 37 C for 24 h. After that time, materials' extracts were collected and transferred to the wells seeded with cells. As a negative control (*K*) cells incubated with DMEM were used. After 24 h of incubation, extracts were discarded and the cells were washed with DPBS, followed by addition of MTT solution (1 mg/ml in DMEM without Phenol Red, 200 ll/well, 4 h, 37 C). Afterwards the MTT solution was discarded and the formed crystals were dissolved by addition of DMSO (400 ll/well) and Sorrensen buffer (50 ll/well). Cell viability was calculated using the following formula:

$$\text{Viability } (\%) = \frac{A570s}{A570k} * 100\% \qquad (1)$$

where A570s is the mean value of the measured absorption of the sample at 570 nm and A570k is the mean value of the measured absorption of the negative control at 570 nm.

3. Results and discussions

3.1. Surface morphology and roughness analysis

The pristine and EUV modified PTFE polymer samples were scanned using AFM in semi-contact mode. The EUV exposure on the polymer surface produced regularly patterned structures. Therefore the structures at the edges and center of modified 10 mm by 10 mm area were scanned using AFM. The average surface roughness values expressed in this section were averaged from three measurements from one sample. For polymers treated with 50 EUV shots, it was observed over the 25 lm by 25 lm area that the average surface roughness increased from 45 nm (pristine) to 54 nm at the edges and 126 nm in the center as measured from AFM, see Fig. 1. For the large area (50 lm 50 lm), the average surface roughness increased from 53 nm (pristine) to 137 nm (EUV center region). Line scans of the AFM data are shown in Fig. 2.

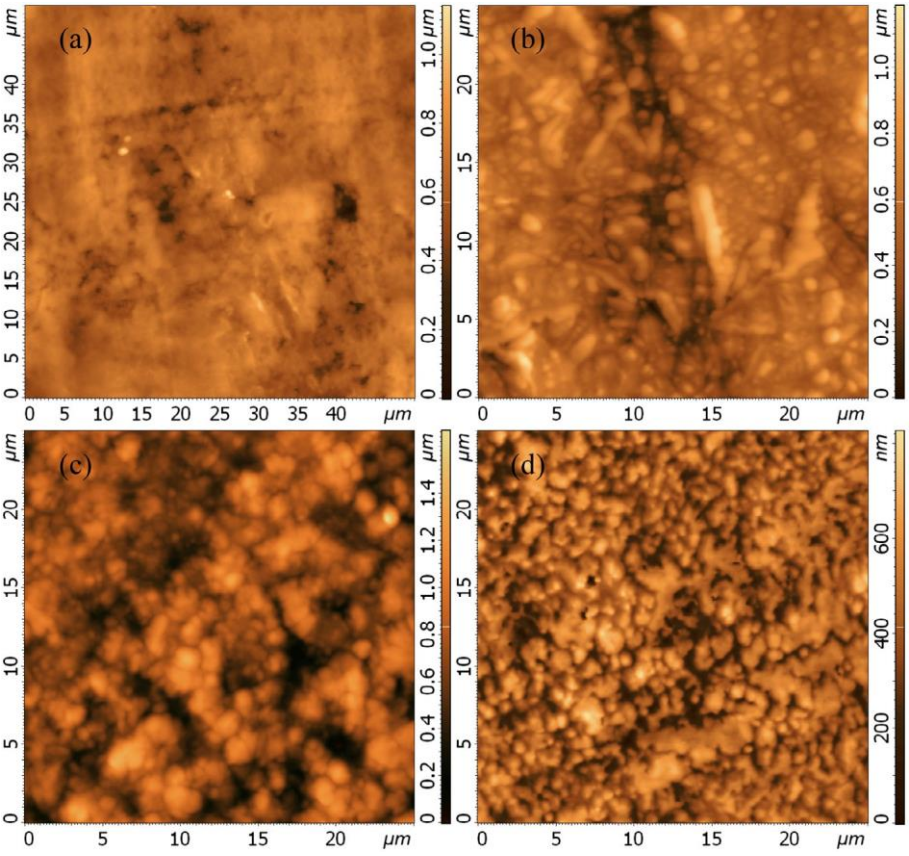

Fig. 1. AFM images of PTFE samples (a) pristine, (b) 50 EUV shots, (c) 200 EUV shots, and (d) 300 EUV shots

