NRC Publications Archive Archives des publications du CNRC

Effect of sterilization on non-woven polyethylene terephthalate fiber structures for vascular grafts

Dimitrievska, Sashka; Petit, Alain; Doillon, Charles J.; Epure, Laura; Ajji, Abdellah; Yahia, L'Hocine; Bureau, Martin N.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1002/mabi.201000268>

Macromolecular Bioscience, 11, 1, pp. 13-21, 2011-01-01

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=0e43094e-0cae-4804-8411-2b63c0d930da>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=0e43094e-0cae-4804-8411-2b63c0d930da>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

DOI: ((insert here when preparing revised/final version))

Article Type: ((insert here))

Effect of Sterilization on Nonwoven Polyethylene Terephthalate Fiber Structures for Vascular Grafts

Sashka Dimitrievska^A, Alain Petit^B, Charles J. Doillon^C, Laura Epure^B, Abdellah Aji^D, L'Hocine Yahia^E and Martin N. Bureau^{A}*

A. Industrial Materials Institute – National Research Council Canada, 75 de Mortagne, Boucherville, J4B 6Y4, Canada
Fax: (((450) 641-5105)); E-mail: ((Martin.Bureau@cnrc-nrc.gc.ca))

B. Division of Orthopaedic Surgery, McGill University, Lady Davis Institute for Medical Research, 3755 Chemin de la Côte Ste-Catherine, Montreal, H3T 1E2, Canada

C. Oncology and Molecular Endocrinology Research Center, 2705 Boulevard Laurier, Quebec City, G1V 4G2, Canada;

D. Chemical Engineering Department, École Polytechnique de Montréal, 2500 Chemin de Polytechnique, Montréal, H3T 1J4, Canada

E. Laboratoire d'Innovation et d'Analyse de Biopformance (LIAB), École Polytechnique de Montréal, 2500 Chemin de Polytechnique, Montréal, H3T 1J4, Canada

Abstract

Nonwoven polyethylene terephthalate (PET) fibers produced via melt blowing and compounded into a 6-mm diameter 3D tubular scaffold were developed with artery matching mechanical properties. This work compares the effects of ethylene oxide (EtO) and low temperature plasma (LTP) sterilization on PET surface chemistry and biocompatibility. As seen through X-ray photoelectron spectroscopy (XPS) analysis LTP sterilization led to an increase in overall oxygen content and creation of new hydroxyl groups. EtO sterilization induced alkylation of the PET polymer. The *in vitro* cytotoxicity showed similar fibroblastic viability on LTP- and EtO-treated PET fibers. However, TNF- α release levels, indicative of macrophages activation, were significantly higher when macrophages were incubated on EtO-

treated PET fibers. Subcutaneous mice implantation revealed inflammatory response with foreign body reaction to PET grafts independent of the sterilization procedure.

Introduction

Current therapies to treat occluded vessels include surgical bypass and autologous grafting, or implantation of intravascular stents. Synthetic vascular prostheses, such as polyethylene terephthalate (PET), have performed well in large peripheral arteries under high-flow low-resistance conditions. However, no material has been demonstrated suitable for small-diameter (< 6 mm) applications because of significant problems associated with mechanical compliance mismatch and thrombogenicity. We previously developed an innovative nonwoven PET scaffold with mechanical properties similar to those of small arteries.^[1] Beyond the immediately targeted small diameter vascular grafts replacement, our nonwoven PET scaffold is also an attractive candidate in regenerative medicine and tissue engineering as its mechanical properties are custom tailorable to bone or vascular modulus, offering properties equivalent to the replacing tissue.^[1-3] Further, the 3D nonwoven PET scaffolds provide structural integrity similar to natural extracellular matrix (ECM) resulting in increased osteoblastic stem cell differentiation when seeded with human Mesenchymal Stem Cells (MSC).^[3] In other words, our novel nonwoven micro-fibers PET structure enhances the diffusion rates to and from the centre of the scaffold facilitating vascularisation, oxygen, nutrient supply, and waste removal through its interconnected pore network structure and has demonstrated suitability as a tissue regeneration scaffold and stem cells osteoblastic differentiation.^[1-3]

