This is a postprint version of the following published document:

Teno, J., González-Gaitano, G. & González-Benito, J. (2017). Poly (ethylene-co-vinyl acetate) films prepared by solution blow spinning: Surface characterization and its relation with E. coli adhesion. *Polymer Testing*, vol. 60, pp. 140–148.

DOI: [10.1016/j.polymertesting.2017.03.020](https://doi.org/10.1016/j.polymertesting.2017.03.020)

© 2017 Elsevier Ltd.

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](https://creativecommons.org/licenses/by-nc-nd/4.0/).

**Poly (ethylene-co-vinyl acetate) Films Prepared by Solution Blow Spinning:
Surface Characterization and its Relation with *E. coli* Adhesion**

Jorge Teno^a, G. González-Gaitano^b and J. González-Benito^{a*}

*To whom all correspondences should be addressed

Address: Av. Universidad 30, 28911 Leganés (Madrid – Spain)

e-mail: javid@ing.uc3m.es

Abstract

Solution blow spinning, SBS, a quite novel processing method, was used to obtain poly (ethylene-co-vinyl acetate), EVA, films with controlled surface properties. The influence of the surface characteristics of EVA films on the adhesion of *DH5 α* *Escherichia coli* was studied. In particular, the initial concentration of the EVA solution to be blow spun was varied in order to get different surface topographies. Considering the potential use of EVA based materials in applications such as food packaging or scaffolds for tissue engineering all factors affecting proliferation of microorganisms on their surfaces should be studied and understood. Structural, morphological and surface characterizations based on the use of infrared spectroscopy, FTIR, scanning electron microscopy, SEM, and contact angle measurements were performed to ascertain the main factor influencing the *E. coli* adhesion on the EVA films. Roughness data were determined at different scales from 3D surfaces obtained using a stereoscopic reconstruction of SEM images. It was concluded that, depending on the conditions of the SBS process, only variations of topography were found on the EVA films, being therefore the unique cause of different adhesion capacity of *E. coli* cells. A correlation between roughness and the number of attached *E. coli* cells showed that the higher the roughness at microscale level the higher the biofilm development, demonstrating that, apart from specific interactions at nanoscale surface, heterogeneity at microscale can greatly modify the antibacterial action.

Keywords: Poly (ethylene-co-vinyl acetate); solution blow spinning; topography; bacteria adhesion.

1. Introduction

In the last decade plastic industry has been extended to the development, preparation and commercialization of new products with applications in agriculture (from green houses, silage bags and much films to drip tape/tubing) and food industry (containers, coatings and thin films for packaging) [1,2]. A particular application within this framework belongs to the group of active packaging, or those that interact with food in order to extend its expiration date [3,4]. Proliferation of bacteria is something expected to be avoided in many of these plastics. These microorganisms form communities so called “biofilms” that, apart from the possibility of damaging the material and food, when formed by a high number of cells usually act as protecting barriers against the action of external agents. A possible solution to this problem may be the modification of the polymer by adding particles or other substances capable of acting against the proliferation of bacteria, either because of their intrinsic biocide character or simply by altering the surface properties of the final materials to avoid the adhesion of microorganisms. In principle, the change of surface properties by the sole modification of processing conditions would be an easier and more affordable way of reducing or even preventing the proliferation of microorganisms.

The bacterial adhesion depends on the ability of cells to attach to the surface in order to properly feed and reproduce. Thus, to understand the mechanism of bacteria adhesion, as well as its influence on the materials acting as substrates is very important. The surface topography is one of the main characteristics affecting the microorganisms adhesion [5,6] since the heterogeneities at nanoscale may influence adhesive forces. Although different mechanisms of bacterial attachment have been described, the initial stages of attachment are driven by van der Waals attractive forces, electrostatic forces, and surface hydrophobicity [5–7].

A very simple process to generate films formed by micro or nanofibers mats that can be directly deposited on practically any kind of substrate is the so-called solution blow spinning method (SBS). This method was firstly described by Medeiros et al. to produce micro and nanofibers of several thermoplastics [8]. The spinning system consisted of concentric nozzles through which a polymer solution and a pressurized gas are simultaneously ejected. One of the main advantages of this method is the high rate of deposition which would allow covering large areas of substrates, of utility for packaging purposes, for example.