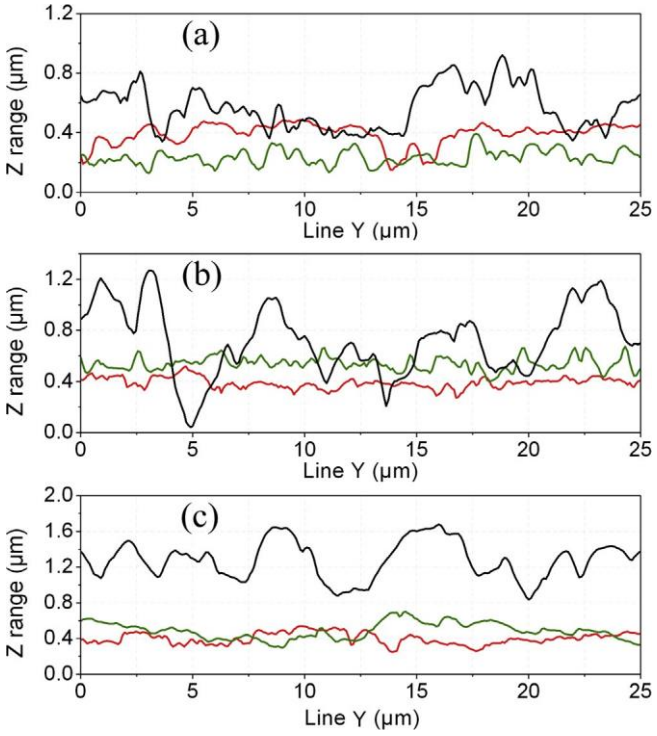

Fig. 2. Cross section analysis of PTFE irradiated with (a) 50 EUV shots, (b) 200 EUV shots, and (c) 300 EUV shots.

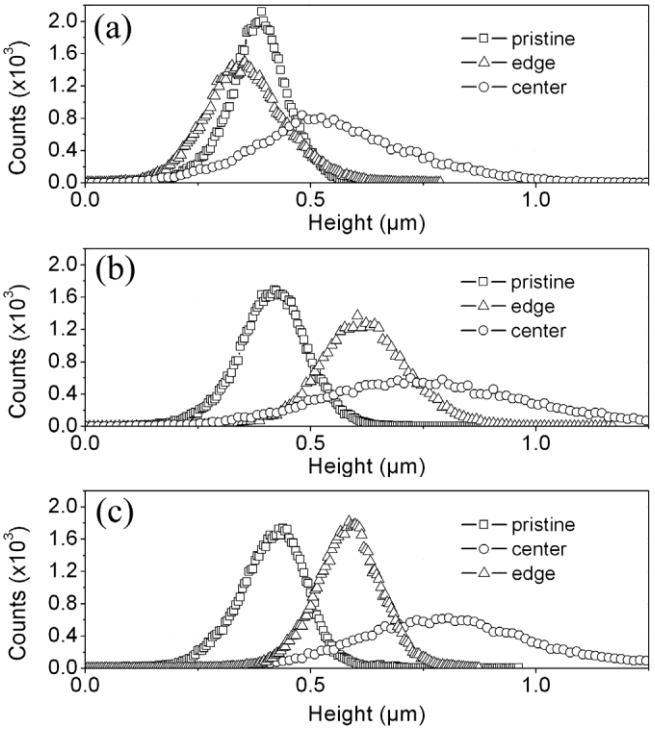

Fig. 3. Histogram of PTFE irradiated with (a) 50 EUV shots, (b) 200 EUV shots, and (c) 300 EUV shots.