One of the essential preconditions for further clinical application is the ability of the nonwoven PET scaffold to withstand sterilization. Since literature presents contradictory results about sterilization effects on polymeric materials physico-chemical properties and the consequent biocompatibility response, we investigated the effect of low temperature non invasive: ethylene oxide (EtO) and low temperature plasma (LTP) sterilizations on the novel

nonwoven PET fibers in terms of surface chemistry and *in vitro* and *in vivo* biocompatibility.^[4]

EtO is the most commonly used low temperature sterilization technique due to its adequate bacterial effectiveness at low temperature, high penetration, and compatibility with a wide range of materials.^[5] Nonetheless, it produces toxic residues and can react with polymeric functional groups rendering an innocuous polymer toxic.^[5] The toxic effects of residual EtO have been extensively documented in humans, allowing the American National Standard ANSI/AAMI ST27-1998 to establish strict regulations concerning EtO sterilization (*e.g.*, < 25 ppm of residues for implantable devices).^[6] Despite this precaution, levels of EtO residues above the FDA/AAMI standards have been detected in polymeric biomaterials sterilized by EtO.^[7] To compensate for EtO shortcomings, the LTP sterilization technology was introduced in 1992 as a low-temperature sterilization alternative with faster turnaround times.^[8] However, relatively little is known about the influence of LTP sterilization on the physico-chemical properties of polymers and whether this affects the biocompatibility of polymeric devices.^[4] The handful of scientists that have examined LTP effects on polymers can be quickly summarized: 1) Tabrizan *et al.* have shown that LTP sterilization induced surface oxidation in different types of polymers tested;^[9] 2) Bathina *et al.* have discovered mechanical damage at an insulation-electrode interface tested;^[10] and 3) Lerouge *et al.* detected oxidation at the near surface layer of polyurethane as well as oligomer alteration.^[7]

LTP is also commonly used to functionalize polymeric surfaces for improved interaction with stem cells. Introducing OH-functionalized surfaces on CH₃ and COOH polymeric surfaces changes the conformation of adsorbed fibronectin leading to altered integrin binding, such that stem cell differentiation is significantly upregulated^[4]. The reactive oxygen species in the currently used LTP sterilization method may possibly introduce -C-OH functionalizations on the PET surface. There is considerable interest in using such simple chemistry to improve stem cell responses in favour of clinical applications.

Due to the primary importance of the sterilization process in the development of medical devices,^[11] the aim of the present study was to evaluate the effects on LTP and EtO sterilization on the nonwoven PET fibers surface chemistry and consequent *in vitro* and *in vivo* biocompatibility. Gamma-ray sterilization was not used in this study due to health-related concerns with the use of radiations in our laboratory.

Experimental Part

Nonwoven PET structures

The nonwoven fiber structures were produced from a neat PET grade (Dupont, Wilmington, DE) with an inherent viscosity of $1\text{cm}^3\text{g}^{-1}$ using a melt blowing process. Briefly, this process consisted of extruding PET through a multi-hole die grid of 230 holes of $300\text{ }\mu\text{m}$ in nominal diameter. Extruded strands of PET were blown by air at very high speed (close to the speed of sound) through a narrow gap sideways of the die, which allowed for fiber stretching at various levels, depending of the flow rate of the molten polymer. Resulting veils of nonwoven fibers were then used to fabricate the nonwoven structures by stacking several fiber veils onto a metallic plate as previously described.^[2] The plate was inserted into an autoclave with controlled temperature for consolidation for an optimum time [patent pending]. The mechanical properties of these structures were presented elsewhere.^[1] Their compliance, measured on tubular structures, was $8.4\pm 1.0\times 10^{-2}\text{ }\%\text{ mmHg}^{-1}$, very similar to those reported for a variety of human arteries ($\sim 8\times 10^{-2}\text{ }\%\text{ mmHg}^{-1}$) and about 10-fold higher than those obtained with commercial Dacron grafts or 1000-fold higher than those obtained with polytetrafluoroethylene (PTFE) grafts.^[1]