Poly (ethyl-co-vinyl acetate), EVA, is a polymer with wide use in agriculture and packaging. EVA is a copolymer with acceptable mechanical strength, barrier properties and biocompatible. In addition, it has low friction coefficients and high adhesiveness, being used in multilayer films as the internal layer to promote self-sealing in food packaging [9]. Thus, to study the effect of topography of EVA films on the development of biofilms must be an important issue since that information will be helpful to smartly select the best conditions to finally produce the materials with the required characteristics to lead to the best performance against bacteria adhesion.

Srinivasan et al. [10] demonstrated how the surface morphology of a blend of poly(methyl methacrylate) (PMMA) with 1H,1H,2H,2H-heptadecafluorodecyl polyhedral oligomeric silsesquioxane, using an air brush with a pressurized nitrogen stream, can be tuned as a function of polymer concentration and molecular weight, changing their final surface properties. Other investigations have demonstrated that the polymer concentration in the SBS process may greatly affect the final topography of the materials prepared and their surface properties [11–13]. One of the most important factors is rate of solvent evaporation, governed by solvent choice and spinning conditions, most importantly working distance to collector and concentration. The working distance can largely determine if films are formed, or interconnected webs of fibres are formed as opposed to individualised fibres.

On the other hand, when certain materials present such heterogeneous surfaces, to obtain topographic images with good lateral resolution may be difficult. Interferometric methods provide great accuracy in heights but sometimes, depending on the information required, poor lateral resolution. Therefore performing studies where roughness has to be obtained at different scales and where, at least, one of them implies a few micrometres squared of surface, might not be carried out. The use of atomic force microscopy (AFM) can be one option. However, the surfaces obtained by SBS are usually in the form of fibres, and AFM may produce irreproducible scans and poor imaging if the scanning tip gets stuck with the fibres. Thus, other experimental options must be considered in order to overcome the later. An easy strategy might be obtaining 3D surfaces from a simple stereoscopic reconstruction of scanning electron microscopy, SEM, images.

The aim of this work is to study the influence of EVA polymer concentration of a solution to be blow spun on the final surface morphology, topography and structure of the films obtained. A correlation between surface properties with *E. coli* adhesion was established, trying to understand the main contributions affecting the biofilm development. Among other things, roughness parameters were calculated from data taken at different scales because roughness greatly depends on the scale from which their representative parameters are determined [14,15]. Therefore, it is expected to find out from what scale topography changes lead to the highest variations in the bacteria adhesion. It is important to highlight here that although for several authors the first intention is the creation of fibers by the use of SBS, here one of the main objectives is just to change the morphology of the polymer films surfaces in a controlled way and finally to characterize them to understand its influence in the bacterial adhesion. Therefore, this article is focused to test the material more than to the creation of proper material.

2. Experimental

2.1 Materials

Poly (ethylene-co-vinyl acetate), EVA (25 % wt/wt in vinyl acetate, density 0.933 g·cm⁻³ at 25 °C, Vicat temperature ASTM D 1525 ¼ 65 °C, and melting point 95 °C, batch # MKAA0470) was supplied by Sigma-Aldrich. Chloroform

(Aldrich, HPLC grade) was used as solvent for the solution blow spinning process.

2.2 Polymer film preparation

EVA films were prepared by solution blow spinning, SBS, from solutions of EVA in chloroform at 50 °C (1 %, 2 %, 5 %, 7 % and 10 % by weight), using a home-made device inspired on the Medeiros' patent [8,16]. The SBS device consisted of an air compressor with a pressure regulator (4 bar), a plastic syringe coupled in a pump (NE 1000 X, New Era System, Inc., Farmingdale, NY) to control the injection rate of the polymer solutions (0.5 mL/min), a nylon tube through which the solution flows into a concentric nozzle, and a rotating cylindrical collector at a working distance of 20 cm. The nozzle geometry comprises a perforated aluminum cylinder (1.0 mm inner diameter) with a hole in the lateral part (4.0 mm of diameter) to introduce the pressurized air. Along the aluminum cylinder a glass tube (inner and outer diameters, 0.5 and 0.7 mm respectively) is introduced and positioned protruding 2 mm beyond the aluminum external cylinder. The polymer films were collected on a rotating drum (6.5 cm of diameter) wrapped with aluminium foil at a rotation speed of 270 rpm. Samples (Figure 1) were finally stored in a desiccator. As a control sample a film of EVA obtained by simple casting from a 10% wt/wt solution in chloroform was used.

