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Design of Biologically Active Surfaces Based on Functionalized Polysulfones by Electrospinning †

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Abstract: The development of a new generation of electrospun polymeric materials designed to accomplish multi-performance in a bioinspired integrated system represents the main goal of the present research. Polysulfones promise to become such nanosized fibrous structures with attractive properties associated with biomedical applications by the development of a new "construct". Therefore, the homogeneous solutions of the functionalized polysulfones with quaternary ammonium groups (PSFQ) dissolved in various solvents were processed by electrospinning to create polymeric scaffolds that can modulate cellular behavior. Additionally, the antibacterial activity of PSFQ fibers against Gram-negative and Gram-positive bacteria has indicated the potential of these scaffolds for biomedical use.

Keywords: quaternized polysulfones; electrospinning; nanofibrous scaffolds; biomedical applications

1. Introduction

The topic of nanofibers has attracted attention both in the scientific community and various industries because of their unique performances. Literature [1–3] indicates different techniques for the fabrication of nanomaterials, including drawing-processing, template-assisted synthesis, self-assembly, solvent casting, phase separation, and electrospinning techniques. With the increasing knowledge about nanotechnology, electrospinning has become the preferred technique over solvent casting and phase-separation because nanofibers achieved with electrospinning possess a high surface area to volume ratio and large number of inter-/intra fibrous pores [4]. Current trends in industrial research on such new materials have opened the way for advancements in diverse fields such as biomedicine, bioengineering, and environmental protection [5–8]. The notable applications include tissue engineering, biosensors, filtration, wound dressings, drug delivery, and enzyme immobilization [9]. In this direction, research awareness is rapidly growing and attracting greater attention to fabricate nanostructures from various types of raw materials, ranging from natural and synthetic polymers to composites, which can find applications in almost every field. The nanofibrous mats produced by this technique mimic the nanoscale properties of native extracellular matrix components much more closely compared to the conventional techniques. It is well-known that almost all tissues and organs, i.e., skin, cartilage, and bone, etc., show a kind of similarity to nanosized fibrous structures. In this context, polysulfones promise to developed of a new generation of nanofibrous scaffolds with attractive properties associated with biomedical applications, designed to achieve multi-performance in a bioinspired integrated system.

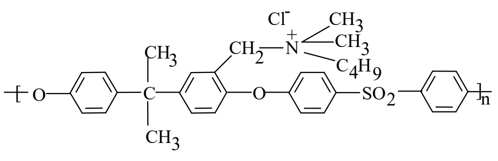
A variety of polysulfones (PSFs) have been molecularly designed [10–12] to enhance their performance, becoming biologically inert and nontoxic compounds exhibiting stable mechanical, morphological and adhesion characteristics during their exploitation. In this context, the cationic polyelectrolytes containing quaternary ammonium side groups (PSFQ) belong to this group of compounds which have many useful characteristics, such as hydrophilicity and high water permeability (of a special interest for biomedical applications) [13], biocompatibility and antibacterial activity [14,15], and better separation [16]. Based on the above-mentioned properties, it has been found that these polymers have numerous applications as biomaterials and semipermeable membranes in a variety of fields, including textile, food, cosmetics, chemical, and pharmaceutical industries.