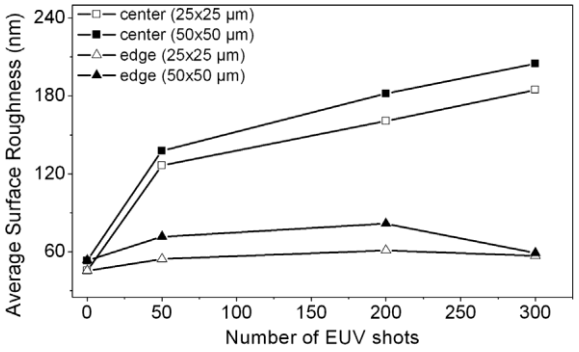

Fig. 4. Summarized surface roughness results from 25 lm 25 lm and 50 lm 50 lm images of pristine and EUV modified PTFE samples.

These show the presence of micropatterned structures with some degree of regularity. The histogram demonstrating the height of structure peaks relative to the number of counts of peaks with a specific height provided interesting results. It was observed that in the case of the pristine PTFE sample, the maximum number of counts occurred for the peaks having a height about 0.3 lm whereas in the EUV modified sample, the most abundant peak height was about 0.5 lm (Fig. 3). The surface roughness analysis of EUV treated PTFE samples are summarized in Fig. 4. Detailed analysis of PTFE films irradiated with 200 and 300 EUV shots by AFM demonstrated increased average surface roughness up to 160 nm and 184 nm, respectively. Similarly the average heights of the peaks also increased. Most importantly, the regularity of the structures improved with increasing number of EUV shots as observed by the line scans analysis. Such wall type structures mimic biological surfaces, thus potentially providing good sites for focal adhesion. This ability to tune surface roughness could therefore allow for control of surface wettability and in turn cell attachment.

From the AFM analysis it can be concluded that progressive building of regular and pronounced wall type structures were obtained with the increasing number of EUV shots irradiated onto the polymer samples. It was observed that the average surface roughness increased from 45 nm to 184 nm from the unprocessed surface to the surface processed with 300 EUV pulses.

3.2. Chemical analysis

The PTFE samples were irradiated with EUV photons in the presence of nitrogen gas. Chemical modifications were investigated using X-ray photoelectron spectroscopy (XPS). PTFE is a fluorocarbon-based polymer [$(C_2F_4)_n$]. XPS scans were made for the detection of binding energies ranging from 0 eV to 700 eV. Therefore emitted electrons from carbon, nitrogen, oxygen and fluorine were easily detected. The results from pristine and EUV irradiated PTFE XPS scans are summarized in Table 2. The source used in this study produces EUV photons with maximum intensity around 10 ± 1 nm wavelength corresponding to photon energy of 112 eV. Therefore a single EUV photon induces breakdown of several bonds as it has energy way above the binding energies of C–C (3.6 eV), C–N (3.2 eV), C–O (3.7 eV) N–N (1.7 eV) and C–F (5.0 eV) bonds. It can be observed that defluorination in the specimen increases with increasing number of EUV shots. The breakings of C–F bonds, which causes defluorination result in carbon–carbon bonding and impact the carbon–fluorine bonding. The carbonization increased with increasing number of EUV shots. The EUV ablation not only modifies the content ratio of carbon–fluorine, but also incorporates nitrogen atoms into the upper layer surface of PTFE. It can be observed that PTFE specimen irradiated with 300 EUV shots incorporates nitrogen atoms [1.10 at.%]. The vacuum in the sample EUV interaction chamber was kept around 10^{2} mbar. The oxygen present in the environment also incorporates into the upper layer surface of sample during EUV treatment. The O/C ratio is 0:1 for pristine sample. For EUV irradiated samples, it is 0.018:1, 0.015:1 and 0.021:1 for 50, 200 and 300 EUV shots respectively. Therefore the oxygen contents (O/C ratio) in all the samples are too low to influence the polymer performance. In addition to the incorporation O and N, reconstruction or more appropriately reorganization of the structure of the polymer chain at the thin upper layer of the EUV treated PTFE sample observed. For the pristine sample we obtained maximum C1s bandwidth (–CF2–C′F2–CF2–) at about 292 eV and F1s (–CF2–CF2′–CF2–) at about 689 eV. The shape of the C1s and F1s band suggests that EUV radiation interaction with the polymer also strongly reduces the ratio of F/C on the polymer surface (from 3.2:1 for pristine sample to 2.2:1 for EUV irradiated sample). For this reason, C1s (Fig. 5a) and F1s (Fig. 5b) bands extend in the direction of the lower binding energy, and peaks shifted to the binding energy corresponding to –CF2–C′F–CF2– (289 eV), –C–C′F–C– (288 eV), –C–C′–C– (285 eV) in C1s band, and –C–CF′–C– (687 eV) in F1s band, can be distinguished. In fact, multiple peaks can be distinguished in the C1s and F1s bands associated with many different transformations of the polymer chain: detachment of fluorine, polymer crosslinking, polymer chain shortening, surface carbonization, C@C bond formation, and the formation of bonds between the C, O and N.