EtO and LTP sterilization treatments

Equal amount of samples (2 g) of PET scaffolds were cleaned by a 2-step 10 min cycle of ultrasonication involving 99.9% ethanol and 98.9% acetone. The samples were then wrapped

in plastic sterilization pouches and sterilized using one of the two different methods (EtO or LTP). EtO sterilization was carried out in SteriVac with a 4-h cycle at 54.4°C followed by 24 h of aeration to remove residual EtO. LTP sterilization was performed at room temperature using a 2.45-GHz surface-wave discharge operated at the 100-W power level in an in-house built apparatus extensively explained by Moisan *et al.* at a gas flow rate (one standard l/min of N₂ plus % of added χ O₂).^[12] Briefly, the plasma reactor based on an inductively-coupled plasma (ICP) consists of a cylindrical tube with top and bottom windows made of quartz glass. Below the bottom window, the ICP antenna is placed, which is powered at 13.56MHz. The gas flow is controlled by mass flow controllers and injected through the sidewalls of the reactor via a ring shower. Plasmas with powers of up to 500 W, gas pressure of 0.1 to 20 Pa, and gas flow from 1 to 50 sccm (standard cubic centimeters per minute) can be generated.^[12]

Chemical Characterization

X-ray photoelectron spectroscopy (XPS)

XPS measurements were acquired on an ESCALAB MKII spectrometer using non-monochromatised Al K α radiation ($h\nu = 1486.6$ eV) from a twin Mg/Al anode operating at 15 kV and 300 W. The operating system pressure during the scans was $\sim 8 \times 10^{-9}$ Torr over a sample area of 3×2 mm². For each sample, the survey spectra (0–1200 eV) were recorded at pass energy of 50 eV, and for the high-resolution scans of O_{1s} and C_{1s}, the spectra were recorded at pass energy of 20 eV. The resolution of the spectrometer was 1 eV for survey scans and 0.7 eV for high-resolution scans. All spectra have been corrected for sample charging, with the adventitious C_{1s} peak (284.7 eV) used as an internal reference.

Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize the presence of specific chemical groups in the PET materials (Perkin-Elmer, Paragon 1800) in the region

from 500 to 4000 cm^{-1} . During the measurement, the sample chamber was purged with nitrogen gas to reduce moisture content. Results are the average of 1024 scans at a resolution of 4 cm^{-1} .

Differential scanning calorimetry (DSC)

DSC measurements were carried out with a Perkin Elmer DSC-7 calorimeter under a nitrogen atmosphere. Samples were scanned from 20°C to 300°C with a heating rate of 10°C min^{-1} . The glass transition temperature (T_g) was taken as the mid-point of the step in heat flow. The melting temperature (T_m) and enthalpy (ΔH_m) were also determined using the Perkin Elmer DSC-7 calorimeter.

Effects of EtO and LTP sterilization on in vitro biocompatibility

Cell cultures

L929 fibroblast cell lines (ATCC, Rockville, MD) were used in this study. Cells were grown at 37°C in a 5% CO_2 humidified atmosphere in low-glucose Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin (Gibco Laboratories, Burlington, ON). The *in vitro* effect of EtO and LTP sterilizations on the biocompatibility (cell viability) of nonwoven PET structures was assessed in indirect and direct contact assays.

RAW 264.7 macrophages cell lines (ATCC, Rockville, MD) were also grown at 37°C in a 5% CO_2 humidified atmosphere in supplemented high-glucose DMEM. The effect of sterilization processes on the activation of macrophages (TNF- α release) was assessed in indirect and direct contact assays.

Indirect contact assay

Extracts were prepared from the EtO- and LTP-treated nonwoven PET structures in agreement with the ISO specification 10993-5.^[13] Briefly, the EtO- and LTP-sterilized nonwoven PET structures were independently immersed in supplemented DMEM at a ratio of 0.2 g/ml and incubated for 24 h at 37°C under constant agitation (250 rpm). Supplemented DMEM incubated for 24 h at 37°C without samples was used as negative control. The cytotoxicity of undiluted extracts was evaluated against L929 fibroblasts (1×10^5 cells/cm²) for up to 72 h while the effect of extracts on cellular activation (TNF- α release) was evaluated for up to 48 h in RAW 264.7 macrophages (2×10^5 cells/cm²).

Direct contact assay

The EtO- and LTP-sterilized nonwoven PET structures were soaked overnight in supplemented DMEM at 37°C in a 5% CO₂ humidified atmosphere prior to L929 fibroblast seeding (1×10^4 cells/cm²). Fibroblast viability was assessed for up to 14 days in culture. RAW 264.7 macrophages were also seeded (2×10^5 cells/cm²) on supplemented DMEM pre-soaked EtO- and LTP-sterilized scaffolds. TNF- α release was assessed up to 48 h in culture.

