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Functionalization of nanochannels by radio-induced grafting polymerization on PET track-etched membranes

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HIGHLIGHTS

- Irradiated PET foils with swift-heavy ions were etched and grafted in a step-by-step process.
- Grafting polymerization was performed on the remaining active sites after etching.
- Track-etched PET membranes were fluorescently labeled by chemical functionalization.
- Functionalized track-etched PET membranes were analyzed by fluorescence and confocal microscopy.

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ABSTRACT

The application of swift-heavy ion bombardment to polymers is a well-established technique to manufacture micro- and nanopores onto polymeric films to obtain porous membranes. A few years ago, it was realized that, during ion bombardment, the high energy deposition along the ion path through the polymer reached cylindrical damage regions corresponding to the core trace and the penumbra. After the etching procedure, there are still enough active sites left in the penumbra that can be used to initiate a polymerization process selectively inside the membrane pores.

In this study, we report the grafting polymerization of glycidyl methacrylate onto etched PET foils to obtain functionalized nanochannels. Grafted polymers were labeled with a fluorescent tag and analyzed by different fluorescence techniques such as direct fluorescence, fluorescence microscopy and confocal microscopy. These techniques allowed identifying and quantifying the grafted regions on the polymeric foils.

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1. Introduction

In the last decade, research in the nanotechnology field has emerged strongly. Radiation research has studied its fundamentals in the atomic and subatomic range since its beginning. In this way, the application of swift-heavy ion bombardment to polymers is a well-established technique to manufacture micro- and nanopores onto polymeric films to obtain porous membranes (Apel et al., 1998).

Membrane technology has had a high impact on the chemical and biotechnology industries in the last 30 years. Its main features and advantages come from the low energy consumption and ability to develop a sustainable process. Today, the most important

industrial market segments are 'medical devices' and 'water treatment' (Ulbricht, 2006). However, novel applications involving adsorptive membranes, catalysis or sensor systems are emerging (Ulbricht, 2006; Vlasiouka et al., 2009; Ventura et al., 2008).

Track-etched membranes are a special kind of membranes where pores are built 'one by one' by swift-heavy ion bombardment onto polymeric films. Latent tracks made by particle bombardment are subsequently etched with specific chemical reagents according to the chemical structure of the trunk polymer. These membranes have specific applications in the biological field as a consequence of their unique properties of very narrow pore size distribution and controlled pore number per area. Track-etched membranes are current commercial products useful for cell separation, air monitoring, cell biology, and liposome preparation, among others (Apel, 2001; www.oxyphen.com).

Novel applications to nanotechnology such as preparation of nanowires, nanofilters and sensors for special usage have been recently reviewed (Waheed et al., 2009). Furthermore, single-ion

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nanochannel and nanowire fabrication is currently possible (Spohr et al., 2010).

The basic requirements to develop functional materials are the acquisition of nanopores of different shapes and the tailor-made control of the chemical functionalization of the internal wall of the nanochannels. The pioneering work of Martin describes nanopore functionalization through thiol-gold chemistry by using electroless gold deposition (Martin et al., 2001). Later on, the straightforward polymer functionalization approach by wet chemistry was also applied. The internal walls of track-etched membranes are shielded with carboxylic acid groups which can be selectively chemically modified (Alem et al., 2008; Friebe and Ulbricht, 2007; Rerat et al., 2010; Smuleac et al., 2004). However, specific chemical reagents are required leading to by-products presence which limits the applications relative to medicine. In addition, protocols include several step reactions.

A few years ago, it was realized that, during ion bombardment, a high energy deposition along the ion path through the polymer reached not only a cylindrical damage region corresponding to the core trace, but also a penumbra region corresponding to the damage caused by the secondary electrons (delta electrons) coming from ion–electron interaction. The extension of this damage zone can be estimated through theoretical calculations (Waligorski et al., 1986) and in general its diameter is one and two orders of magnitude longer than the core trace diameter.

The chemical etching process can remove material from the core and penumbra region by hydrolyzing the polymer chains, reaching pore diameters in the range of nano- to micrometer scale on the material. In most cases, after the etching procedure on the irradiated polymer, there are still enough active sites left that can be used to initiate a polymerization process selectively inside the pore. This process has been demonstrated for acrylic acid polymerization onto track-etched polypropylene (PP) and polyvinylidene fluoride (PVDF) membranes (Mazzei et al., 2005, 2006; Cuscito et al., 2007). It allows to graft locally in the tracks and not on the surface in a one-step reaction without using any hazardous chemicals.

