Low-Temperature Air Plasma Modification of Electrospun Soft Materials and Bio-interfaces

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Abstract For several decades, plasma processing has been employed in the areas of food processing, manufacturing, and agriculture. Plasma processing has also been recognized as greatly beneficial in the field of tissue engineering for the modification of biomaterials. Polyethylene terephthalate (PET) has been employed as a vascular graft material but fails in small diameter applications. In this work, a multifaceted approach combining electrospinning to produce nano- and microscale fibers from PET blended with polybutylene terephthalate (PBT) added for flexibility and plasma modification for enhancing the surface chemistry is demonstrated to be an efficient approach to increase the biocompatibility as evidenced by enhanced fibroblast growth. The analysis of the surface chemistry shows an increase in oxygenated surface functionality, while the bulk analysis shows no significant changes. Thus, an efficient methodology for producing PET/PBT-based grafts that are easily modified with low-temperature plasma and show enhanced biocompatibility for vascular tissue engineering applications is reported.

Keywords Plasma modification · Biomaterials · Electrospinning · Fibroblasts · Vascular grafts

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Introduction

Cardiovascular disease (CVD) is implicated as one of the leading causes of death and the associated costs are near $330 billion dollars per annum [1]. For the case of arterial occlusion leading to vessel damage, the treatment options include either autografts or xenografts to replace the damaged vascular section. Due to various considerations such as size mismatch, autografts are not always the ideal solution; thus, synthetic vascular xenografts whether degradable to non-degradable must be used. Vascular grafts constructed from various non-degradable biomaterials such as expanded polytetrafluoroethylene (ePTFE) and polyethylene terephthalate (PET or Dacron) have been demonstrated for large diameter (>6 mm) applications for decades [2]. Despite success in the large diameter arena, these materials, among many others, have failed in small diameter (<4 mm) applications [2].

The trend in recent decades has been to incorporate a tissue engineering approach that combines the mechanical properties of synthetic materials and surface treatments to enhance endothelial cell adhesion and proliferation to form a confluent endothelial layer on the intimal surface which provides anti-thrombogenic properties to resist occlusion [3]. The electrospinning technique, known since 1934 employed only for niche applications for many years, experienced a large resurgence in the literature for the production for nano-fibrous polymeric constructs [4]. The importance of the fibrous structure was recognized and employed as a methodology to mimic native extracellular architecture, thus enhancing and promoting cell/cell interaction and cell/scaffold interaction [5–8]. However, the typical procedures of chemical surface treatments to functionalize the surfaces of these fibrous scaffolds can result in material property degradation and may also leave undesirable residues; therefore, a far superior surface modification technique is low-temperature plasma modification [8].

Plasmas are characterized as quasi-neutral, compressible fluids that exhibit collective behavior which spans a continuum of behavior from high-temperature equilibrium behavior to low-temperature non-equilibrium behavior that includes a mixture of ground state, excited, and ionized atoms/molecules in addition to free electrons and can initiate a variety of chemistries [8, 9]. Low-temperature plasmas (where the neutral species temperature is around room temperature or 300 K) are dominated by particle collisions since they are partially ionized [9]. Furthermore, the electron temperature is much higher (around 22,000 K or around 2 eV) and causes a variety of phenomena including atomic/molecular ionization, atomic/molecular radical formation, and photon formation [9]. All the foregoing processes result in a medium that is highly reactive to the surface of biomaterials, and yet remains benign enough to limit degradation. Although it is possible to over modify surfaces and eventually degrade biomaterials with long treatment times, much experimentation has shown the 1–10 min time frame as very suitable for improving the surface characteristics without noticeable degradation effects.

Cell/scaffold interaction is mediated by both surface chemistry and surface morphology [10]. Patterning of the chemical functional groups as well as patterning of the surface morphology at both the micro- and nanoscales has important effects on

the in vitro culturing of cells, particularly fibroblasts, which is useful for estimating the in vivo response; additionally, fibroblasts are a known component of the outer layers of vascular members [10, 11].

PET films have been extensively modified with plasma for decades with a variety of feed gases and conditions [12–14]. Furthermore, electrospun PET has been explored for tissue engineering but curiously the surface modification is typically undertaken with outdated wet chemistry techniques such as formaldehyde to yield R–OH chemical groups on the surfaces [15]. Even current approaches where other components such as fibroin are incorporated suffer from less than ideal surface chemistry or rely solely on grafting techniques; plasma modification offers a far more efficient and green solution for surface chemistry tuning than the current reported methods for vascular graft engineering [16–18].