2.3 Analytical methods

To study possible structural changes in the polymer, infrared attenuated total reflectance was performed with a FTIR-ATR Nicolette Avatar 360 spectrometer (resolution of 2 cm⁻¹ and 32 scans per spectrum).

The morphological studies, including the direct observation of cell adhesion, were 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. Five images per sample were obtained at different tilt angles (0 °, 5 °, 10 °, 15 ° and 20 °) that will be used for ulterior 3D reconstructions. As the samples were not conductive, they were coated with a very thin layer of gold using a low vacuum coater Leica EM ACE200.

Roughness (arithmetic mean height, R_a , and the root mean square height, R_q) was studied after reconstruction of the 3D images using the SEM module of the MountainsMap® software (Digital Surf Surface Intelligence). A stereoscopic reconstruction operator was used to get 3D images from a stereoscopic pair of SEM images collected from the sample at two different angles of view.

Surface free energies of the different materials were obtained from contact angle data. An OCA-15 KRÜSS GmbH tensiometer based on the drop method was used. The contact angle for each testing liquid was obtained from the average of four drops (3 µL) in different locations of the same surface, and the surface free energy was calculated using the Owens-Wendt method [17,18]. As the testing liquids for the contact angle measurements, distilled and deionised water,

glycerol and diiodomethane were chosen. Table 1 gathers the values of some important parameters of the testing liquids used.

Figure 1.

Table 1

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 of the Table 1 were taken from bibliography data [19].

2.4 Culture of bacteria

To culture the bacteria DH5 α , a strain of the bacteria *E. coli* was used. From the different materials prepared on aluminum foil square pieces of about 8×8 mm were cut out and glued with an epoxy adhesive on stainless steel disks with the non coated part of the aluminum. Then, materials were disinfected spraying with ethanol 70 % (v/v). By adding a 3 % 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 (5 mL of volume), 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 8 h 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 carried out. 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.

3. Results and discussion

FTIR spectra of all the samples are presented in Figure 2. As expected, differences were not found either in the position, shape or absorbance ratio of the bands, pointing out that the structure of the polymer is not affected by the mild processing conditions considered in this work. By contrast, clear differences were observed in terms of topography and surface properties. SEM images of the blow spun samples are shown in Figure 3. As can be seen, the topography became more heterogeneous the higher the concentration of the solution to be blow spun was. When a 1 % EVA solution was used a non-fully coated surface was obtained formed by globs or drops of polymer on the aluminium foil (Figure 3a).

Figure 2.

Figure 3.

Domingos et al. [12] stated that solution viscosity and surface tension increased the higher the polymer concentration was, demanding higher drag forces to take the polymer solution to the collector, leading to thicker polymer fibres.

Besides, previous studies showed that lower surface tension could reduce the formation of beads and avoid the production of interconnected fibres and continuous film [13] as happened in the present work with the less concentrated solution. When the concentration of the solution was increased to 2 % of EVA an almost continuous film formed by globs or drops of polymer was obtained (Figure 3b). The behaviour seemed similar to that of a 1 % solution but in the case of 2 % with enough amount of polymer to fully coat the aluminium foil. When the concentration of EVA was increased up to 5 % clear formation of interconnected fibres was observed (Figure 3c), in accordance with results of Oliveira et al. [13] Finally, for higher concentrations, 7 % and 10 %, the fibres became so thick that in many regions of observation over the polymer surface their interconnection resulted in a practically continuous film (Figures 3d and e). Therefore, it seems clear that by simply changing the concentration of the polymer solution it is possible to tailor the topography of the final blow spun polymer and therefore their surface properties, which might have important consequences on the cell adhesion. It is clear here that more setting SBS conditions might have been explored to obtain different morphologies like more

defined micro and nanofibers; however, this article is more focused to test the material more than to the proper creation of the material.

In order to extract quantitative topographical information from the SBS produced surfaces, roughness determinations were carried out. Topography or 3D images (Figure 4a) were obtained from a stereoscopic pair of SEM images collected from the sample at two different view angles (Figure 4b). After that, the software MountainsMap® was used to obtain different parameters associated to the roughness using 5 roughness profiles (Figure 5) from the 2D reconstructed maps and the ISO 4287 standard. Values of the roughness parameters were averaged taken the results obtained from the 3D maps available after their construction from the 5 SEM images taken at different tilts.