To our knowledge, no grafting onto the remaining active sites has been reported for polyethylene terephthalate (PET) membranes. In addition, the classical gravimetric method and infrared analysis are at the limit of detection to study these systems and several experimental setup rearrangements, such as increasing the track-fluency occurrence and/or increasing the pore diameter, should be made to measure the modifications (Mazzei et al., 2005; Cuscito et al., 2007).

As mentioned by Apel a decade ago, track-etched membranes have unique optical properties which can be explored for novel studies and/or development of novel materials (Apel, 2001). Taking this property into account, highly sensitive fluorescence techniques were applied to follow and characterize grafted track-etched membranes.

Here, we report grafting polymerization on the remaining active sites on etched PET foils. We used the advantage of the chemically reactive monomer, glycidyl methacrylate (GMA), to label the grafted polymer with a fluorescent tag. This procedure was useful to follow the grafting process and to characterize the material by different fluorescence techniques.

2. Materials and methods

Glycidyl methacrylate (GMA) and cysteamine were from Sigma Chemical Co., USA. Fluorescein isothiocyanate (FITC) was from Merck. All reagents were used without further purification. All solvents were of analytical grade or higher, obtained from Anedra (Buenos Aires, Argentina) and used without further purification.

Swift heavy ion irradiations were performed at GANIL in collaboration to CIMAP and LSI, France. PET foils, 12 μm thick, were irradiated with $^{86}\text{Kr}^{31+}$ ions (8.66 MeV/amu, fluence of $7 \times 10^7 \text{ cm}^{-2}$). Foils were kept at room temperature.

The etching procedure was performed on $5 \times 5 \text{ cm}^2$ foils. Samples were previously washed with distilled water and ethanol and dried at 40 °C. UV sensitization was carried out by irradiation for 1 h on each side of the foils, using a UV lamp of 302 nm wavelength. Samples were held in a homemade frame for immersing in fresh prepared etching solution. The etching step was run in 2 N NaOH solution at 50 ± 1 °C. The grafting procedure was run immediately after etching. Samples were removed from the etching solution, dried with a tissue paper and soaked in 10% GMA monomer solution (ethanol/water, 1/1 v/v ratio) under a nitrogen atmosphere at 62 °C for different periods of time. After grafting, samples were washed in water and ethanol in alternate cycles and dried in an oven at 40 °C overnight.

The samples were chemically modified onto epoxide pendant groups of grafted polyGMA. Samples of $0.5 \times 1 \text{ cm}^2$ were soaked in buffer Tris 50 mM, pH 7.5 containing 10 mg/ml cysteamine. The reaction was developed at room temperature and constant stirring overnight. Samples were washed with water and buffer in alternate cycles and dried in an oven at 40 °C overnight. Fluorescently labeled samples were prepared by soaking in FITC 0.25 mg/ml solution in buffer carbonate 0.1 M, pH 9.0, and then washed and dried as previously.

Fluorescence was measured in a Nanodrop 3300 micro-volume full-spectrum fluorospectrometer (Thermo Scientific, USA). Relative fluorescent units (RFU) for fluorescein were acquired at 508 nm during sample excitation with a blue LED. The fluorescence signal corresponds to a cylindrical section of 0.1256 mm^2 area, according to the optical fiber diameter. The amount of grafting per area was calculated according to this area of analysis.

Pore diameters of grafted and etched membranes were determined by statistical analysis of FESEM pictures performed by Zeiss Supra 40 (Carl Zeiss NTS, LLC).

2.1. Confocal microscopy

Fluorescence and confocal pictures were captured with a fluorescence microscope Nikon Eclipse TE2000 (60.0x/1.40/0.13, numerical aperture 1.4, pinhole 33.3 μm). Small sample sections of labeled membranes were placed on a microscope slide, added with a few drops of water, and covered with a coverglass. The membrane was always handled using surgical gloves and tweezers.

3. Results and discussion

Track-etched membranes were made by an etching process onto latent tracks coming from swift-heavy ion bombardment of polymeric foils. A few years ago, researchers envisioned that residual radicals, remaining from the delta electrons-polymer interaction, could be still available after the etching process and those they could be used as initiators of a grafting polymerization process (Betz, 1995). Therefore, in a further step after etching, pore wall modification can be directly achieved by polymerization grafting (Mazzei et al., 2006). Fig. 1 shows a scheme of the nanopore functionalization steps, including the chemical derivatization and the fluorescent labeling. The epoxy groups from the grafted polyGMA were chemically modified with cysteamine and finally labeled with fluorescein. The fluorescence of labeled membranes was measured directly onto the foils by Nanodrop® 3300 (fluorometer) and quantified as RFU, in a membrane section of 0.1256 mm^2 area.

