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Nanofibrous Polysulfone/TiO₂ Nanocomposites: Surface Properties and their Relation with *E. coli* Adhesion

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ABSTRACT

Thermoplastic nanocomposite materials with potential antibacterial properties were prepared. Solution blow spinning was used to prepare fibrous films of based on polysulfone, PSF, filled with well dispersed TiO₂ nanoparticles. PSF/TiO₂ nanocomposites were prepared with different nanoparticles content (0%, 1%, 2%, 5%, and 10% by weight). A deep characterization was carried out focusing on the morphology at the nanoscale, roughness, contact angles and surface free energy. Cell adhesion was studied by simple inspection by scanning electron microscopy, SEM. A uniform dispersion of the nanofiller was achieved because the nanoparticles could be embedded in the polymer along the fibers when they were created during the blow spinning process. TiO₂ content influenced the topography of the films, most likely due to a direct effect on the solvent evaporation rate. All the results obtained pointed out that an increase of the surface hydrophobicity as a result of the increased roughness induced by the presence of TiO₂ nanoparticles was the main contribution to the reduction of DH5 α *E. coli* cells adhesion.

KEYWORDS: Polysulfone; TiO₂; blow spinning; nanocomposites; cell adhesion.

INTRODUCTION

Each time more plastics are being used for biomedical applications such as prosthesis, catheters, scaffolds for tissue engineering, etc. Many times, apart from having the required properties for their final use it would be interesting to be able to prepare them in-situ, or directly deposited on a substrate, as in the case of the field of tissue repairing or wound healing. Lastly, mats and films of thermoplastics with complex topographies are showing great interest due to their potential use as materials for drugs delivery¹, wound dressings²⁻⁴, tissue engineering^{5,6}, etc. Perhaps, the most extended way of preparing this kind of materials is by the use of electrospinning, a process in which a drop of polymer is subjected to an electric field

which deforms it to generate nanofibers⁷. However, this method of processing uses high voltages in electrically conductive targets where the fibers are deposited, showing in addition low deposition rates which sometimes suggests the necessity of finding other methods, at least when direct dispensation of the films is required in certain applications as surgeries or in-situ creation of wound dressings. Solution blow spinning, SBS, might be a good alternative. Medeiros et al. developed SBS to produce micro- and nanofibers of several thermoplastics⁸, the spinning is carried out with a device formed by, among other things, concentric nozzles through which a polymer solution is ejected by the action of pressurized gas also introduced in the nozzles.

In particular, in materials for biomedical applications, apart from the ability of being easily processed, to have good mechanical and optical properties, to avoid proliferation of harmful bacteria is a very interesting property. To fulfil the above mentioned, to fill the thermoplastic polymers with nanoparticles having highly specific properties such as bactericidal effect might be a good option. Among the thermoplastic polymers to prepare the final nanocomposites with the desired properties, polysulfones, PSF, may be a good choice since they are biocompatible semitransparent, high performance amorphous engineering thermoplastic, highly resistant to a wide range of pH's and good mechanical properties even at high temperature due to its high glass transition temperature ($T_g = 185\text{ }^\circ\text{C}$)⁹. They have great deal of applications particularly in medicine (its high hydrolysis stability allows its use in medical applications requiring autoclave and steam sterilization), food, processing equipment, electrical and electronics components^{10,11}. On the other hand, TiO_2 nanoparticles can be selected as the nanofiller because of its potentiality as biocidal material^{12–17}. Besides, the nanoscopic character of the particles allows them to be added in low proportion because their large surface to volume ratio implies the creation of so large interphase as to significantly change the polymer matrix properties.

Although the antibacterial effect can be attributed to a direct influence of a certain material on the bacteria metabolism (biocide effect), many times it is associated to an inhibition of biofilm development by poor cell adhesion to the material. To clarify this, deeper investigation should be carried out. In other words, before facing the description and explanation of biofilms development, data about interactions between a surface of a certain substrate and a particular cell should be collected and analyzed.