Figure 4.

Figure 5.

It is important to bear in mind that surface roughness calculation is a scale-dependent process, i.e., it results from undulations and imperfections on the surface of a material in relation to the observed or scanned area.

Therefore, average surface roughness parameters may be different at different scales [14,15]. In order to take into account this, the roughness parameters were obtained at different scales reducing the dimensions of the original image profile by 10 %, 20 %, 30 %, 40 % and 50 %, respectively. The objective of this procedure is to find what scale, in terms of roughness, better correlates with wettability data and, if possible, with subsequent biofilm development.

Data associated to the roughness parameters are gathered in Table 2. As can be observed, there is a clear increase in roughness the higher the concentration of the polymer solution to be blow spun is, increasing by almost 900 % when the concentration of polymer solution changes from 1% to 10%, in accordance to the microscopy results.

Table 2

On the other hand, from a reduction of the scale up to 50 % there are clearly changes in the values of the roughness parameters for the most heterogeneous surfaces but always in the microscale. It is observed that if roughness, represented by the arithmetic mean height, R_a , has values around 2 μm, its reduction falls within the experimental uncertainty range.

The roughness decrease as a function of the scale used to make the roughness calculations has been plotted in Figure 6. From an extrapolation it is striking to see how at about 100 % of scale reduction there is a tendency to obtain the same value of roughness. Therefore, for the system under study, if there is any effect

on the bacteria adhesion due to topography changes that should be attributed to variations at microscale from surfaces elements larger than $\sim 400 \mu\text{m}^2$.

Figure 6.

At this point it is possible to look for correlations between the surface roughness produced by the blow spun process and other properties related to adhesion, as the wettability and/or surface free energy. The wettability of liquids on solid surfaces is governed by the chemical composition and structure of the surface as well as the topographic texture and roughness at the micro/nano-length scales [20–23]. Taking into account that no gross structural changes are observed regardless the conditions of the EVA film preparation (Figure 2), any change in wettability should be due to the topography characteristics.

Water drops on surfaces of the materials studied are shown in Figure 7. It is observed how there is a tendency to increase the contact angle when the concentration of EVA solution increases (Table 3). As a reference, a water drop on the surface of a simple casted 10 % EVA solution has been also included (Figure 7a). In this case the contact angle is only higher than that obtained with the sample prepared by SBS with a 1 % EVA solution (Figure 7b), as expected, since with the 1 % EVA solution the aluminium foil used was not fully coated (Figure 3a). Therefore when the aluminium substrate is not fully covered the structure or the physicochemical properties of the surface directly in contact with the liquid drop should be quite different. In fact, the aluminium regions are more hydrophilic as finally reflected its better wetted surface.

Figure 7.

Considering the data corresponding to the roughness data (Table 2) and the contact angles (Table 3) it is clear that the higher the roughness the higher the contact angle when a hydrophilic test liquid is used, while the opposite occurs with less polar liquids. Therefore, roughness may affect wettability even in opposite ways depending on the physicochemical characteristics of both the liquid and substrate. The system studied shows a typical behaviour in which wettability is influenced by the roughness under the mechanism explained by the Wenzel model [24] when a hydrophobic liquid is used. By contrast, when the liquid is hydrophilic the Cassie-Baxter model [25] explains better the wettability of the blow-spun EVA.

Table 3

The values of surface free energy are shown in Table 4. As expected a clear correlation between the values of the contact angles and surface free energies can be observed; thus, it can be inferred that the surface free energy is highly conditioned by the surface topography. Therefore to get information about the physicochemical properties, just in terms of interactions of the EVA surfaces with *E. coli* cells, using surface free energy values, might be very adventurous unless further explanation were given about the surface properties of bacteria.

Table 4

The final step has been to study the *E. coli* adhesion on the blow-spun surfaces. This has been performed by the direct SEM observation of the single cells remaining over the substrates after the fixation process. In Figure 8, as an example, SEM images of *E. coli* cells dispersed over the surface of two different samples are shown. Single cells (pointed with yellow arrows) can be seen

uniformly distributed all over the surfaces. Although not shown here, the rest of the samples showed similar morphologies, with the only differences associated to the topography of the film. From a careful image examination it was observed (Figure 8) that, when comparing between the samples in terms of *E. coli* adhesion, the higher the EVA concentration of the solution blow spun the higher the *E. coli* adhered to the material.