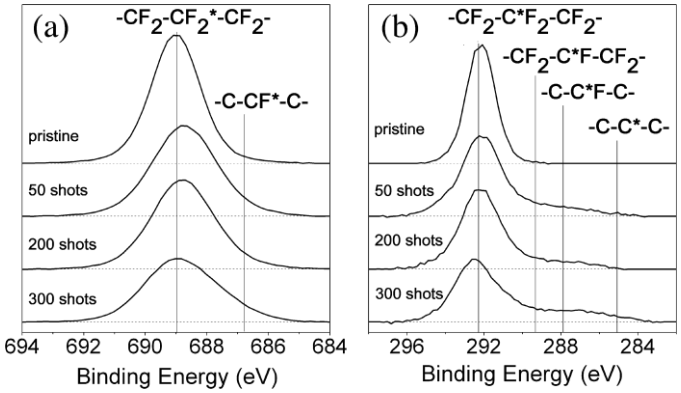

Fig. 5. XPS spectra of pristine and EUV modified PTFE samples (a) C1s band and (b) F1s band.

3.3. Wettability

The water contact angle measurements were obtained for pristine and EUV modified PTFE surfaces. As demonstrated by other groups using the ultraviolet surface modification technique, similar results have been obtained [38]. An increase in contact angle was observed with the increasing number of EUV shots. This increment in contact angle can be associated not only with surface roughness due to micro-texturing but also with incorporation of surface functional oxygen or nitrogen groups. About 11% change in contact angle of PTFE surface irradiated with 50 EUV shots was observed as the WCA was increased from 99 (pristine surface) to 110 . For PTFE surfaces irradiated with 200 EUV shots, the increment in WCA was about 16% and the measure WCA reached 115 . The PTFE surfaces irradiated with 300 EUV shots demonstrated a change of 21% in WCA by reaching the value of 120 (see Fig. 6). Therefore the increment of 21 for the EUV processed sample over the pristine sample was observed indicating that the polymer samples became more hydrophobic with reduced surface energy. The contact angle results indicated that the EUV surface could promote cell adhesion and viability. This was further investigated with the in-vitro cell culture experiments.

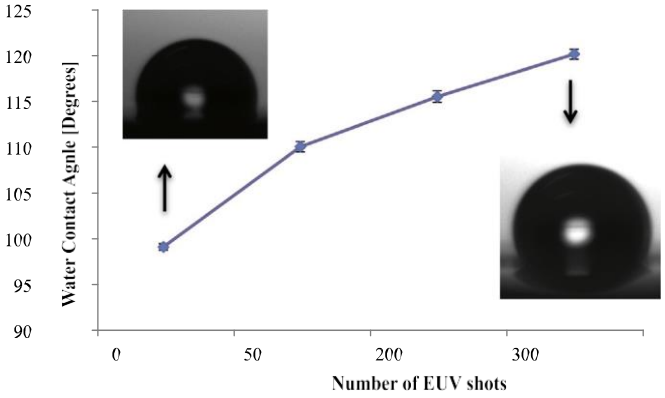

Fig. 6. Summarized water contact angle results for pristine and EUV modified PTFE samples.