Recently, the technology of high-performance materials fabrication has advanced significantly by the development of new materials with nanoscale properties. Thus, electrospun nanofibers play an important role in the biomedical field, this fact being determined by numerous articles that highlight their importance in the area of biomaterials. Additionally, the electrospun nanofibers scaffolds can be tailored in accordance with the purpose of their use. For example, such hybrid nanofibers scaffolds play an important role in providing a familiar environment to the cells, which ultimately results in their better attachment, proliferation, and differentiation [17,18]. Similarly, electrospun nanofiber scaffolds are also used as a drug delivery carrier for carrying drugs to their target sites [19,20]. Besides biomedical application, electrospun nanofiber has also found application in protection of the environment (both air and water) as an affinity membrane [21,22]. Electrospun nanofiber could also be used for producing high surface area chemical and biological nanosensors [23,24]. Furthermore, ultrafine electrospun nanofiber scaffolds have also been used for the preparation of nanotubes, which are of prime importance in various industries [25]. Consequently, the production of nanomaterials (nanofibers) via electrospinning has led to significant improvement of the design materials used and, implicitly, of achieved nanomaterials. In this context, the solutions of the functionalized polysulfone, with a tunable density of quaternary ammonium functional groups, were processed by electrospinning to create new materials with high-performance characteristics—biologically active surfaces—designed to be integrated in a bioinspired system in medical therapy. Therefore, the present study includes two challenges, namely the processing of quaternized polysulfones solutions by electrospinning to obtain fiber scaffolds, and extending the possible applications of these materials in the biomedical field. Thus, we examine the effects of solutions, processing parameters and solvent properties on fiber morphology and the impact of this efficient technology in the biomedical field.

2. Experimental

2.1. Materials

Commercial aromatic polysulfone in powder form (UDEL-3500—PSF, Union Carbide Company, Houston, TX, USA) (Mn = 39,000 g/mol; Mw/Mn = 1.625) was used in the synthesis of functionalized polysulfones [10–12]. Thus, the cationic polysulfone containing quaternary ammonium side groups (PSFQ, Mn = 28,000 g/mol) was synthesized by the reaction of chloromethylated polysulfone (CMPSF with a content in chlorine of 7.42% and Mn = 29,000 g/mol) with a tertiary amine, *N*,*N*-dimethylbutylamine (DMBA) [12,26]. The quaternary polymer was isolated from the reaction medium by precipitation in diethylether, washed three times with diethylether, and dried for 48 h under vacuum, at room temperature. The general chemical structure of the quaternized polysulfone is illustrated in Scheme 1.



Scheme 1. Chemical structure of the monomer units of the polysulfone with quaternary groups (PSFQ).

2.2. Processing of PSFQ Solutions by Electrospinning

The PSFQ homogeneous solutions for electrospinning were prepared by dissolving the polymer in *N*,*N*-dimethylformamide (DMF) and 2-Methoxyethanol (2-MOEt) at room temperature. The polymer solution's concentration of 40% was chosen to obtain fibers with sizes in the sub-micrometer range. Electrospinning was carried out at normal laboratory conditions. Around 3 mL PSFQ solution was electrospun under an applied voltage potential of 15–20 kV with a needle tip-collector distance of 15 cm and flow rate of the solution through the syringe of 0.75 mL/h. An aluminum rotary disc was used as collector for PSFQ fibers.

2.3. Scanning Electron Microscopy

The morphology of electrospun fibers was investigated using an environmental scanning electron microscope (ESEM), Quanta 200 operating at an accelerating voltage of 20 kV with secondary electrons in low vacuum mode.

2.4. Antibacterial Testes

The in vitro antibacterial activity of electrospun PSFQ assessed on two bacteria strains, by the diffusion method (Kirby-Bauer) certificated by the National Committee on Clinical Laboratory Standards (NCCLS), was evidenced by the occurrence of an inhibition zone. The Gram-positive *Staphylococcus aureus* ATCC 25,923 (*S. aureus*) and the Gram-negative bacteria *Escherichia coli* ATCC 10,536 (*E. coli*) were used as test microorganisms. The preincubation conditions of the test bacteria were 37 °C for 18 h.

Plates with Müeller-Hinton-Broth medium (pH 7.3 at 25 °C) were uniformly inoculated with the test microorganism using a sterile cotton swab. Circular polysulfones fibers 10 mm in diameter were applied on the surface of the medium. The plates were incubated at 37 °C for 24 h. The diameter of the inhibition zone (mm) depends both on the polymer present on the medium as well as by the microorganism susceptibility. Finally, the diameter of the inhibition clear zone was measured with a ruler.