Figure 8.

Single cell counting has been performed taking 5 images per sample. The results are gathered in Table 5 in which the standard deviations are provided in a separate column to give them more the idea of width of a distribution than an error. It is confirmed how, in general, the higher the concentration of the EVA solution, the higher the number of E-coli cells adhered. Since the main difference between the surfaces of the samples is the change in their topography, this must be the main factor controlling the cell adhesion. In order to see more clearly this effect the average number of cells adhered as a function of the roughness of the EVA samples has been represented in Figure 9. Except for the sample EVA-1 there is a monotonically increase of adhered cells when the roughness increases.

Table 5

However, the physico-chemical information given by the values of the contributions to the surface free energy (Table 4) and the results shown in Figure

9 also would point out the hydrophobic character of the surfaces as the driving force of the *E. coli* adhesion since, the more hydrophobic surface the higher *E. coli* adhesion. To confirm the later the *E. coli* strain used in this work should have mainly a hydrophobic surface character usually characterize by the presence of dispersive interactions when they are in contact with any substrate. However, that is not the case as it will be seen later.

Cell-surface hydrophobicity assay of bacteria DH5 α was already performed by Christopher W. Reid et al. [26] 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. [27] From the later, cell surface hydrophobicities of *E. coli* DH5 α was determined: BATH (%) = 31.1 $\pm$ 2.76

Assuming the possibility of estimating the surface tension of any bacteria from considering the weighted contributions of each solvent used in the BATH method (water and hexadecane in this case) one could say that:

$$\begin{aligned}\gamma_d(\text{bacteria}) &= \phi_1 \cdot \gamma_d(1) + \phi_2 \cdot \gamma_d(2) \\ \gamma_p(\text{bacteria}) &= \phi_1 \cdot \gamma_p(1) + \phi_2 \cdot \gamma_p(2)\end{aligned}\tag{1}$$

where the subscripts (1) and (2) refer to the solvents 1 and 2 respectively (water and hexadecane) and ϕ refers to the 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

$$\begin{aligned}\gamma_d(\text{bacteria}) &= (1-0.311) \cdot 21.8 + (0.311) \cdot 27.5 = 23.6 \text{ (mN/m)} \\ \gamma_p(\text{bacteria}) &= (1-0.311) \cdot 51.0 + (0.311) \cdot 0.00 = 35.1 \text{ (mN/m)} \\ \gamma_t(\text{bacteria}) &= 23.6 + 35.1 = 58.7 \text{ (mN/m)}\end{aligned}$$

These results are in accordance with bacteria cells having a surface mainly polar, pointing lower *E. coli* adhesion the higher the dispersive contribution to the surface free energy. However, the experiment results showed just the opposite (Table 4 and Figure 9). Therefore in this kind of EVA based systems the *E. coli* DH5 α adhesion must be more controlled by the topography than by the proper specific interactions.

Figure 9.

The only exception in the tendency corresponded to the sample prepared from a 1 % EVA solution, in which despite the roughness is clearly low, the number of adhered bacteria is the highest. However, this sample has not a pure EVA surface. Once again this result can be explained taking into account that the aluminium foil used to prepare the EVA materials was not completely coated, conferring to the surface of this substrate different physico-chemical properties arising from the aluminium with quite higher polarity. This result again reinforces the conclusion given above, when the physico-chemical properties in terms of specific interactions do not change, as it happens in EVA systems under consideration, the topography since to be the main cause affecting the *E. coli* DH5 α adhesion.

4. Conclusions

EVA films with controlled surface properties were prepared by solution blow spinning, SBS. The initial concentration of the EVA solution to be blow spun was the variable considered to get different topographies on the surfaces. A correlation between surface characteristics, particularly the roughness, with *E. coli* adhesion was established, trying to understand, among other things, the contribution of surface topography on the biofilm development. As FTIR spectra reflected the structure of the EVA polymer does not seem to be affected by the mild processing conditions considered. By contrast, topographic, morphologic analysis and contact angle measurements shown big differences on the surface characteristics, indicating that by simply changing the concentration of the initial polymer solution it is possible to tailor the topography of the final blow spun polymer and therefore their surface properties. It was found that the change of topography for the system under study, where the physico-chemical properties do not change, was the main factor controlling the *E. coli* cell adhesion. Besides, the scale for roughness calculation which better correlates wettability data was explored. Data extrapolation shown that when reducing the scale up to 100 % the

roughness tend to the same value of about $R_a = 1.2 \mu\text{m}$. This result pointed out that the bacteria adhesion controlled by topography changes seems to be due to variations at microscale from surfaces elements larger than $\sim 400 \mu\text{m}^2$.