On the other hand, the fabrication of micrometer and sub-micrometer sized

nonwoven meshes/mats, so as the ability to tailor the morphology/topography, have high interest in a number of applications including sensors, filtration, drug release, tissue engineering scaffolds, and liquid-repellent fabrics^{18–23}. Besides, obtaining surfaces and coatings with high hydrophobicity or hydrophilicity, promoting low or high surface tension liquid repellence may have a number of potential applications, including fabrics highly resistant to wetting by different sort of liquids, surfaces for avoiding biofouling or fluid frictional drag and separation of oil/water dispersions. Due to this, controlling topography of surface coatings is of primary importance.

Focused on the above mentioned, a systematic investigation into the effect of the surface chemistry on the bacterial adhesion has been reported²⁴. In particular, a number of relevant physicochemical factors when defining the surface at the molecular level were assessed for their effect on the adhesion of *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *E. coli*^{25–30}, concluding that, for a particular substrate-bacteria system, detailed knowledge about the surface features (morphology, roughness, surface free energy) and cell adhesion is necessary to understand the possible antibacterial properties and/or cell proliferation.

Although different mechanisms of bacterial attachment have been described, the initial stages of that process are driven by Van der Waals attractive forces, electrostatic forces, and surface hydrophobicity. The distance between the microorganism and the surface of the material is another relevant factor to be considered. That is why the surface topography has been widely discussed as a parameter influencing microbial adhesion^{31–33} since heterogeneities at nano-scale govern adhesive forces. However, there is still certain controversy in the literature regarding the real contribution of all these surface characteristics

as, for example, the degree of roughness favouring bacterial adhesion³⁴.

Different types of interactions are involved in various interfacial phenomena such as biofouling and microbial adhesion. Many investigations into the fundamental mechanisms governing this phenomenon have shown the occurrence of electrostatic, Van der Waals interactions, as well as lone pair (Lewis acid-base) effects^{35–37}. To reduce or prevent microbial adhesion it is therefore essential to know, among other factors, the surface properties of microorganisms from the physicochemical point of view. In the present work the well known DH5 α strain of *E. coli* has been used (it is not a pathogen, and was developed for laboratory cloning use). The cell-surface hydrophobicity assay of bacteria DH5 α was already performed by Christopher W. Reid et al.³⁸ Surface hydrophobicity of cells grown of *E. Coli* DH5 α was assessed using the bacterial adhesion to hydrocarbon (BATH, particularly hexadecane) assay of Rosenberg et al.³⁹ From the later, cell surface hydrophobicities of *E. coli* DH5 α was determined: BATH (%) = 31.1 ± 2.76 . Therefore for this particular strain it is expected a quite hydrophilic behaviour.

One way of performing this kind of studies is, apart from the corresponding surface characterization, the direct observation of the microorganisms proliferation from their initial stages. Different microscopic techniques for biofilm monitoring including scanning electron microscopy (SEM) have been proved to be suitable in order to follow the study of adhesion stage and biofilm formation^{40–42}. In particular, because of its high resolution and the possibility of working at relatively low acceleration voltage scanning electron microscopy in its modality of field emission, FESEM, should be an ideal tool to study the biofilm development and cell adhesion.

In this work, solution blow spinning, SBS, has been used to prepare nanocomposites based on polysulfone, PSF, filled with well dispersed TiO₂

nanoparticles with potential antibacterial activity. As a function of the nanoparticles content morphology studies based on the observation of the dispersion of the nanoparticles within the polymer have been performed. Besides, in-depth characterization of the surface of the materials (morphology, topography, wettability and surface free energy) was carried out to explore and establish correlations with data coming FESEM about the cell adhesion and biofilm development of *E. coli* cells onto the nanocomposites surface.

EXPERIMENTAL

Materials

LATI 95-15T auto-lubricated polymer based on Polysulfone, PSF (commercial name Lasulf, supplied by Lati ibérica) was used as the matrix for the composites. TiO₂ nanopowder (mixture of rutile and anatase with 99.9% of purity), with mean diameter < 100 nm (BET) and < 50 nm (XRD) nm was purchased from Sigma-Aldrich, and used as the filler. Chloroform and acetone (HPLC grade), from Aldrich, were used as solvents for the solution blow spinning process.