Materials and Methods

For electrospinning, the working solutions of 80:20 PET/PBT (Scientific Polymer Products, Ontario, NY 14519) in 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) (Oakwood Chemical, Estill, SC 29918) at a concentration of 20% w/v were made by dissolving with strong stirring at RT for 3 d. Bubble- and residue-free aliquots were loaded into 3 mL syringes equipped with 25 G needles and electrospun using apparatus previous reported [8]. Briefly, working distances of 15–20 cm and lateral displacement of 200 cm at 40 mm/min rate with a total potential of 16–18 kV and 1.0 mL/h flow rates were used to spin onto a steel mandrel (4 mm diameter) precoated with a water-soluble layer to aid in removal of the scaffolds. Finished scaffolds were dried/degassed 48 h over $CaCl_2$ and under vacuum.

Plasma modification was accomplished by exposure to low-pressure (300–800 mTorr) RF plasma (13.56 MHz, 45 W) produced in a Harrick (Ithaca, NY 14850) PDC-001 Chamber equipped with dual analog flow meters (up to 50 SCCM) and manual control for the RF source. Feed gas comprised of normal room air.

Video contact angle measurements were accomplished via a custom-made video contact goniometer (f4 lens webcam, 720p) and Image J (US NIH) for analysis. 5 μL of 99.5% glycerol (Humeco Austin, TX 78701) was dropped via calibrated pipette in accordance with the sessile drop methodology [8]. Images were taken within 1 s of surface contact.

XPS analysis was accomplished via Phi 5000 Versaprobe (Phi Electronics, Chanhassen, WI 55317) equipped with an Mg Kα source at 300 W and BaO neutralizer. Step and pass energies were 0.5 eV and 187.5 eV, respectively, with averaging of multiple scans (4 for survey and 8 for short pass).

SEM analysis was conducted using a Quanta FEG 650 (FEI/Thermo Scientific, Hillsboro, Oregon 97124) operating at 20 kV. The samples were sputter coated with Au/Pd at 40 mA current for 45 s under argon prior to loading in the chamber.

FTIR measurements were taken with a Nicolet 4700 (Thermo Electron, Waltham, MA 02451) running OMNIC software and fitted with a Smart Orbit ATR. 400–4000 wavenumbers were scanned/averaged 64 times at 0.2 wavenumber resolution. Noise reduction and baseline correction were each run once for each compiled spectrum using the OMNIC software.

DSC measurements were taken with a Q100 DSC (TA Instruments, New Castle, DE 19720) with nitrogen purge at 50 mL/min and aluminum hermetic pans. TA Universal analysis was used to mark thermal features in the range of temperatures from –10 to 300 °C and standard mode ramp rate at 10 °C/min was used for all measurements.

For the biocompatibility study, IMR-90 fibroblasts were incubated 48 h at 37 °C in minimum essential media (Gibco, Thermo Fisher, Waltham, MA 02451) and trypsinized to detach from the flask and centrifuged for 5 min at RT and 1300 RPM to collect cells. The scaffolds were sterilized by 20% antibiotic solution at RT for 24 h and then rinsed 3× with PBS. Small 4-mm-diameter discs of PET/PBT scaffold were cut and seeded with 250,000 cell/disc and then rocked for 30 min at RT to distribute the cells. Seeded scaffolds were incubated at 37 °C for 48 h and then rinsed gently with PBS, stained with Hoechst nuclear stain and imaged via fluorescent microscope.

Results and Discussion

Electrospinning produces a highly porous construct that aims to mimic the extracellular environment (pores can range from approximately 100 nm^2 up to micron level). The plasma treatment process is extremely energetic with respect to the electron temperature [9]. Therefore, etching through ablation is well known to be one of the effects of plasma treatment; however, by limiting the time frame (1–10 min), this effect is minimized. As shown in Fig. 1, the fibers exhibit the same morphology both before and after plasma treatment, thus keeping their original as-spun configuration.