Evaluation of Cytotoxicity

The cytotoxicity of PET extracts was evaluated against L929 fibroblasts using the methyl tetrazolium (MTT) assay as described by the manufacturer (Sigma-Aldrich, Oakville, ON). Absorbance was measured at 550 nm on an ELISA microplate reader. The cellular proliferation of L929 fibroblasts in direct contact with the PET fibers was monitored using the Alamar BlueTM assay as specified by the manufacturer (Biosource, Nivelles, Belgium). Wells without cells were used as blanks and fibroblasts grown on Tissue Culture Plates supplemented with DMEM were used as a negative control. Absorbance was measured on an ELISA microplate reader at 570 nm and 600 nm.

Macrophages activation

RAW 264.7 macrophage activation was assessed by the release of TNF- α . The levels of TNF- α in the supernatants from both the indirect and the direct assays were evaluated with a TNF- α specific sandwich enzyme linked immunosorbent assays (ELISA) as described by the manufacturer (Biosource). Supplemented DMEM was used as a negative control while 10 μ g/ml of lipopolysaccharide (LPS, *E. coli*; Sigma-Aldrich) was used as a positive control. The optical density was then determined using a microplate reader set to 450 nm and corrected at 570 nm. The minimum detectable levels were 3 pg/ml.

Statistical analysis of in vitro biocompatibility assays

Results are the mean $\pm$ standard deviation of three (3) experiments performed in triplicate. Numerical data were analyzed statistically using Student's T tests and statistical significance was considered at $p < 0.05$.

Effects of EtO and LTP sterilization on *in vivo* biocompatibility

Implantation of the PET structures was performed in CD1 mice (Charles River, Montreal, QC) after approval by our local institutional Animal Care Committee and in agreement with the guidelines of the Canadian Council for Animal Care. Under anesthesia, the polymeric implants, 1 cm in diameter, were inserted in a subcutaneous pocket created in the thigh close to the inguinal fat pad. The skin incision was done laterally to the pocket and then sutured with polypropylene monofilament. For each condition, six implants were investigated. After 30 days, implants were retrieved with their nearby adherent tissues and fixed in formaldehyde to be processed for histological sections. Tissue sections were stained with hematoxylin, eosin, and safran (HES stain). This staining is routinely used to distinguish nuclei, cytoplasm, cell shape, permitting to identify cell types such as inflammatory cells, stromal cells and

endothelial cells as well as the extracellular matrix including blood and extravasation. Tissue reaction was evaluated by two Pathologists independent to the study.

Results

Chemical characterization of EtO and LTP induced surface modifications

EtO- and LTP-treated nonwoven PET structures were characterized by XPS and compared with the untreated nonwoven PET fibers XPS spectra with the O_{1s} spectra on the left hand side and the C_{1s} spectra on the right hand side of **Figure 1**. For a better comprehension, the XPS results are also presented quantitatively in **Table 1**.

Following the EtO treatment, the deconvolved C_{1s} and O_{1s} spectra indicated that the C_{1s} peak width remains unchanged but its overall height is lowered significantly, with the C-C aromatic peak (benzene ring) intensity lowered (Fig. 1B, Right). The ratio between the C-O and C=O components, separated by a fitting procedure, also increased after EtO treatment compared to the control indicating an intensity reduction of the carbonyl group. The O_{1s} spectra separated by a fitting procedure as well visually demonstrates the same carbonyl decrease, revealing a preferential location of the EtO-induced bond breaking (Fig. 1B, Left). In addition, following EtO treatment a new peak also appeared at 287 eV in the hydroxyl range (Fig. 1B, Left), which is quantitatively attributed to an increase C-O/C-OH bonds, as listed in Table 1. Although the values of Table 1 supports the idea of EtO as an oxidizing agent of the fiber surface, the shape of the C_{1s} peak indicates that the additional C-O/C-OH is resident EtO rather than reacted C-O. In addition the O_{1s} overall shape and fitting procedure support the same argument as there is no significant O_{1s} peak widening or new peak apparitions.