3. Results and Discussion

3.1. Effects of Parameters on Electrospinning

The literature [27,28] mentions that there are several factors that affect the electrospinning process. These factors are classified as electrospinning parameters (the applied electric field, distance between the needle and collector, flow rate, and needle diameter), solution (the solvent, polymer concentration, viscosity, and solution conductivity) and environmental parameters (relativity humidity and temperature). All of these parameters directly influence the electrospinning process, affecting the generation of smooth and bead-free electrospun fibers. Therefore, to gain a better understanding of the electrospinning technique and fabrication of polymeric nanofibers, it is essential to thoroughly understand the effects of all of these governing parameters.

The electrospinning process begins when electric charges move into the polymer solution via the metallic needle. This causes instability within the polymer solution as a result of the induction of charges on the polymer droplet. At the same time, the reciprocal repulsion of charges produces a force that opposes the surface tension, and ultimately the polymer solution flows in the direction of the electric field (Figure 1). A further increase in the electric field causes the spherical droplet to deform and assume a conical shape. At this stage, ultrafine nanofibers emerge from the conical polymer droplet (Taylor cone), are collected on the metallic collector and kept at an optimized distance.

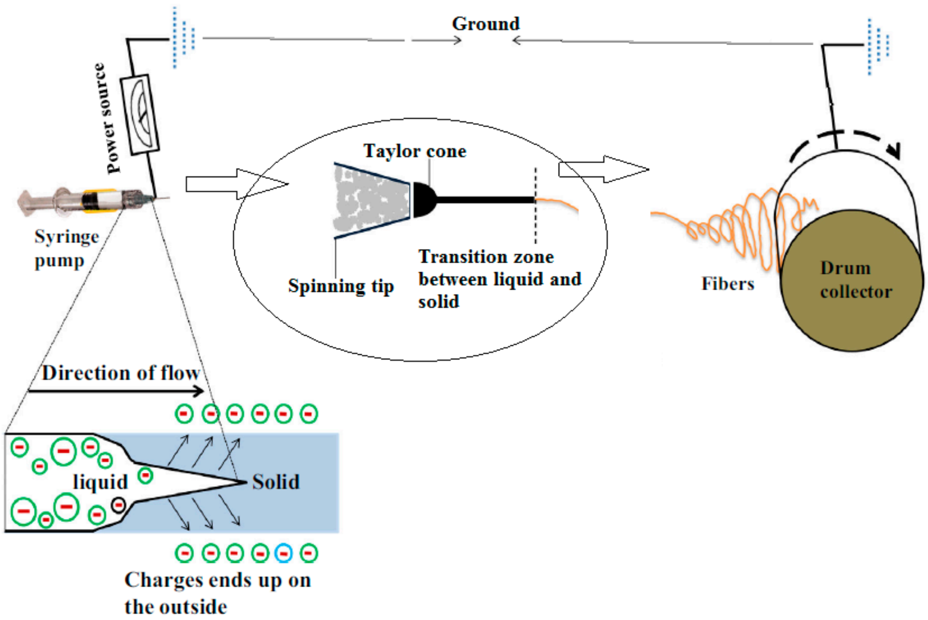


Figure 1. Schematic depicting electrospinning setup and phenomenon of electrospinning.

A stable charge jet can be formed only when the polymer solution has sufficient cohesive force. During the process, the internal and external charge forces cause the whipping of the liquid jet in the direction of the collector. This whipping motion allows the polymer chains within the solution to stretch and slide past each other, which results in the creation of fibers with diameters small enough to be called nanofibers [29,30].

Based on these remarks, in particular, the electrospinning of the PSFQ solutions was carried out and continuous cylindrical fibers were obtained in the experiments. The morphology of the PSFQ electrospun fibers was examined by SEM, with representative images shown in Figures 2 and 3.