Acknowledgements

Authors gratefully acknowledge financial support from the Project MAT2014-59116-C2 (Ministerio de Economía y Competitividad). We also greatly appreciate the technical support from the Tissue Engineering and Regenerative Group (Department of Bioengineering and Aerospace Engineering) of the Carlos III University of Madrid, particularly to Angélica Corral and José Luis Jorcano.

5. References

- [1] T. V. Duncan, Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors, *J. Colloid Interface Sci.* 363 (2011) 1–24. doi:10.1016/j.jcis.2011.07.017.
- [2] H.M.C. de Azeredo, Nanocomposites for food packaging applications, *Food Res. Int.* 42 (2009) 1240–1253. doi:10.1016/j.foodres.2009.03.019.
- [3] L. Vermeiren, F. Devlieghere, M. Van Beest, N. De Kruijf, J. Debevere, Developments in the active packaging of foods, *Trends Food Sci. Technol.* 10 (1999) 77–86. doi:10.1016/S0924-2244(99)00032-1.
- [4] P. Appendini, J.H. Hotchkiss, Review of antimicrobial food packaging, *Innov. Food Sci. Emerg. Technol.* 3 (2002) 113–126. doi:10.1016/S1466-8564(02)00012-7.
- [5] R.J. Crawford, H.K. Webb, V.K. Truong, J. Hasan, E.P. Ivanova, Surface topographical factors influencing bacterial attachment, *Adv. Colloid Interface Sci.* 179–182 (2012) 142–149. doi:10.1016/j.cis.2012.06.015.
- [6] E. Preedy, S. Perni, D. Nipiç, K. Bohinc, P. Prokopovich, Surface Roughness Mediated Adhesion Forces between Borosilicate Glass and Gram Positive Bacteria., *Langmuir*. 30 (2014) 9466–9476. doi:10.1021/la501711t.
- [7] H. Tang, T. Cao, X. Liang, A. Wang, S.O. Salley, J. McAllister, et al., Influence of silicone surface roughness and hydrophobicity on adhesion and colonization of *Staphylococcus epidermidis*, *J. Biomed. Mater. Res. - Part A*. 88 (2009) 454–463. doi:10.1002/jbm.a.31788.
- [8] L.H.C.M. E.S. Medeiros, G.M. Glenn, A.P. Klamczynski, W.J. Orts, Solution blow spinning: a new method to produce micro- and nanofibers from polymer solutions, *J. Appl. Sci.* 113 (2009) 2322–2330. doi:10.1002/app.30275.
- [9] R. Coles, M.J. Kirwan, D. McDOWELL, *Food Packaging Technology*, Blackwell Publishing Ltd., London, 2003.
- [10] S. Srinivasan, S.S. Chhatre, J.M. Mabry, R.E. Cohen, G.H. McKinley, Solution spraying of poly(methyl methacrylate) blends to fabricate microtextured, superoleophobic surfaces, *Polymer (Guildf)*. 52 (2011) 3209–3218. doi:10.1016/j.polymer.2011.05.008.
- [11] A. Abdal-Hay, P. Vanegas, J.K. Lim, Air jet spray of nylon 6 membrane structures for bone tissue engineering, *Mater. Lett.* 125 (2014) 51–55. doi:10.1016/j.matlet.2014.03.138.
- [12] D. Domingos da Silva Parize, J. Elvis de Oliveira, M. Mitsuyuki Foschini, J.