Materials preparation

The PSF/TiO₂ materials with different amounts of TiO₂ (0%, 1%, 2%, 5% and 10% by weight) were prepared in the form of films by solution blow spinning, SBS, using a commercial airbrush and compressed air. A suspension of TiO₂ nanoparticles in a PSF solution of 10% wt/v in a chloroform/acetone was prepared and then blow spun onto a glass substrate. Finally, the films were taken off from the glass substrate by the simple use of tweezers.

In all cases TiO₂ suspensions were prepared using solutions of PSF (10% wt/v) made with a mixture of chloroform/acetone in a proportion 8:2 v/v. The process can be described as follows:

- a) 1g of PSF was dissolved in 6 mL of chloroform (solution).

- b) A suspension of TiO_2 in a mixture of 2 mL of chloroform and 2 mL of acetone was prepared adding the required amount of nanoparticles, as to have a particular composition in the nanocomposite, and sonicating it for 30 min to improve disaggregation of nanoparticles (suspension 1).
- c) The suspension to be blow-spun (suspension 2) was prepared mixing the solution and suspension 1, stirring for 15 min and sonicating for 15 min in an ultrasound bath at room temperature. Then, the mixture was maintained under agitation until SBS process was carried out. The actual solvent used to prepare the suspensions was then a 20% v/v solution of acetone in chloroform.

The conditions to perform the SBS were, nozzle diameter: 0.5mm; fluid cup capacity: 5 cm³; pressure: 4 bar and working distance: 4-5 cm.

Samples of the resulting films prepared by SBS are shown in Figure 1. As it can be seen the higher the amount of TiO_2 particles the more opaque the material is.

FIGURE 1 Films of the PSF/ TiO_2 nanocomposites prepared by SBS with different amounts of TiO_2 nanoparticles.

Equipments

The morphological studies of the composites were carried out using a field emission scanning electron microscope Zeiss Ultra 60 (FE-SEM) equipped with a system of X-Ray energy-dispersive spectroscopy for microanalysis (XEDS, with detector Oxford 80 mm² X-Max silicon drift). As the samples were not conductive, they were coated with carbon using

a high resolution ion beam coater GATAN Model 681 (Extrad Instruments). Direct observation of cell adhesion was carried out using a TENEO field emission scanning electron microscope, FESEM (FEI). The acceleration voltage was 2.0 kV and an Everhart Thornley detector was used taking the signal coming from secondary and backscattered electrons. In this later case the samples were gold coated using a low vacuum coater Leica EM ACE200.

The roughness studies were performed by white light interferometry using a NewView™ 7300 3D Optical Surface Profiler (Zygo).

Surface free energy of the different materials was obtained from contact angle measurements. An OCA-15 KRÜSS GmbH tensiometer based on the drop method was used. The contact angle for each testing liquid was the average of four drops, and the surface free energy was obtained by the Owens-Wendt method^{43,44}. As the testing liquids for the contact angle measurements, distilled and deionized water, glycerol and diiodomethane were chosen. Table 1 gathers the values of some important parameters of the testing liquids used.

TABLE 1 Values of densities and contributions to the surface tension of the liquids used to carry out the contact angle measurements.

Liquid	δ (g/cm ³)	γ_d (mN/m)	γ_p (mN/m)	γ_t (mN/m)
Water	0.998	21.8	51.0	72.8
Glycerol	1.259	37.0	26.4	63.4
Diiodomethane	3.220	50.8	0.00	50.8
Hexadecane	0.770	27.5	0.00	27.5

Where δ is the density of the liquid, γ_d and γ_p are the dispersive and polar components of the surface tension of the liquids, and γ_t the total surface tension or the sum of the polar and dispersive components. The surface tension values were taken from Stöm et al.⁴⁵.

Culture of bacteria

To culture the gram negative bacteria DH5 α , a strain of *E. coli* was used. Circular pieces of about 5 mm of diameter of the different materials were cut out and glued with an epoxy adhesive on stainless steel disks. Then the materials were disinfected by spraying with ethanol 70% (v/v). By adding a 3% by weight of *E. coli* to Luria Bertani (LB), used as growth medium, a stock suspension was prepared by culturing at 37 °C for 12h with agitation. Then, a 1/100 dilution of the stock suspension was used as the medium to immerse the materials and generate the biofilm on their surfaces.