Results suggested that long, uniform and continuous fibers can be obtained without any beads structure, and exhibit a smooth surface with variations in diameters. Fibers morphology, by different forms and dimensions, can be explained in terms of the different solution properties, (i.e., the boiling point of the solvents, the viscosity, and the surface tension) and intramolecular repulsive interactions between the ionized groups (i.e., ammonium groups). Thus, SEM images show that the morphology of fibers electrospun from PSFQ solution in DMF for a concentration of 40% (Figure 2a) with different forms and dimensions (average fibers diameters was 1.60 μm) can be explained in terms of the various solution properties, namely the boiling point of the solvents, the viscosity, and the surface tension. On the other hand, the fibers obtained from PSFQ solution of 40% in 2-MOEt have a wide distribution of diameters, ranging from 0.65 μm to 2.40 μm (Figure 2b). This fact shows that changes of the surface and characteristics of nanofibers (e.g., increases in the diameter) are attributed to the effect of the concentration/viscosity of polymer solutions, including the surface tension and conductivity, according to Fong et al. [28].

Therefore, usually two things need to be kept in mind before selecting the solvent; firstly, the preferred solvents for the electrospinning process have polymers that are completely soluble, and second, the solvent should have a moderate boiling point [31]. Thus, the effects of the solvent and/or solvents system in conjunction with the applied voltage affect the morphology of nanofibers (Figures 2 and 3). Moreover, the formation of smaller-diameter nanofibers is attributed to the stretching of the polymer solution in correlation with the charge repulsion within the polymer jet, following with an increase in the applied voltage [32].

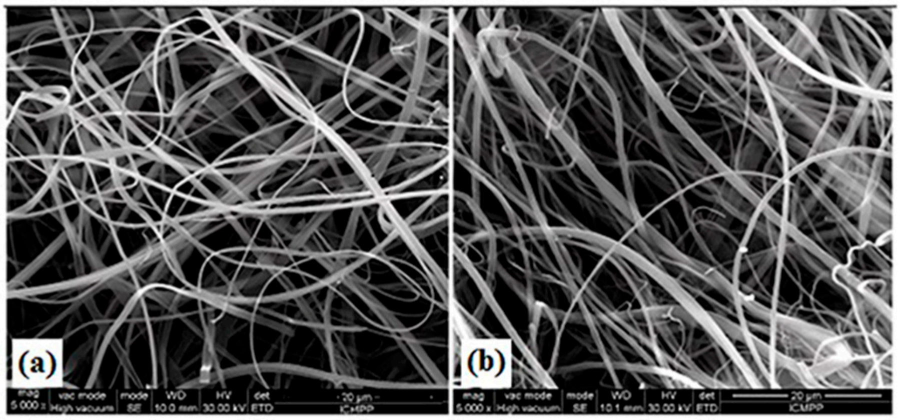


Figure 2. SEM images of PSFQ fibers obtained in: DMF (**a**) and 2-MOEt (**b**) at 40% polymer concentration for 50 μm scan area and magnification of 2000×.

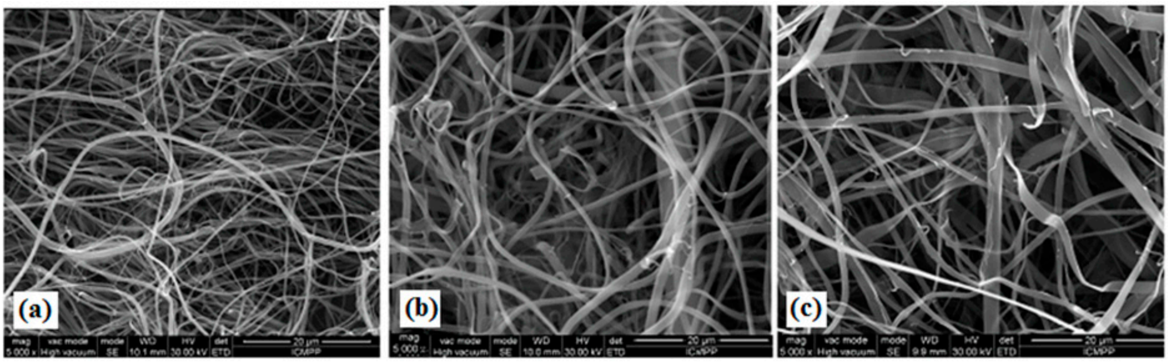


Figure 3. SEM images of PSFQ fibers obtained in various mixing ratios of solvents: 75DMF/25 2-MOEt (**a**), 50DMF/50 2-MOEt (**b**), and 25DMF/75 2-MOEt 2-MOEt (**c**) at 40% concentration for a 50 μm scan area and magnification of 2000×.

