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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.

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

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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 host-induced 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 micro- and nano-textured 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 X-ray 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].