To prepare the biofilms a multi-well plate was used by introducing one sample per well, and then pouring 2 mL of the diluted suspension into each well. The plate was then agitated for 8 h at 37 °C, until adhesion of the bacteria to the surfaces of the materials and generation of the biofilms. After 8h of culture the suspension was removed by aspiration to eliminate the non-adhered bacteria and the resulting materials rinsed with a sterile solution of sodium chloride 0.9 wt%.

In order to prepare the samples for SEM visualization a fixation process was necessary. 1 mL of glutaraldehyde 2.5 wt% was added to each well and left for 30 minutes at room temperature to fix and kill the bacteria onto the materials. After 30 minutes, glutaraldehyde was removed and samples were rinsed 3 times with PBS to remove the remaining glutaraldehyde. After fixation, the samples were dehydrated in four 10-minutes steps of increasing ethanol concentration (30, 50, 70 and 100%). Finally, ethanol was removed and the samples left in the laminar flow hood for their complete drying.

SEM images ($\times 1000$ of magnification) of each sample cultured with DH5 α *E. coli* were taken and then every single cell observed was counted to give the number of adhered cells per surface unit. Finally, from the results of 5

specimens an average value and the corresponding standard deviation was obtained.

Data analysis

The software MountainsMap® Universal, version 7.2.7481 (Digital Surf Surface Intelligence) was used to obtain different parameters associated to the roughness from the data obtained with the white light interferometer by mapping the 3D surface, using the ISO 4287 standard with a Gaussian filter of 0.08 mm. The parameters thus obtained were: maximum peak height, R_p ; maximum pit height, R_v ; maximum height R_z ; arithmetic mean height, R_a and root mean square height, R_q .

RESULTS AND DISCUSSION

To study at nanoscale the morphology of the materials SEM images were obtained at different magnifications using the signal coming from secondary, SE, and backscattered, BSE, electrons. It is worthy emphasizing here that topography is highly dependent on the side of the sample film. The top side corresponds to the surface exposed directly to air while the bottom is the surface in contact with the glass substrate used to deposit the material during the SBS process. In Figure 2, as an illustration, the top and bottom surfaces of the same film are shown for a sample of neat PSF. It can be clearly observed how the bottom surface is much smoother than the top one. The top surface reflects the way the material is deposited on the substrate. Nano and submicrometer fibers of PSF can be seen over a portion of continuous material that probably is formed if solvent evaporation does not completely occur during the time of flight when the SBS process is taking place. In fact, the bottom surface simply reflects how the material is spread on the extremely flat surface of the glass plate. The rest of the composites, regardless the TiO₂ content, showed similar topographies when comparing the top surfaces with the bottom ones.

FIGURE 2 Left: SEM (SE signal) representative images of the top (a) and bottom (b) surfaces of blow spun neat PSF. Right: 3D images.

On the other hand, when comparing the top surfaces of the materials prepared with different TiO_2 nanoparticles content, some topographic differences can be observed (Figure 3). Regardless the composition, the materials show a fibrous morphology, with diameters of fibers ranging from 50 to 200 nm, which, in general, arises from an arrangement of fibers in the form of bundles (Figure 3f). When adding nanoparticles small accumulations similar to coils are formed which sizes increase the higher the amount of TiO_2 nanoparticles. Besides, the amount of fibers so as their diameters seem to decrease with the TiO_2

nanoparticles content. A similar observation was done for a system formed by polyvinylidene fluoride and TiO_2 nanoparticles⁴⁶. In that case the explanation given pointed to the different solvent evaporation rates influenced by the TiO_2 nanoparticles content. If there were competing interactions, for instance, polymer-solvent and polymer-nanoparticles, an increase of polymer-nanoparticles interactions might lead to more freedom for solvent molecules, favouring their evaporation process⁴⁶.