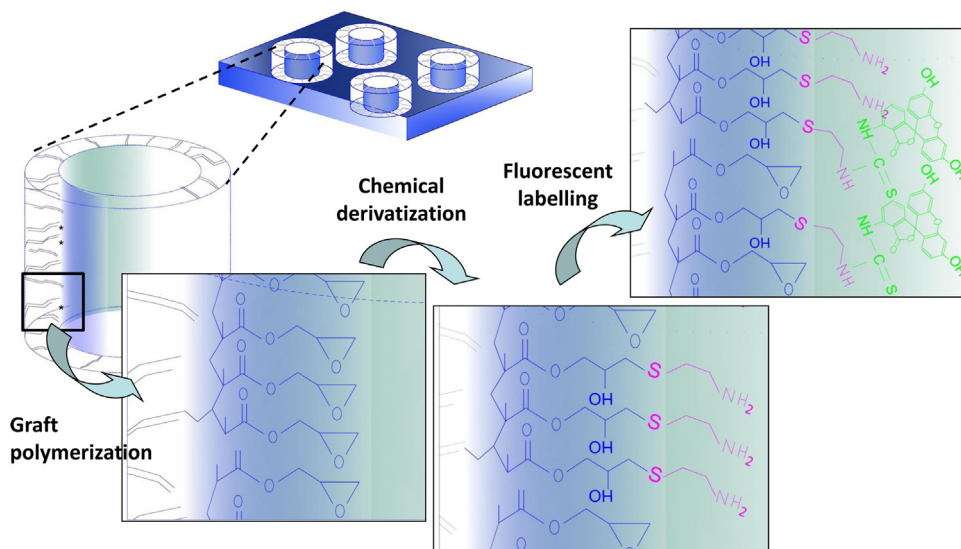


Fig. 1. Scheme of grafting, chemical derivatization and labeling of nanochannels built in track-etched PET foils.

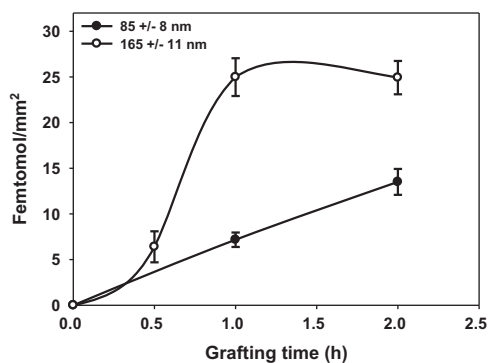


Fig. 2. Grafting amounts for two different PET track-etched membranes versus grafting time at 62 °C and 10% GMA in water/ethanol mixture (1:1). The grafting was calculated from the fluorescence signal at 508 nm corresponding to fluorescein emission.

As mentioned above, this technique has been previously used to modify etched PVDF and PP irradiated with swift-heavy ions. In the case of the former, free-radicals are available in a minute-scale lifetime. Thus, membranes should be grafted immediately after etching (Cuscito et al., 2007). In the case of irradiated PP, it is believed that peroxide or hydroperoxide functional groups are responsible for the grafting process (Mazzei et al., 2006). In the case of irradiated PET, the situation could be similar to the latter. However, according to EPR and Raman spectral information, it has been described that there are free radicals coming from graphite-like structures and/or delocalized π -electrons of the carbon clusters of the damaged regions (Vilensky et al., 2007). Therefore, in this study, we chose a grafting temperature of 62 °C to cover both possibilities, by free radicals or peroxide activation. Irradiated PET foils were etched in an alkaline solution and immediately grafted by soaking them in a degassed 10% GMA solution. The chemical modification protocol and labeling were previously optimized by using polyethylene foils surface-grafted with polyGMA and applied to freshly grafted PET membranes. The optimum chemical reaction condition is described in Section 2.

The kinetics of the grafting process is plotted in Fig. 2 for membranes with two different pore diameters. The amount of grafted polymer was measured directly by fluorescence detection. Calibration curves were previously run using standard solutions of fluorescein. The increases in fluorescence with the grafting time

can be clearly detected in grafted membranes of the same fluence respect to the etched ones for two different pore sizes (165 nm and 85 nm pore diameter). Membranes with 165 nm pore-diameter had higher amounts of grafted polymer and reached this size in a shorter time. The grafting kinetics for this membrane had a similar evolution as that of the GMA-grafted polyethylene membranes prepared by radiation-induced graft polymerization (Grasselli et al., 2003). In the case of membrane with 85 nm pore diameter, it showed a grafting kinetics compatible with a diffusion controlled process.