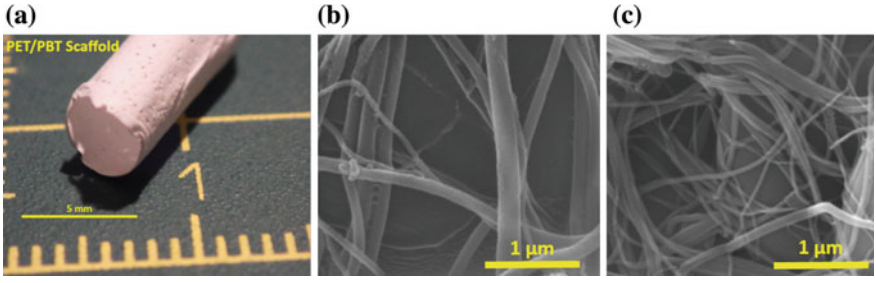

Fig. 1 The representative photograph of the electrospun tubular constructs **a** showing the macroscopic geometry. In SEM, the nanoscale fibers are clearly shown for the control **b** and plasma modified **c** with 5 min exposure. Scale bar in **a** is 5 mm and bars in **b** and **c** are 1 µm

Table 1 XPS analysis and contact angle measurements for various plasma treatment conditions

Plasma source	Gas/Feed rate/Pressure	Power/Time	C/O % (XPS integration)	Contact angle (degrees ± SD)
None (control)	n/a	n/a	69.9/30.9	92 ± 2.2
Harrick	Air/50 sccm/500 mTorr	45 W/1 min	63.1/36.9	64 ± 4.0
Harrick	Air/50 sccm/500 mTorr	45 W/5 min	60.8/39.2	48 ± 2.0

Exposure to plasma has a large effect on the surface chemistry of the surfaces subjected to bombardment by the energetic species [8, 9, 12, 13]. The excited species present in air plasmas such as hydroxyl radical, ozone, as well as free electrons can act to break surface bonds while adding alcohol, aldehyde, and carboxyl functional groups [8]. These groups act to increase the wettability, thus decreasing the contact angle, of the surface through the polar/polar interactions and hydrogen bonding that can occur in a media rich in hydroxyl groups (water, glycerol, cell media, extracellular matrix, etc.) These changes are evidenced by the increase in surface oxygen as determined by XPS (see Table 1). It is well known that the analysis by characteristic energies of photoelectrons is only from the first 10 nm of surface; deeper photoelectrons are absorbed by the substrate. The inference is that only the surface is modified and the deeper layers do not show the characteristic increase in oxygen-containing groups. This difference is further supported by the FTIR analysis.

FTIR analysis provides chemical information but at greater depths than is accessed via XPS (typically around 0.5–5.0 μm for ATR windows). The spectra show the characteristic absorption for aliphatic hydrocarbon (around 2900 wavenumber) and carbonyl from the ester linkages (around 1700 wavenumber) in addition to the myriad of absorption bands at wavenumber less than 1500 associated with other modes such as $–CH_2–$ bending (around 720 wavenumber). In comparison with the XPS data, the FTIR shows almost no change among the control or treated samples other than a slight change of baseline. There are no shifts of peak nor significant increase or decrease in the peak heights. From the comparison of data from XPS and FTIR, the overall inference is that the modification process produces dramatic changes on the surface while leaving the bulk of the substrate unmodified given the time constraints of less than 10 min plasma processing time. This result is consistent with both previous work and the literature regarding plasma modification of polymer films. If bulk changes are present, they are unable to be measured currently (Fig. 2).

DSC measurement yields a similar trend to the FTIR analysis, whereas the thermal properties of the scaffolds are not changed due to plasma treatment. This data shows that although there is presumably restructuring (due to new chemical functional groups) of the surface it does not have an impact on the overall thermal characteristics. This can be overcome with longer treatment times where local heating of the scaffolds can induce changes but this risks damaging or possibly fusing the fibers. The SEM images show undamaged fibers before and after treatment which is consistent with the DSC data that shows similar thermal properties among the treated and control scaffolds (Fig. 3).