In comparison following the LTP treatment, the observations from the deconvolved C_{1s} and O_{1s} spectra were that the C_{1s} peak widens significantly and changes in appearance (Fig. 1C, Left). Similarly as with the EtO treatment, the quantitative analysis reveals a decrease in C-C

aromatic peak at the expense of an overall oxygen increase (Table 1). Although the quantitative analysis trend is similar for both treatments, the overall C_{1s} peak shape change indicates a formation of new bonds after LTP. Following a fitting procedure of the C_{1s} peak, a new bond at 287 eV was found (Fig 1C, Left), indicative of the emergence of a new chemical component: C-OH hydroxyl. The O_{1s} spectra separated by a fitting procedure as well visually demonstrates the appearance of hydroxyl at 536eV (Fig 1C, Right). The C-C decrease, at the expense of hydroxyl group increase, is significant of C-C bonds breakage under the plasma bombardment. The C-O/C=O bonds creation after LTP is 20% greater following the LPT treatment compared to after the EtO treatment, indicating a clear carbonyl group reduction into the hydroxyl group, and supporting the creation of an new C-OH bond (Table 1). The O/C ratio also indicates a 40% overall total O increase in the LTP-treated fibers, as compared to both reference and EtO-treated nonwoven PET fibers. The high oxygen ratio is also in part due to bond breaking sites (free radicals) reacting with the air oxygen and water further forming C-OH groups, increasing the overall oxygen content in the fibers surface. The different observations of C_{1s} peak shape change before and after EtO and LTP sterilizations are also clearly distinguishable in **Figure 2** superimposed C_{1s} peaks.

The purpose of FTIR usage in this study was to verify the degree of surface modification of EtO and LTP treatments as XPS has a low surface penetration ≤ 10 nm and FTIR has a penetration depth of ≤ 1 μ m, allowing a in depth characterization. Both LTP- and ETO-treated nonwoven PET fibers had an FTIR spectrum fully overlaying over the untreated nonwoven PET fibers (**Figure 3**). As both LTP an EtO treatments modify mainly the carbonyl and hydroxyl contents, attention was brought to O-containing chemical bonds in the carbonyl 1730-1695 cm⁻¹ and 1150-980 cm⁻¹ region. However, even under a larger scale, no significant modifications were seen through FTIR analysis on the nonwoven PET scaffolds following the two different sterilization treatments.

The LTP sterilization treatment did not affect significantly the thermal characteristics of the PET fibers (**Figure 4**). The pristine and LTP-treated fibers showed the same glass transition at 75°C, and almost superimposed recrystallization and melting peaks at respectively 130°C and 254°C (both peaks showed less than 2.5% difference in area). Similar to the latter, the EtO sterilization treatment did not affect significantly the recrystallization and melting peaks (same small difference in peak area of less than 2.5%). However, the glass transition of EtO-treated fibers is affected, with a far more marked and shifted glass transition near 85°C. This higher temperature shift in glass transition is attributed to the temperature at which the EtO treatment is performed (54.4°C), causing thermal relaxation of the polymer chains similar to an annealing treatment, therefore increasing glass transition to a temperature above the annealing temperature.

Effect of sterilization method on *in vitro* cell cultures biocompatibility

In terms of cellular growth, the EtO- and LTP-sterilized scaffolds had no toxic effect on cells in direct and indirect contact assays. The EtO- and LTP-treated nonwoven PET structures and their degradation products (indirect contact) had no significant effect on short term fibroblastic viability as the viability remained constant around 100% independent on the scaffold sterilization treatment (**Figure 5A**). However, the number of cells is decreased after 3 days of direct contact with EtO- and LTP-treated PET compared to control (**Figure 5B**). After 14 days, the different treatments had no effect on cell viability. The difference between EtO- and LTP-sterilized nonwoven PET scaffolds *in vitro* cellular response was seen on TNF- α release by macrophages (**Figure 6**). With both direct and indirect macrophage contact assays, a significant increase in TNF- α release following the EtO-sterilized fibers incubation and no significant change following the LTP-treated fibers incubation was seen. After 24 h, EtO-sterilized nonwoven PET structures extracts stimulated the release of 359 pg/ml TNF- α versus 62 pg/ml for LTP-sterilized nonwoven PET structures. At 48 h, the same trend of

significantly higher TNF- α release following EtO sterilization was observed. As in presence of the extracts, macrophages plated on EtO-sterilized nonwoven PET released 466 pg/ml of TNF- α after 24h as compared to 88 pg/ml for the LTP-sterilized PET0. As a positive control, LPS stimulated TNF- α release at least 20 times more than the negative control at all times.