FIGURE 3 Blow spun nanocomposites based on PSF with different TiO_2 nanoparticles content: a) 0%; b) 1%; c) 2%; d) 5% and e) 10% wt. Image f) shows a detail of a bundle of nanofibers created during the SBS process.

Another very important point to consider is the nanofiller dispersion within the polymeric matrix. A uniform dispersion of the nanoparticles is the only way to ensure homogeneity in terms of properties. In Figure 4 it is shown a SEM image obtained from a combination of SE and BSE signals (50%) of the top surface of a PSF sample with a 10% of TiO_2 nanoparticles. Small bright tiny spots (< 100

nm) and small regions (< 400 nm) well dispersed in a dark grey matrix can be observed. In principle, the largest regions observed in Figure 4 suggest the presence of some agglomerates due to the relatively high concentration of nanoparticles (10% wt) which makes more probable the contact between them. Besides, bright spots located within the nanofibers all along them (see the arrow) were detected.

These spots and regions can be assigned to the presence of heavier elements which enhance the BSE signal. X-Ray microanalysis on these regions confirmed the presence of titanium from the TiO_2 nanoparticles. It can be concluded, at least for the system formed by PSF and TiO_2 nanoparticles, that SBS is a good method to prepare nanocomposites with a dispersion of nanoparticles quite uniform, which get trapped throughout the fibers when they are formed during the blow spinning process.

FIGURE 4 SEM image obtained from a combination of the SE and BSE signals (50%) of the top surface of a sample of PSF with a 10% of TiO_2 nanoparticles.

Apart from the morphology, additional surface characteristics should be studied to interpret cell adhesion results. Topographic information was obtained using white light interferometry. From the average of 5 roughness profiles (Figure 5), taken from the interferometric images of the different materials, roughness parameters were extracted and gathered in the Table 2. Only the top sides of the samples were considered as they present more interesting fibrous morphology in terms of their potential final use in medical applications such as, for instance, scaffolds for tissue engineering. The inspection of the typical parameters associated to the roughness, R_a and R_q , reflect, accordingly with the SEM observations, that there is an initial increase in the heterogeneity of the topography when a small amount of TiO_2 nanoparticles are added to the PSF up to 2% wt and then remains almost constant (Table 2). Apart from changes in the topography the influence of the presence of TiO_2 nanoparticles on the surface features of the materials can be reflected in the physico-chemical interactions with particles deposited on them as can be bacteria cells. One way to study these interactions is by contact angle measurements and surface free energy determinations.

FIGURE 5 Topographic map obtained by white light interferometry for PSF + 10% of TiO_2 , and roughness profile.

TABLE 2 Roughness parameters from averaging five linear profiles in each sample (Figure 6).

Material	R_q (μm)	R_p (μm)	R_v (μm)	R_z (μm)	R_a (μm)
PSF-0%	0.59 ± 0.20	2.23 ± 0.95	1.10 ± 0.19	3.33 ± 1.10	0.39 ± 0.08
PSF-1%	1.25 ± 0.35	3.11 ± 0.76	2.52 ± 0.522	5.63 ± 0.95	0.97 ± 0.32
PSF-2%	1.39 ± 0.40	3.28 ± 0.60	3.15 ± 1.12	6.43 ± 1.57	1.09 ± 0.36
PSF-5%	1.31 ± 0.42	4.67 ± 0.79	2.13 ± 0.90	6.79 ± 1.33	0.91 ± 0.37
PSF-10%	1.24 ± 0.29	4.83 ± 0.58	1.80 ± 0.49	6.63 ± 0.82	0.88 ± 0.25

The values of contact angle obtained with the three test liquids used are collected in Table 3. In general, it was obtained that, when the polarity of the test liquid used is high or relatively high contact angles decrease the smoother the sample is, however, the opposite can be observed when the dispersive contribution of the test liquid is quite high and the polar one very low. This result is easily confirmed when comparing between contact angles of drops deposited on top and bottom sides of samples (Table 3). Thus, there is an important contribution to the contact angle that must arise from the surface roughness. In particular, the data of Tables 2 and 3 show tendencies that suggest that the surface gets more hydrophobic the higher the roughness. The impact of roughness on wettability of a solid substrate is well known⁴⁷. The surface free energy is obtained from the Young's equation which assumes a chemically homogenous and

topographically smooth surface. However, this is not true in the case of real surfaces, which are characterized by a range of contact angles instead of having a particular one. Wenzel defined the relationship between roughness and wettability considering that an increase of surface roughness enhances the wettability caused by the chemistry of the surface⁴⁷. For example, if the surface is chemically hydrophobic, it will become even more hydrophobic when surface roughness is increased accordingly with the results of the present work.