- Manoel Marconcini, L. Henrique Capparelli Mattoso, Poly(lactic acid) fibers obtained by solution blow spinning: Effect of a greener solvent on the fiber diameter, *J. Appl. Polym. Sci.* 43379 (2016) 1–10. doi:10.1002/app.43379.
- [13] J.E. Oliveira, E.A. Moraes, R.G.F. Costa, A.S. Afonso, L.H.C. Mattoso, W.J. Orts, et al., Nano and submicrometric fibers of poly(D, L -lactide) obtained by solution blow spinning: Process and solution variables, *J. Appl. Polym. Sci.* 122 (2011) 3396–3405. doi:10.1002/app.34410.
- [14] A.M. Homola, J.N. Israelachvili, P.M. McGuiggan, M.L. Gee, Fundamental experimental studies in tribology: The transition from “interfacial” friction of undamaged molecularly smooth surfaces to “normal” friction with wear, *Wear*. 136 (1990) 65–83. doi:10.1016/0043-1648(90)90072-I.
- [15] P.J. Blau, Scale Effects in Steady-State Friction, *Tribol. Trans.* 34 (1991) 335–342. doi:10.1080/10402009108982042.
- [16] E.S. Medeiros, G.M. Glenn, A.P. Klamczynski, W.J. Orts, L.H.C. Mattoso, *Solution Blow Spinning*, 1 (2014).
- [17] D.K. Owens, R.C. Wendt, Estimation of the surface free energy of polymers, *J. Appl. Polym. Sci.* 13 (1969) 1741–1747. doi:10.1002/app.1969.070130815.
- [18] D.H. Kaelble, Dispersion-Polar Surface Tension Properties of Organic Solids, *J. Adhes.* 2 (1970) 66–81. doi:10.1080/0021846708544582.
- [19] B.R. Göran Ström, Monica Fredriksson, Per Stenius, Kinetics of steady-state wetting, *J. Colloid Interface Sci.* 134 (1990) 107–116. doi:10.1016/0021-9797(90)90256-N.
- [20] A. Lafuma, D. Quéré, Superhydrophobic states., *Nat. Mater.* 2 (2003) 457–60. doi:10.1038/nmat924.
- [21] A. Marmur, A. Marmur, The Lotus Effect : Superhydrophobicity and Metastability The Lotus Effect : Superhydrophobicity and, *Langmuir*. 20 (2004) 3517–3519. doi:10.1021/la036369u.
- [22] D. Quéré, Wetting and Roughness, *Annu. Rev. Mater. Res.* 38 (2008) 71–99. doi:doi:10.1146/annurev.matsci.38.060407.132434.
- [23] M. Callies, D. Quéré, On water repellency, *Soft Matter*. 1 (2005) 55. doi:10.1039/b501657f.
- [24] R.N. Wenzel, Resistance of solid surfaces to wetting by water., *J. Ind. Eng. Chem. (Washington, D. C.)*. 28 (1936) 988–994. doi:10.1021/ie50320a024.
- [25] A.B.D. Cassie, S. Baxter, Wettability of porous surfaces, *Trans. Faraday Soc.* 40 (1944) 546. doi:10.1039/tf9444000546.
- [26] C.W. Reid, N.T. Blackburn, A.J. Clarke, The effect of NAG-thiazoline on morphology and surface hydrophobicity of *Escherichia coli*, *FEMS Microbiol. Lett.* 234 (2004) 343–348. doi:10.1016/j.femsle.2004.03.047.
- [27] M. Rosenberg, D. Gutnick, E. Rosenberg, Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity, *FEMS Microbiol. Lett.* 9 (1980) 29–33. doi:10.1111/j.1574-6968.1980.tb05599.x.

Figure captions

Figure 1. Films of EVA obtained by SBS with solutions of different concentration. From left to right 1 %, 2 %, 5 %, 7 %, and 10 % wt/wt.

Figure 2. FTIR spectra of blow-spun samples of different EVA concentrations (% wt/wt in chloroform).

Figure 3. SEM images of EVA films. Concentrations (wt/wt) of the blow spun solutions: a) 1 %; b) 2 % c) 5 %; d) 7 % and e) 10 %.

Figure 4. Example of a calculated 3D image (a) using a stereoscopic pair of SEM images (b). Film obtained from an EVA solution 10 %.

Figure 5. (Up) 2D map reconstructed from the 3D image of Figure 5a (dashed lines correspond to the profiles chosen for the make roughness calculation). (Bottom) Example of one of the five roughness profiles chosen.

Figure 6. Roughness values for blow spun EVA samples of different concentrations as a function of the reduction in the scale used to perform the roughness calculations.

Figure 7. Representative water drops on the surfaces of the blow-spun EVA samples: a) 10 % EVA water-casted; b) SBS of 1 % EVA solution; c) SBS of 2 % EVA solution; d) SBS of 5 % EVA solution; e) SBS of 7 % EVA solution and f) SBS of 10 % EVA solution.