Effect of sterilization method on *in vivo* biocompatibility after subcutaneous implantation

After retrieval, the EtO- and LTP-sterilized nonwoven PET implants had similar appearance without any obvious fibrotic capsule around the implants. However, histological sections revealed an inflammatory response in both EtO- and LTP-sterilized nonwoven PET implants (**Figure 7**). Inflammation was accompanied by a foreign body reaction composed of giant cells (fused macrophages) often directed to the nonwoven PET fibers. The degrees of foreign body reaction varied depending on the specimens. Blinded observation revealed no difference between the nonwoven PET implants sterilized by EtO or LTP after 30 days. In addition, some areas of the tissue response to implants, particularly in the EtO-treated implants, had areas of fibrotic tissues with elongated cells (*i.e.*, fibroblasts) and a fine extracellular matrix deposition. The distribution of the fibrotic reaction was randomly dispersed between the nonwoven PET fibers in areas in which the inflammatory response was limited. In the LTP-treated implants, fibrotic tissue was present at a less degree, and foci of isolated inflammatory cells were often seen in these implants.

Discussion

Our nonwoven PET scaffold with custom tailorable mechanical properties is an attractive candidate in regenerative medicine and tissue engineering. Its stem cell differentiation potential has been previously demonstrated with human MSC and its porosity optimized for

diffusion rates facilitating vascularisation, through its interconnected pore network structure suitable as a tissue regeneration scaffold and stem cells differentiation support.^[1-3]

For the further successful clinical implantation, the nonwoven PET scaffolds have to be efficiently sterilized before use. The contradictory results on sterilization effects of polymeric devices have motivated this sterilization effect study on our novel nonwoven PET vascular implants.^[7-10] An inadequate sterilizing method can cause morphological and chemical damage reducing the biological performance of our novel nonwoven PET vascular graft.^[7-10] Results of the present study suggest that the most important change is the surface oxidation, *i.e.* benzene (C-C) groups decrease in favor of C-OH/-COOH groups, after both sterilization methods.

Surface oxidation has been described in literature as an advantageous polymeric surface modification as it benefices surrounding cells by increasing the affinity between proteins and polymeric matrix and increases stem cell adhesion.^[14] In terms of the amount of oxidation, XPS qualitative results suggest that both methods were equally beneficial in terms of oxygenation increase. However, the visual C_{1s} and O_{1s} peak appearance and Gaussian fitting clearly show that oxygenation is only induced in the LTP-treated nonwoven PET fibers. The C_{1s} peak shape alteration indicates the formation of C-O bonds and breakage of C-C bonds after LTP sterilization, whereas the C_{1s} peak shape after EtO sterilization suggests surface alkylation artifact. As LTP mechanisms of action is based on a radical mechanism and EtO sterilization is based on an alkylating mechanism, the surface modification results were expected.^[15] More precisely, the reactive oxygen species in LTP sterilization primarily used for their bactericide efficacy offered the added C-OH functionalization benefit.^[7] Furthermore, peak fitting of the C_{1s} region LTP-treated nonwoven PET fibers, indicated the formation of highly oxygenated carbon suggesting bond breaking sites have at least partially reacted with air oxygen and water to form C-OH groups, further increasing the overall oxygen content in the fiber surface. This oxygenation of the fiber surface probably induced a change

in the interfacial properties of the fibers increasing their overall hydrophilicity, which as previously explained increases the cell adhesion.^[15] In regards to XPS spectra after EtO sterilization, the presence of alkylation artifacts is not surprising since alkylation is the known EtO mechanism of action. In the present case, alkylation by EtO is most likely of the carbonyl functional group (in agreement with lowering C=O-related peak on XPS C_{1s} spectra and O_{1s} spectra). However, residual EtO presence in the macromolecular network could also increase the C-O character in the XPS spectra.^[7]

As shown by FTIR, the EtO and LTP sterilizations induced surface modifications limited to the outermost 10 nm while DSC results showed that sterilization did not affect the crystallinity of the nonwoven PET fibers. The sterilization effects were limited to changes in the nonwoven PET fibers glass transition which is not pertinent to their overall biocompatibility.

In terms of *in vitro* toxicity, despite its strong alkylation, the EtO-sterilized nonwoven PET fibers did not show any cellular toxicity despite the known toxic effect of EtO residues.^[16] This suggests that EtO residues reacted with components of the media (proteins, amino acids) reducing EtO bioavailability.^[17] However, EtO-sterilized nonwoven PET fibers induced a higher *in vitro* TNF- α release than LTP-sterilized ones, which can be explained by EtO residues increasing the macrophages activation levels despite the innocuous effect on cellular toxicity. Indeed, solely a decreased TNF- α release *in vitro* was observed as a positive *in vitro* outcome from the beneficial increased hydroxyl content in the LTP-sterilized nonwoven PET fibers. The lack of *in vitro* macrophage stimulation by LTP-sterilized nonwoven PET fibers suggests only favorable macrophage reactions due to the higher hydrophilic character of the nonwoven PET fibers.