3.2. Antibacterial Activity

Growing resistance of microorganisms—disease-causing microbes—to drug therapy is an increasingly serious public health problem. For this reason, a great interest towards investigating bactericidal properties of polymeric materials has been renewed as an alternative. Therefore, there is an urgent need to develop new bactericides. In this sense, quaternary ammonium groups represent one of the most popular types of antimicrobial agents that affect structural organization and integrity of the cytoplasmic membrane [33]. Their biological activity depends on their structure and physicochemical properties, which affects the interaction with the cytoplasmic membrane of bacteria and influences their cell metabolism.

In particular, the antibacterial activity of PSFQ fibers obtained in DMF and 2-MOEt for 40% polymer concentration, was investigated against Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli* bacteria. Tests performed to assess the antibacterial activity of fibers electrospun against the two types of bacteria showed significant antibacterial activity (Figure 4). Results were assessed semi-quantitatively by the agar diffusion method against both bacteria, with a higher activity against *Staphylococcus aureus* as compared to *Escherichia coli*. This fact can be explained by taking into account the specific compositions of the cell wall of Gram negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria that induce different antimicrobial activity. Thus, from the literature [34], it is known that the component of Gram-positive bacteria cell walls (peptidoglycan) confers the hydrophobic character of *S. aureus*, while the major constituent of Gram-negative bacteria cell walls (peptidoglycan, together with other membranes, such as lipopolysaccharides and proteins) ensures the hydrophilic character of *E. coli*. Therefore, inhibition of the hydrophilic *E. coli* to the hydrophilic PSFQ fibers is lower than the inhibition of hydrophobic *S. aureus* cells. Moreover, the PSFQ fibers obtained in DMF, with smaller diameters (around 1.60 μm), inhibit the growth of microorganisms with the inhibition becoming stronger against *S. aureus*.

Consequently, all these aspects indicate that the antibacterial activity depends not only on the substituent groups of the quaternized polysulfones and fiber organization during the electrospinning process, but also on the bacterial cell membrane characteristics.