To confirm that the grafting is localized in track regions, labeled membranes were visualized by fluorescence microscopy. The negative control corresponds to labeled non-grafted membranes. In order to reduce the non-specific adsorption, a proper washing procedure was applied on the labeled samples. The green dots in Fig. 3 correspond to the grafted pores in the clear field and dark field microscopy images. According to FESEM measurements this membrane has pore of 240 nm diameter.

The confocal microscopy images, with a z-axis resolution of 0.8 μm , were analyzed to demonstrate that pore modification is achieved also through the nanochannel path. Fig. 4 shows a confocal microscopy image of a labeled membrane in the x–y plane and the z axis reconstruction in the right and bottom sides of the picture. The z reconstruction shows fluorescent lines corresponding to the modified nanochannels. It can be noticed that fluorescence intensity decreases along the z-axis. This is an artifact coming from fluorescein photobleaching as a consequence of the high laser intensity during data acquisition. From the statistical analysis of the images of grafted and etched membranes, we found 100% of labeled pores. Hence, this grafting procedure is highly robust to obtain functional membranes.

4. Conclusions

Here, we showed the application of a fluorescent labeling technique to follow and characterize the grafting process into nanochannels. Irradiated PET foils with swift-heavy ions were etched and grafted in a step-by-step process with GMA monomers. Derivatization and labeling procedures were achieved under mild conditions with a high selectivity and homogeneity according to fluorescence microscopy analysis. Direct fluorescent measurement

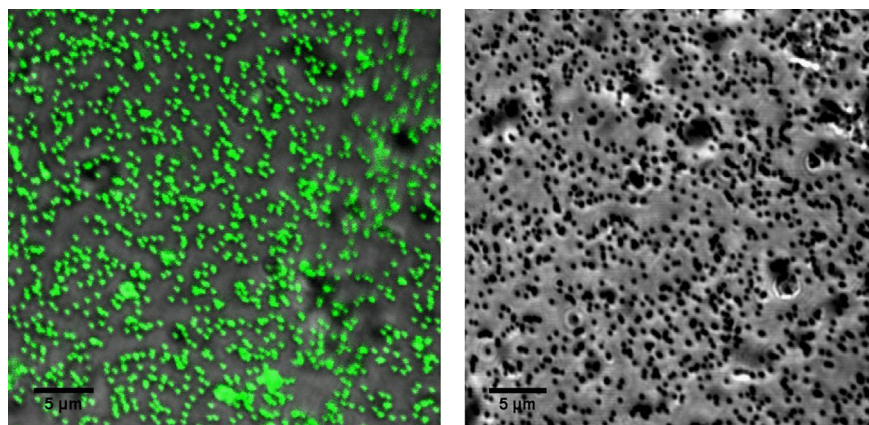


Fig. 3. Fluorescence microscopy images in clear field corresponding to labeled grafted-etched membrane (left) and etched membrane (right). Scale bar corresponds to 5 μm .

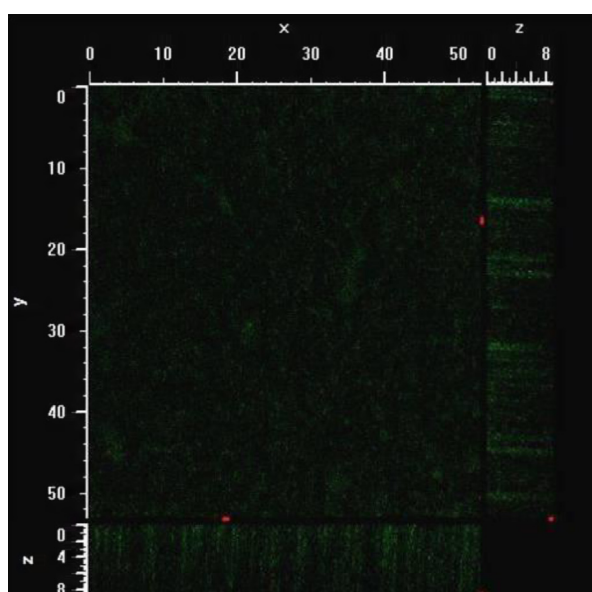


Fig. 4. Confocal microscopy image corresponding to a fluorescein-labeled grafted-etched membrane. Right side and bottom correspond to the fluorescence signal on the z axis. Scale rule corresponds to microns.

allowed us to quantify the grafted polymer onto the nanochannels with a very low detection limit.

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