It was anticipated that *in vivo*, as previously shown,^[17-18] the surrounding cells will benefit of the higher surface oxygen content of the LTP-treated nonwoven PET fibers. However, this was not confirmed after 30 days of implantation in our mice subdermal model. In fact, newly

formed fibrotic tissue apparition was seen between the inflammatory foci after both treatments, which may facilitate wound tissue ingrowth. This observation also suggests that the induction of TNF- α release observed *in vitro* after EtO sterilization may occurred earlier during the implantation period than the time point of implant retrieval. This remains to be investigated. It can also be speculated that both treatments induced a slight chronic inflammatory reaction independently of a transient TNF- α release. Nevertheless, it is difficult to evaluate, at the moment, what is the cause of those inflammatory reactions. *In vivo* the subdued inflammatory reaction characterized *in vitro*, was not seen on our subdermal implants, and no clear advantage was seen from LTP-treated favorable surface modifications, *i.e.* hydrophilic surface due to added hydroxyl groups. Although the TNF- α release assay is an indirect method to predict inflammatory reaction, other factors are involved in the *in vivo* tissue reaction as seen in both implants. The physicochemical surface properties of PET may also attract and activate platelets and leukocytes which do not depend on TNF- α activation, but further trigger inflammatory cells. In addition, the observation of foreign body reactions was the result of unspecific response in which activated monocytes (macrophages) fused together into giant cells, unable to eliminate synthetic polymer implants. Moreover, it is unlikely that direct immune response with antigen presentation is implicated towards synthetic polymer.

Conclusion

Our nonwoven PET scaffold with custom tailorable mechanical properties is an attractive candidate in regenerative medicine and tissue engineering with observed stem cell differentiation potential. For their further clinical implantation the effect of LTP and EtO sterilizations on the nonwoven PET vascular grafts in terms of chemical modifications and *in vitro* and *in vivo* biocompatibility was evaluated. Results of surface modification suggest that

LTP approach is more suitable for the sterilization of nonwoven PET fibers. However this did not translate into a clear *in vivo* biocompatibility advantage when scaffolds were implanted in mice.

Acknowledgements: The authors gratefully acknowledge *Natural Sciences and Engineering Research Council of Canada (NSERC)* for their financial support, our regretted friend and colleague *Dr. Kenneth Cole* for his FTIR guidance, *Dominique Desgagnés* for her FTIR technical support, *Nicole Cote* for her DSC technical support, and *Dr. Johanne Denault* for her DSC guidance.

Keywords: Biocompatibility; Biological applications of polymers; Cold plasma; Functionalization of polymers; Polyethylene (PE)

References

- [1] M.J. Moreno MJ, A. Ajji, D. Mohebbi-Kalhari, M. Rukhlova, A. Hadjizadeh, M.N. Bureau, *J. Biomed. Mater. Res. Part B*. in press, DOI: JBMR-B-09-0495.
- [2] S. Dimitrievska, A. Petit, A. Ajji, M.N. Bureau, L. Yahia, *J. Biomed. Mater. Res. Part B*. **2008**, *1*, 44.
- [3] S. Dimitrievska, M. N. Bureau, J Antoniou, F Mwale, A Petit, R. S. Lima, B. R. Marple, , *J. Biomed. Mater. Res. Part A*. in press, DOI: JBMR-A-10-0109
- [4] R. Dorothee R, A. Lendlein, M.A. Schmidt, S. Kelch, A. Roehlke, R. Fuhrmann, R.P. Franke, *J. Biomed. Mater. Res. Part B*. **2003**, *67B*, 722.
- [5] P.R. Marreco, M.P. Luz, G.S. Candelária, A.M. Moraes, *J. Biomed. Mater. Res. Part A*. **2004**, *71B*, 268.