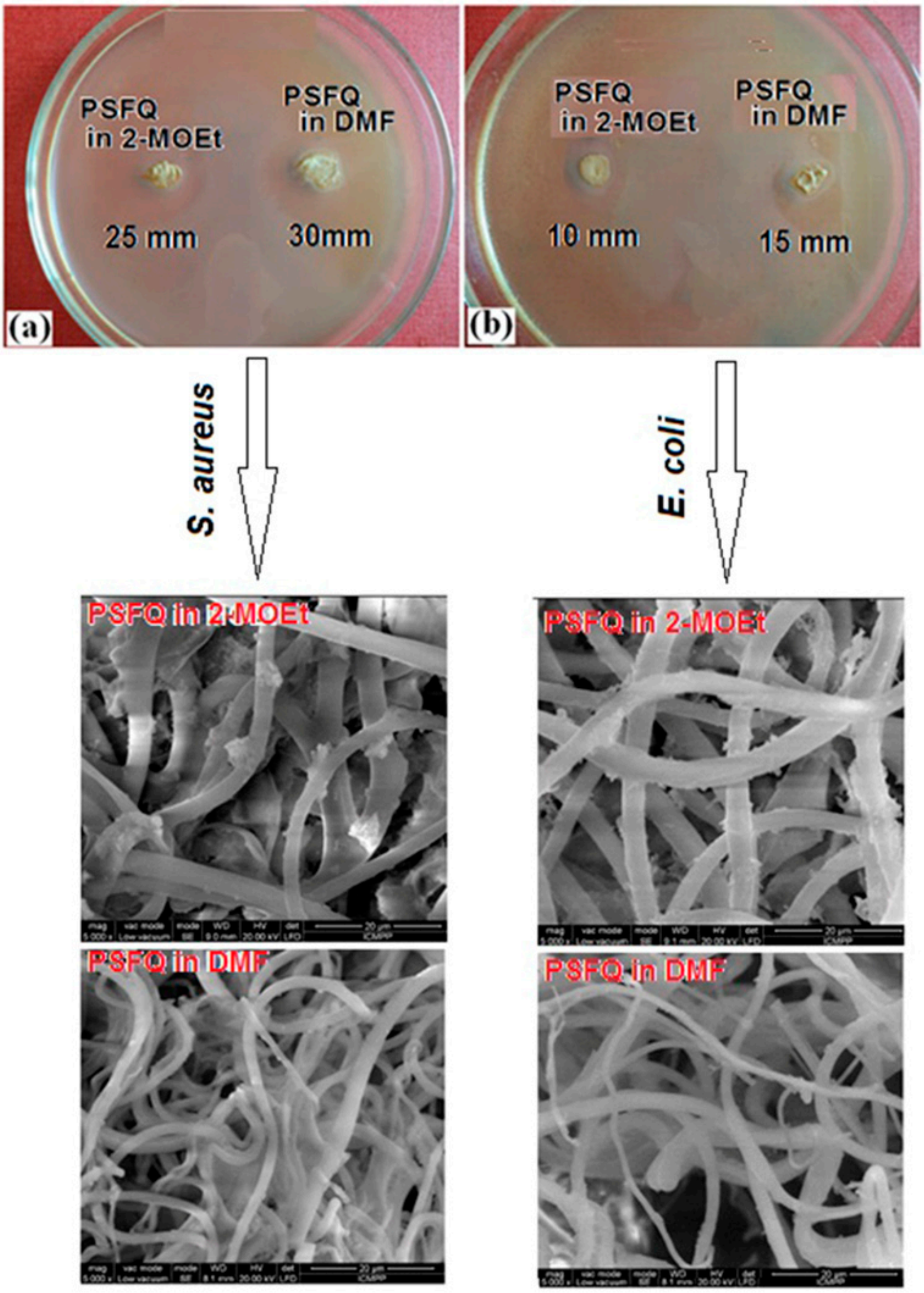


Figure 4. Antibacterial tests for PSFQ fibers obtained from solutions in DMF and 2-MOEt at 40% polymer concentration, against *S. aureus* (**a**) and *E. coli* (**b**). SEM images of PSFQ fibers after being inoculated for bacteria for a 50 μm scan area and magnification of 2000×.

4. Conclusions

Processing of the functionalized polysulfones with quaternary ammonium groups (PSFQ) solutions to obtain new materials with high-performance characteristics—biologically active surfaces—designed to be integrated in a bioinspired system in medical therapy, was carried out by electrospinning. Thus, long, uniform and continuous fibers without any beads structure, which exhibit a smooth surface with variations of diameters, were obtained. Fibers morphology with different forms and dimensions can be explained in terms of the various electrospinning parameters, solution properties and environmental parameters, as well as the intramolecular repulsive interactions between the ionized groups (i.e., ammonium groups). Additionally, certain inhibitory effects of PSFQ electrospun fibers on the growth of *S. aureus* and *E. coli* bacteria have been observed.

The antimicrobial activity of polysulfones with quaternary ammonium groups is considered to be one of the most important properties and directly related to new possible applications, and this indicates that the PSFQ fibers are potential materials for biomedical applications. Consequently, the electrospinning proved to extend the possible applications of quaternized polysulfones by preparing continuous fine fibers with characteristics that recommend them for applications in biomedical fields.

Author Contributions: A.F. and N.O. have designed the experiments (processing of polysulfones solutions by electrospinning), discussed and validated the obtained data and wrote the paper. F.D. has performed the scanning electron microscopy measurements. S.D. has investigated the effectiveness of nanofibers as biocides through antibacterial assays. C.L. has accomplished the characterization of the synthesized polysulfones. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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