3.4. Cell culture

L929 fibroblasts were used to investigate the behavior of cells cultured on pristine and EUV modified PTFE surfaces. The morphology of cells provide vital information about healthy status of a particular cell type. Fibroblastic cells are elongated and spindle-like in shape. The cells contacted with pure and EUV modified PTFE surfaces presented normal morphology (Fig. 7). It can be observed that comparing to pure PTFE surface, fibroblasts on the EUV modified PTFE samples were more elongated in shape (Fig. 7a–d).

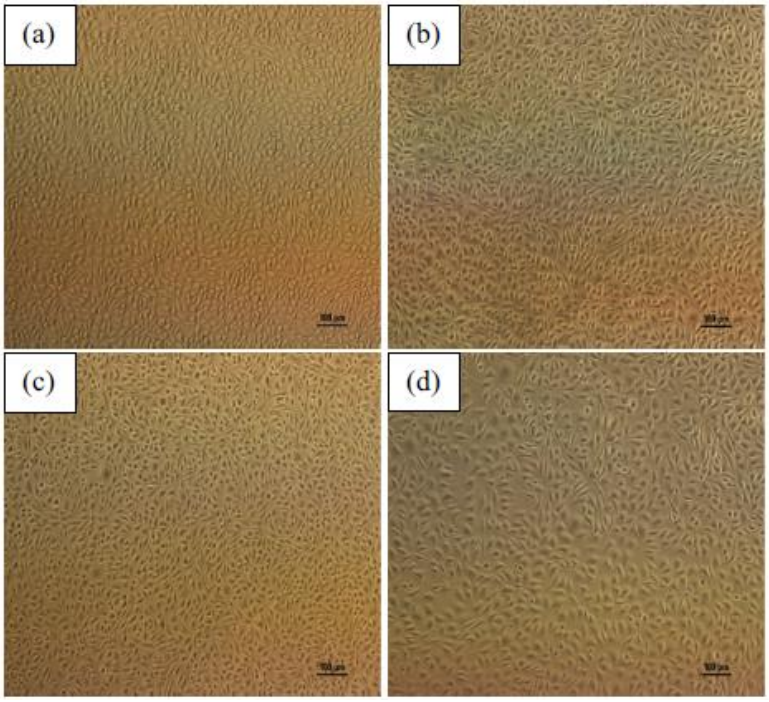

Fig. 7. PTFE cells morphology after 24 h of direct cell–material contact (a) pristine sample, (b) 50 EUV shots, (c) 200 EUV shots, and (d) 300 EUV shots.