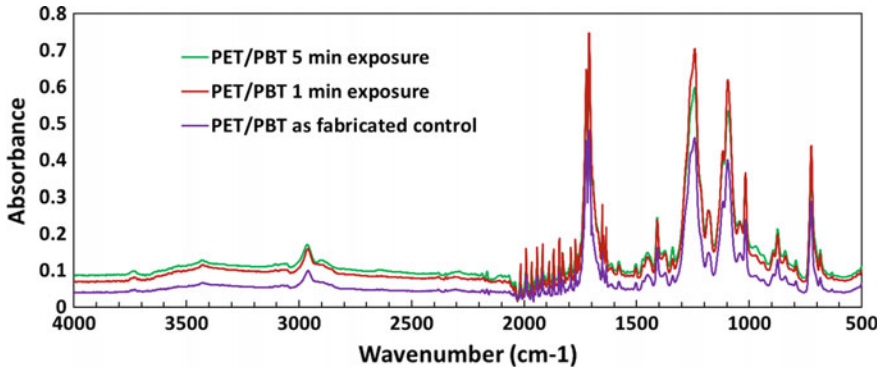

Fig. 2 FTIR spectra of control and plasma-treated scaffolds exposed for 1 and 5 min

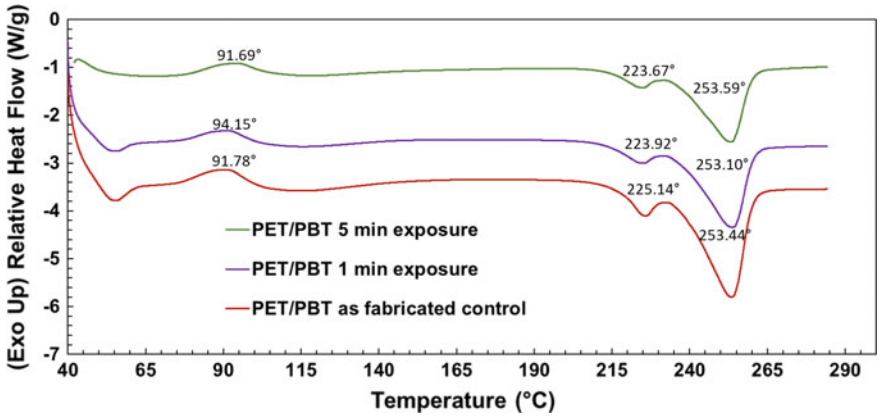

Fig. 3 Overlaid DSC curves of control and plasma-treated scaffolds. The curves are shifted for clarity

The fluorescent nuclei (after staining with Hoechst) are striking indicators of enhanced biocompatibility (Fig. 4). The control scaffolds, although not inherently toxic, do not offer a good interface with the aqueous media due to its hydrophobic nature as evidenced by its contact angle. Therefore, it is not surprising that the cells, although present, are in low density; they still grow but are not preferentially attracted to the fibrous construct. In contrast, the treated scaffolds, offering a more hydrophilic surface as evidenced by decreased contact angle, show enhanced fibroblast attachment and growth. The scaffold morphology is essentially the same as shown by SEM; the bulk properties are essentially the same as shown by FTIR and DSC. However, the surface chemistry is dramatically different as shown by XPS and contact angle, is a direct consequence of the plasma exposure, and is an inference to be responsible for the increased fibroblast proliferation that is observed.

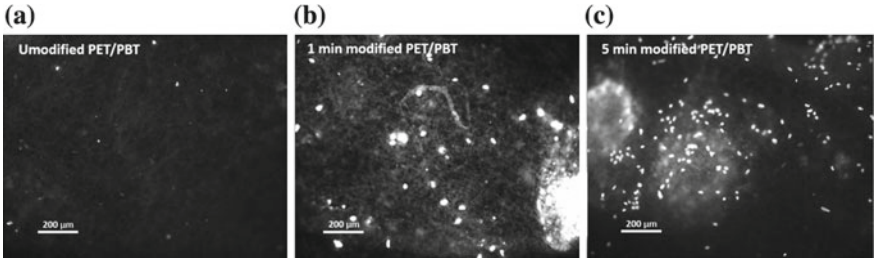

Fig. 4 Fluorescent microscope images of IMR-90 fibroblasts grown on control (**a**) and plasma-modified scaffolds for 1 min (**b**) and 5 min (**c**) treatment times. All scale bars are 200 μm

Conclusions

The implementation of PET in vascular graft engineering has been demonstrated but requires improvement for the small diameter arena. The use of plasma has been demonstrated as an effective surface modification tool. Using a time-restricted domain (1–10 min), air plasmas can create new hydrophilic, oxygen-rich surface functionality of electrospun PET/PBT fibers while leaving the morphological features and bulk chemical/thermal properties essentially unchanged. This treatment has an effect of enhancing fibroblast growth and the evidence supports the conclusion that air plasma modification enhances biocompatibility. This work represents a practical method toward the development of small diameter vascular xenografts constructed from electrospun PET/PBT fibers.

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