From the data of Table 3, surface free energy values and their polar and dispersion contributions were obtained using the Owens-Wendt method^{43,44} (Table 4).

TABLE 3 Values of contact angle, CA, obtained when water is used as the test liquid.

Sample	CA in Water (°)		CA in Glycerol (°)		CA in Diiodomethane (°)	
	Top	Bottom	Top	Bottom	Top	Bottom
PSF	107 ± 2	92 ± 6	103 ± 5	96 ± 5	50 ± 1	42 ± 4
PSF-1% TiO₂	124 ± 12	84 ± 5	97 ± 3	78 ± 1	28 ± 7	30 ± 5
PSF-2% TiO₂	134 ± 4	99 ± 5	100 ± 2	81 ± 3	29 ± 4	36 ± 8
PSF-5% TiO₂	111 ± 5	96 ± 5	97 ± 3	73 ± 2	33 ± 7	35 ± 6
PSF-10% TiO₂	120 ± 5	83 ± 4	93 ± 3	78 ± 4	32 ± 6	40 ± 2

TABLE 4 Values of surface free energy obtained from the Owens-Wendt method for both sides of the specimens, top and bottom.

Sample	γ_t (mN/m)	γ_d (mN/m)	γ_p (mN/m)
PSF-0% (Top)	32.7 ± 2.2	32.6 ± 2.2	0.13 ± 0.10
PSF-1% (Top)	50.4 ± 4.3	44.4 ± 3.7	6.0 ± 2.0
PSF-2% (Top)	52.8 ± 2.3	44.8 ± 1.9	8.0 ± 1.2
PSF-5% (Top)	43.8 ± 3.3	41.0 ± 3.1	2.7 ± 1.2
PSF-10% (Top)	46.4 ± 2.7	43.2 ± 2.4	3.3 ± 1.3
PSF-0% (bottom)	37.5 ± 2.5	37.0 ± 2.5	0.5 ± 0.3
PSF-1% (bottom)	41.6 ± 1.7	41.4 ± 1.7	0.2 ± 0.1
PSF-2% (bottom)	40.4 ± 3.0	40.4 ± 3.0	0.01 ± 0.01
PSF-5% (bottom)	42.0 ± 2.2	41.9 ± 2.2	0.1 ± 0.1
PSF-10% (bottom)	41.3 ± 1.3	39.4 ± 1.1	2.0 ± 0.1

The usual values of surface free energy found in the literature for PSF are around 40 mN/m⁴⁸. As it can be observed only for the smoothest surfaces or the bottom sides of the materials under study (TABLE 4), the surface free energy values are similar to the literature ones. However, for the top sides the surface free energies obtained were slightly higher than those taken from the bibliography. Furthermore, the higher the TiO₂ nanoparticles content the higher the surface free energy, mainly due to the increase of both dispersion and polar contributions. These results may seem to be contradictory regarding the hydrophobic behaviour of the samples taking into account the contact angle values (Table 3). In Figure 6 surface free energy (and contributions) as well as roughness are represented as a function of TiO₂ nanoparticles content. Considering the data of Table 3 the most hydrophobic samples are those with the highest polar contribution, as can be seen in Table 4 and Figure 6. However, at the same time changes in the dispersion contribution follow the same trend (Figure 6). A possible explanation of these results may be associated to the consideration that complex topography lead to calculated surface free energy

contributions without any physical meaning which may induce to erroneous interpretations.

FIGURE 6 Surface free energy and contributions and roughness as a function of TiO₂ nanoparticles content.