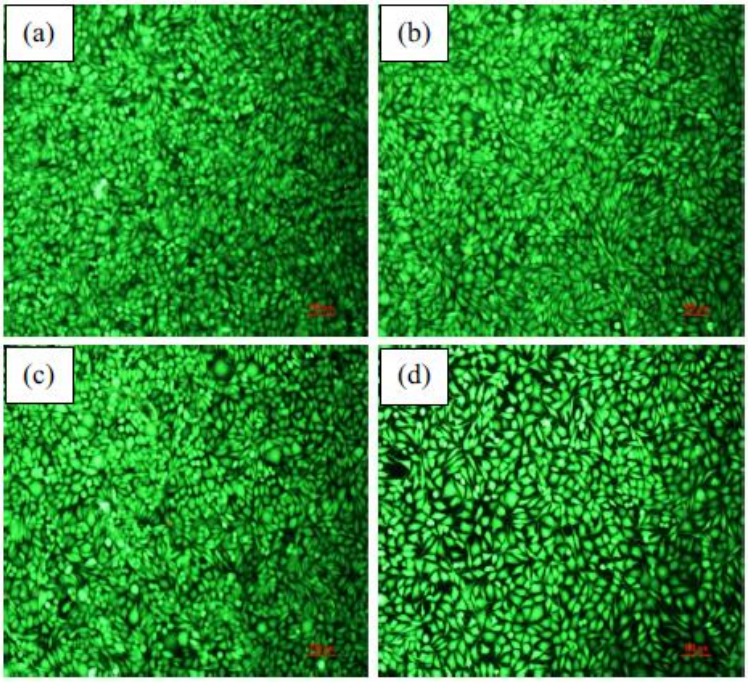

Fig. 8. Cells contacted with material (stained with calcein and iodium propidium) (a) pristine sample, (b) 50 EUV shots, (c) 200 EUV shots, and (d) 300 EUV shots.

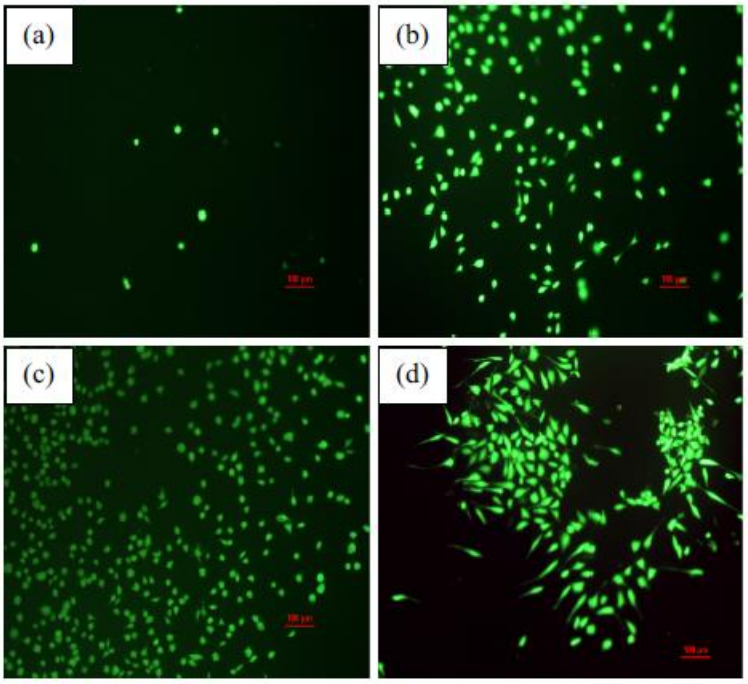

Fig. 9. Cells adhered to material (stained with calcein and iodium propidium) (a) pristine sample, (b) 50 EUV shots, (c) 200 EUV shots, and (d) 300 EUV shots.

To determine the cell viability, calcein and iodium propidium dyes were used to stain the live cells (green fluorescence) and dead cells (red fluorescence) (see Fig. 8). There were no red spots observed in all PTFE samples irrespective of EUV treatment. This simple test confirmed that fibroblasts were happy to stay on PTFE samples and exhibited high viability. Next, the effect of EUV treatment on cell adhesion of fibroblasts was investigated. It has been observed that the cells were able to discriminate smooth PTFE samples and EUV induced microstructures by strongly attached to polymer samples (Fig. 9). As observed from Fig. 9a, cells prefer not to attach to the pristine PTFE samples. As expected, the cells prefer to attach to micro-structured EUV modified PTFE samples. The number of cells adhered to PTFE samples increased with increasing number of EUV pulses irradiation. Moreover the cells exhibited elongated shapes along the EUV induced structures.