Figure 8. Example of E-coli cells distributed over the surface for: a) SBS of a 2 % EVA solution; b) SBS of a 7 % EVA solution.

Figure 9. Average number of E-coli adhered as a function of the roughness of the EVA samples and the initial concentration of the blow spun EVA solutions.

Tables

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)	Supplier
Water	0.998 ^b	21.8 ^b	51.0 ^b	72.8 ^b	Home lab
Glycerol	1.259 ^a	37.0 ^b	26.4 ^b	63.4 ^b	Panreac
Diiodomethane	3.220 ^a	50.8 ^b	0.00 ^b	50.8 ^b	Sigma-Aldrich

^a Data given by the supplier.

^b Data obtained from reference[19].

Table 2.- Roughness parameters obtained using different scale reductions of the original image for different EVA concentrations.

		SAMPLE	EVA-1	EVA-2	EVA-5	EVA-7	EVA-10
Scale Reduction	0%	Ra (μm)	2.13±0.22	2.06±0.51	5.41±0.42	13.03±2.74	20.9±4.93
		Rq (μm)	2.32±0.47	2.45±0.54	6.57±0.54	15.73±2.80	27.8±6.94
	10%	Ra (μm)	2.06±0.33	2.01±0.54	5.27±1.03	11.97±3.13	18.4±7.90
		Rq (μm)	2.52±0.40	2.25±0.63	6.42±1.31	14.53±3.36	23.5±10.2
	20%	Ra (μm)	1.98±0.24	1.97±0.48	5.26±1.07	11.43±3.53	18.1±8.08
		Rq (μm)	2.43±0.29	2.38±0.57	6.38±1.34	14.12±4.22	23.0±10.7
	30%	Ra (μm)	1.96±0.36	2.04±0.60	5.01±1.08	10.40±3.42	14.2±4.44
		Rq (μm)	2.39±0.46	2.43±0.70	6.19±1.41	12.70±3.96	18.5±6.38
	40%	Ra (μm)	1.81±0.30	1.75±0.56	4.05±1.46	9.91±5.50	16.3±8.87
		Rq (μm)	2.22±0.36	2.11±0.65	4.91±1.83	12.16±6.53	21.5±12.8
	50%	Ra (μm)	1.75±0.14	1.46±0.49	2.99±0.50	5.40±0.92	9.66±1.55
		Rq (μm)	2.11±0.10	1.74±0.50	3.59±0.49	6.62±1.04	12.2±2.23

Table 3.- Contact angles obtained for the test liquids used.

Sample	θ (°)		
	Water	Glycerol	Diiodomethane
EVAcasting	95.70 ± 2.80	84.44 ± 2.99	61.17 ± 4.46
EVA-1	83.30 ± 2.28	71.98 ± 2.26	51.38 ± 2.90
EVA-2	102.8 ± 1.20	88.47 ± 1.58	58.83 ± 1.92
EVA-5	115.4 ± 1.50	97.08 ± 1.66	42.70 ± 1.98
EVA-7	111.0 ± 3.21	97.70 ± 4.73	46.34 ± 4.90
EVA-8	110.1 ± 2.79	94.16 ± 4.12	44.34 ± 5.10
EVA-10	133.3 ± 3.25	116.9 ± 4.77	23.69 ± 7.04

Table 4.- Values of surface free energy obtained from the Owens-Wend method.

Sample	γ_t (mN/m)	γ_p (mN/m)	γ_d (mN/m)
EVA-casting	28.4 ± 2.8	1.1 ± 0.3	27.3 ± 2.7
EVA-1	35.4 ± 1.8	2.7 ± 0.7	32.8 ± 1.7
EVA-2	30.9 ± 1.4	0.0 ± 0.1	30.9 ± 1.4
EVA-5	38.4 ± 1.6	1.7 ± 0.4	36.8 ± 1.6
EVA-7	36.4 ± 3.4	0.7 ± 0.6	34.7 ± 3.3
EVA-10	53.0 ± 3.6	9.2 ± 1.6	43.7 ± 3.2

Table 5.- Number of *E. coli* cells per surface unit.

Sample	Average number (bac/mm ²)	Standard Deviation (bac/mm ²)
EVA-1	10554	5007
EVA-2	2472	1829
EVA-5	3764	1806
EVA-7	6107	5059
EVA-10	7971	2729