Another explanation is that the increase of the dispersive contribution is what actually induces the changes observed in the wettability behaviour. An increase of the polar contribution to the surface free energy can be simply due to the presence of highly polar TiO₂ particles whose influence seems to be higher the higher the interface of contact between the substrate and the testing liquid drops. What is clearly observed is that water better wets the surfaces of the materials with lower surface roughness, while the non-polar diiodomethane better wets the surfaces the higher the surface roughness is. Thus, one would expect a better adhesion of hydrophilic particles to the surfaces of the materials with lower roughness.

As *E. coli* cells will be cultured on top of the blow spun materials, to analyse their surface characteristics must be an important issue for further understanding of their adhesion behaviour.

As was mentioned in the introduction surface hydrophobicity of cells grown of *E. Coli* DH5 α was assessed using the bacterial adhesion to hydrocarbon (BATH, particularly hexadecane) assay of Rosenberg et al.³⁹ From the later, cell

surface hydrophobicities of *E. coli* DH5 α was determined: BATH (%) = 31.1 $\pm$ 2.76.

We propose here the estimation of the overall surface tension of bacteria by the weighted contributions of each solvent used in the BATH method (water and hexadecane in this case)^{38,39}. In this way:

$$\gamma_d(\text{bacteria}) = \phi_1\gamma_d(1) + \phi_2\gamma_d(2) \quad (1)$$

$$\gamma_p(\text{bacteria}) = \phi_1\gamma_p(1) + \phi_2\gamma_p(2)$$

where the subscripts (1) and (2) refer to the solvents 1 and 2, respectively (water and hexadecane) and ϕ refers to the weight fraction of bacteria extracted by a certain solvent which well might be the BATH parameter. Therefore, for the *E. coli* DH5 α it would be obtained

$$\gamma_d(\text{bacteria}) = (1-0.311) \cdot 21.8 + (0.311) \cdot 27.5 = 23.6 \text{ (mN/m)}$$

$$\gamma_p(\text{bacteria}) = (1-0.311) \cdot 51.0 + (0.311) \cdot 0.00 = 35.1 \text{ (mN/m)}$$

$$\gamma_t(\text{bacteria}) = 23.6 + 35.1 = 58.7 \text{ (mN/m)}$$

These results are in accordance with bacteria cells having a mainly polar surface, pointing therefore a lower *E. coli* adhesion the higher the dispersive contribution to the surface free energy is. In order to see whether this is the expected behavior, inspection by SEM of *E. coli* cells adhered to the different materials prepared was performed. Figure 7 shows as an example the bacteria distribution on the surface of neat blow spun PSF, in which several bacteria have been green coloured for better visualization. Similar images were obtained for the rest of samples although with clear variations in the average number of bacteria per surface unit. Figure 8 shows representative regions of the SEM images taken from the different cultured materials, which qualitatively

show the bacterial cells adhered as a function of the TiO₂ nanoparticles content.

FIGURE 7 SEM image of the sample PSF without TiO₂ nanoparticles (PSF-0%) cultured with DH5 α *E. coli*. Some individual bacteria have been coloured in green. The inset shows a zoomed region of the central part of the SEM image.

It can be observed that most of the regions inspected indicate that the higher the TiO₂ nanoparticle content the lower the bacteria cells adhered. To be precise, worse bacteria adherence seems to occur when there exists higher surface of composite available or surface with nanoparticles. In order to get quantitative information on the bacteria growth, cell counting was carried out from different SEM images of the materials investigated. Table 5 gathers the average number of adhered bacteria and standard deviation per surface unit for the different compositions of nanoparticles. As can be observed, the trend is just the opposite of both the roughness (Table 2 and Figure 6) and the hydrophobicity (Table 3). This result is completely in agreement with the expected from the surface hydrophilic characteristics of the DH5 α *E. coli* cells.

FIGURE 8 Representative regions of the SEM images taken from the different cultured materials: a) PSF-0%; b) PSF-1%; c) PSF-2%; d) PSF-5%, and e) PSF-10%.