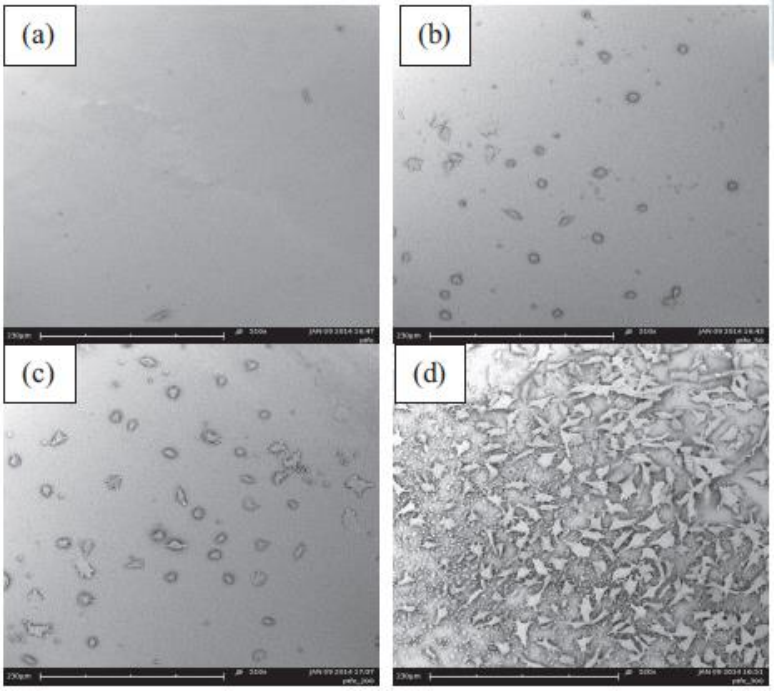

Fig. 10. SEM images of fibroblasts cells adhesion on PTFE films (a) pristine sample, (b) 50 EUV shots, (c) 200 EUV shots, and (d) 300 EUV shots.

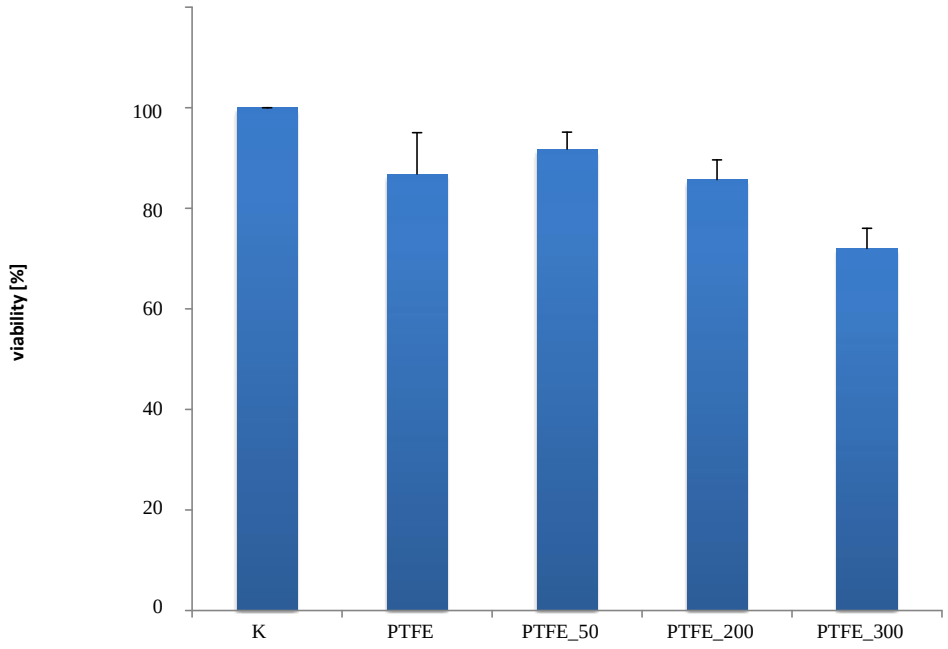

Fig. 11. Fibroblasts cell viability after 24 h on pure and EUV irradiated PTFE samples.

The cells preferentially attached to these micro-size channels or grating type structures patterned by the EUV. Additionally, the cell adhesion on the PTFE samples was investigated by Scanning Electron Microscopy (SEM). The cells were completely washed away from the pristine PTFE sample (Fig. 10a). A few cells were found on PTFE samples irradiated with 50 and 200 EUV shots (Fig. 10b and c). The cells demonstrated very strong adhesion on PTFE sample treated with 300 EUV shots (Fig. 10d).