- [6] ST27-1988, A. A., Guideline for industrial ethylene oxide sterilization of medical devices. *New York: American National Standards Institute* **1988**.
- [7] S. Lerouge, G. Guignot, M. Tabrizian, D. Ferrier, N. Yagoubi, L. Yahia, *J. Biomed. Mater. Res.* **2000**, *52*, 774.
- [8] S. Hury, D.R. Vidal, F. Desor, J. Pelletier, *Lett. Appl. Microbiol.* **1998**, *26*, 417.
- [9] S. Lerouge, M. Tabrizian, M.R. Wertheimer, R. Marchand, L. Yahia, *J. Biomed. Mater. Res.* **2002**, *12*; 3.
- [10] M.N. Bathina, S. Mickelsen, C. Brooks, J. Jaramillo, T. Hepton, F.M. Kusumoto, *J. Am. Coll. Cardiol.* **1998**, *32*, 1384.
- [11] V. Woedtke, T. Schlosser, M. Urban, G. Hartmann, V. Julich, W.D. Abel, P.D. Wilhelm, *Biosens. Bioelectron.* **2003**, *19*, 269.
- [12] M. Moisan, J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian, L. Yahia, *Int. J. Pharm.* **2001**, *226*; 1.
- [13] ANSI/AAMI/ISO, 10993-5. Biological evaluation of medical devices, Part 5: Tests for in vitro cytotoxicity. **1999**.
- [14] M. Kazanci, D. Cohn, G. Marom, H. Ben-Bassat, *J. Mater. Sci.- Mater. Med.* **2002**, *13*, 465.
- [15] D.J. Wilson, N.P. Rhodes, R.L. Williams, *Biomaterials* **2003**, *24*, 5069.
- [16] A.D. Lucas, K. Merritt, V.M. Hitchins, T.O. Woods, S.G. McNamee, D.B. Lyle, S.A. Brown, *J. Biomed. Mater. Res. Part B.* **2003**, *66B*, 548.
- [17] G. Mayer, N. Blanchemain, C. Dupas-Bruzek, V. Miri, M. Traisnel, L. Gengembre, D. Derozier, H.F. Hildebrand, *Biomaterials* **2006**, *27*, 553.
- [18] S. Dimitrievska, M. Moreno, E. Pinney, R. Gendron, M.N. Bureau, Society for Biomaterials. Seattle, Washington, April 21, 2010, STAR award.

Figure 1. XPS C_{1s} signal spectra (left) and O_{1s} signal spectra (right) of a) pristine, b) EtO- and c) LTP-treated nonwoven PET structures.

a) Pristine PET

b) EtO-treated PET

c) LTP-treated PET

Figure 2. Superimposed XPS carbon signal spectra (C_{1s}) of nonwoven PET structures before and after EtO and LTP sterilizations.

Figure 3. FTIR signal spectra of nonwoven PET structures for non-sterilized PET (Pristine), and for EtO-treated and LTP-treated PET.

Figure 4. DSC signal spectra of nonwoven PET structures: PET (pristine) and after EtO (EtO-treated PET) and LTP (LTP-treated PET) sterilizations.

Figure 5. Effect of EtO and LTP sterilizations of nonwoven PET structures on the viability of L929 fibroblasts. The indirect contact effect (A) of EtO and LTP sterilizations was determined through the MTT assay while the direct contact effect (B) was determined through the Alamar Blue assay. Results are the mean $\pm$ standard deviation of 3 experiments performed in triplicate.

A

B

Figure 6. Effect of EtO and LTP sterilizations of nonwoven PET on the release of TNF- α by RAW 264.7 macrophages. The release of TNF- α in the indirect (A) and the direct (B) contact assays was determined by ELISA. Results are the mean $\pm$ standard deviation of 3 experiments performed in triplicate.

Figure 7. HES stain of histological sections of subcutaneous implantation of PET fibers in mice. Sterilization by EtO (A and C) and LPT (B and D) resulted in an inflammatory response with a foreign body reaction (giant cells; arrowheads), often associated to PET fibers (white or birefringent areas). Some fibrotic reactions (asterisks) and microvessels (arrows) were present, particularly in the EtO-treated polymer. Bars = 200 μ m.

Table 1. XPS evaluation of elementary composition of nonwoven PET structures before sterilization and after EtO and LTP sterilizations.

Treatment	C	O	N	C-O/C=O	O/C
	%	%	%		
Non treated PET	70.3	28.5	0.0	0.8	0.4
EtO	68.8	29.5	0.0	1.3	0.4
LTP	58.1	38.8	1.0	1.5	0.7