Two can be the reasons of the antibacterial behavior observed when adding TiO_2 nanoparticles: i) a direct action of the nanoparticles against the bacteria; and ii) changes in the induced topographic characteristics of the blow spun samples that directly affect the hydrophobicity of the materials. If the first reason were the dominant one would expect less adhered bacteria cell in the case of the highest loaded PSF (sample PSF-10), however that was not the case. Thus, the increase of surface hydrophobicity due to augmented roughness induced by the presence of TiO_2 nanoparticles in the blow spun films seems to be the most likely contribution to the reduction of DH5 α *E. coli* adhesion.

Some other works have demonstrated that direct bactericide action might not be necessary in order to avoid biofilm development since, high efficiency of surface properties in terms of adherence against the proliferation of bacteria might be enough. For instance, Wei et al. fabricated transparent slippery hybrid coatings which exhibited outstanding long-term slippery

stability that seemed to be the main cause of inhibiting biofilm formation⁴⁹; Fu et al. prepared and studied liquid-repellent and antibacterial nanocomposite coatings cross-linking a fluorinated copolymer and poly(urea-formaldehyde) nanoparticles containing active methylol groups with hexamethylene diisocyanate⁵⁰ and Wu et al. prepared colored fabrics nanocomposites with durable antibacterial and self-healing superhydrophobic properties via a solution-dipping method blending poly(ethylenimine), silver nanoparticles, and fluorinated decyl polyhedral oligomeric silsesquioxane with cotton fabrics⁵¹.

However, when talking about antibacterial materials the later it is no always the main factor accounting for this effect. In other systems it seems that there is a direct antibacterial action arising exclusively from the particles as Sheikh et al. shown with polyurethane nanofibers containing copper nanoparticles⁵² or as Xu et al.⁵³ shown with materials based on Cu nanoparticles embedded

in PAN/ β -Cyclodextrin (β -CD) composite nanofiber films.

TABLE 5 Average number of adhered bacteria and standard deviation as a function of the nanoparticle content.

Sample	Average number $\times 10^3$ (bac/mm ²)	Standard Deviation (bac/mm ²)
PSF-0%	81.6	16.3
PSF-1%	8.5	4.4
PSF-2%	1.8	0.3
PSF-5%	1.6	0.6
PSF-10%	19.1	8.0

CONCLUSIONS

SBS has been demonstrated to be a good method to prepare nanocomposites with uniform dispersion of TiO₂ nanoparticles in polysulfone matrix, in which TiO₂ nanoparticles get trapped along the fibers when they are formed during the blow spinning process. Regardless the amount of TiO₂ nanoparticles, the materials show fibrillar morphology, with diameters of fibers ranging from 50 to 200 nm, which, in general, arises from an arrangement of fibers in the form of bundles. The amount of fibers as well as their diameters depend on the TiO₂ nanoparticles content, which may influence solvent evaporation rates. An increase of polymer-nanoparticles interactions seems to reduce solvent-polymer and solvent-nanoparticles interactions then favouring solvent evaporation.

The hydrophobic surface of PSF gets more hydrophobic the higher the roughness, in accordance with Wenzel who stated that adding surface roughness will enhance the wettability caused by the chemistry of the surface. It seems that the increase of the dispersive contribution to the surface free energy is what actually induces the changes in the wettability behavior of the PSF based nanocomposites. All the results obtained point out that the increase of

surface hydrophobicity due to increase of roughness induced by the presence of TiO₂ nanoparticles in the blow spun suspensions is the main contribution to the reduction of DH5 α *E. coli* adhesion.

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GRAPHICAL ABSTRACT

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TITLE: Nanofibrous Polysulfone/TiO₂ Nanocomposites: Surface Properties and their Relation with *E. coli* Adhesion

TEXT: Preparation of nanofibrous polysulfone/TiO₂ nanocomposites, study of their surface properties as a function of titania nanoparticles content and their relation with E. coli adhesion.

GRAPHICAL ABSTRACT FIGURE

Preparation of nanofibrous polysulfone/TiO₂ nanocomposites, study of their surface properties as a function of titania nanoparticles content and their relation with *E. coli* adhesion.