Table 3
Effect of EUV treatment on physical and chemical properties of PTFE samples with associated cell culture observations.

EUV treatment	Surface roughness (nm)	Water contact angle (degree)	Chemical changes		Cells adhered to material	Adhesion
			O/C ratio	F/C ratio		
Pristine sample	45	99 ± 0.6	0:01	3.2:1	11	Low
50	137	110 ± 0.9	0.01:1	2.6:1	150	Medium
200	160	115 ± 1	0.01:1	2.6:1	377	Medium to high
300	184	120 ± 0.9	0.02:1	2.2:1	276	High

The MTT assay test was also performed to investigate if there were any cytotoxic effects of EUV surface modification on the fibroblasts. The cell viability of more than 70% in MTT assay test considered as non-cytotoxic result according to ISO 10993-5 stan dard. The average cell viability using MTT assay test for pure PTFE sample and EUV modified samples irradiated with 50, 200 and 300 EUV shots were 87%, 92%, 86% and 72% respectively (Fig. 11). Therefore the pure and EUV modified surfaces demonstrated high cell viability and no cytotoxic effects were observed.

The increased cell adhesion may associate with increased surface roughness and total surface area. The nanostructures could be the cause of increase amount of protein adsorption, which promotes cell attachment. Moreover the size of EUV induced patterns would be expected to influence cell adhesion. The results demonstrated that the cell adhesion was greater in the samples treated with highest EUV intensity (300 shots). Nano-sized structures of the order of dimension of the hydrodynamic radii of proteins (1–5 nm) would also be expected have an influence on cell attachment. In contrast to surface modification of PTFE by other groups, EUV surface modification increased the WCA of the samples [5,7,16,38]. However, another group also observed increased hydrophobicity of PTFE samples, as found in this study, on r.f. plasma modified PTFE [19]. The surfaces with nanometer grooves may provide contact guidance, hence the cell movement could be aligned by a certain direction provided by foreign material. Enhanced cell attachment and strong cell adhesion by PTFE surface modification has been reported by various groups [5,7,16,38]. However EUV surface modification is a single step technique that can be performed easily by a compact laboratory scale tool.

4. Conclusion

Extreme ultraviolet (EUV) induced microand nano-structuring of polymers for improved degree of biocompatibility is a new surface modification technique. The motivation to use EUV photons for polymer processing is that nanometer wavelength range can be used to produce nano-patterned micrometer sized grooves on polymer surfaces, which promote protein and cell adhesion. A basic requirement for a technique to be widely acceptable for the surface modification of materials is that the bulk properties should be retained during the treatment. The photochemical processes employed using plasma or UV photons are capable to penetrate deep inside the polymer surface up to 500 lm which ultimately degrade bulk material [47]. This problem is usually ignored, however recent advancements in the field of organic materials for various applications dramatically increases the requirement of

efficient surface modification technique with no un-desirable effects to bulk material. In the current study, the nanoand micro-structuring of polytetrafluoroethylene (PTFE) in order to control the degree of biocompatibility was successfully demonstrated by EUV surface modification. The effects of EUV surface treatment on physical properties and chemical composition of PTFE samples with associated effects on cell culture studies are summarized in Table 3. Successful grafting of nitrogen atoms, defluroination and carbonization were observed from the XPS scans. The average surface roughness of EUV treated polymer samples increased by up to four times as compared to that of the pristine sample. The higher surface roughness was found to correspond to an increased hydrophobicity of the polymer samples. Pronounced regular micro-channels on the EUV modified surfaces, which provide focal adhesion sites for the fibroblast cell type and hence promoted cell adhesion. Significant improvement in cell adhesion and good cell morphology were observed in EUV modified samples. The preliminary cell culture results strongly indicate the high potential present in EUV surface modification. Further detailed studies are required to exploit this technique in order to optimize the process, prove its efficacy for other cell types, and to provide a robust higher throughput EUV laser surface modification production technique.

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