Obtention of Scaffolds for Grafts on Human Skin with PLA-Nanofibers and Cinnamon Oil-Lanoline Emulsions

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Abstract: The interest in tissue engineering is growing in the scientific and medical communities, as it can solve actual problems regarding donor tissues, wound healing and drug delivery systems. Nanofibers are gaining relevance in this topic thanks to their excellent mechanical properties and similarities concerning the human skin.

This project has explored how combining the nanofibers' membranes created of PLA, made by electrospinning, with a dissolution of lanoline and cinnamon essential oil not only imitates the human skin, as it was demonstrated in a later project, but also obtains an antibacterial character. Analytical techniques such as a spectrophotometer, an electrokinetic analyzer, a scanning electron microscope, a Fourier transform infrared spectroscope, and an optical tensiometer were employed.

Results confirmed successful integration and migration of the cinnamon oil, with antibacterial efficacy achieved against specific bacterial strains, as hypothesized. Notably, scaffolds composed of seven layers exhibited migration behavior closely aligned with theoretical expectations.

Keywords: Electrospinning, Scaffolds, Cinnamon essential oil, PLA, Lanoline.

1. INTRODUCTION

Burns are among the most complex injuries to manage in the medical field due to their high susceptibility to infection. Proper care is essential to prevent infections, promote healing, and minimize long-term complications. In this context, tissue engineering has emerged as an innovative solution to develop alternatives to traditional grafts, particularly with scaffolds. These structures, intended for use as skin grafts, must meet specific requirements, including biocompatibility, a porous structure, mechanical properties similar to human skin, and antibacterial capabilities.

Human skin is composed of the epidermis, dermis, and hypodermis, plays a critical role in protection and regeneration. The outermost layer, the epidermis, includes the stratum corneum (SC), which consists of 15 to 20 layers of flat polyhedral corneocytes surrounded by a lipid matrix that acts as a permeability barrier. The SC serves multiple purposes: it protects against external agents such as bacteria, viruses, and chemicals; prevents water loss; absorbs ultraviolet radiation; and reduces friction and wear [1]. This matrix resembles wool wax lanoline, a substance composed of 87% high molecular weight esters and 11% aliphatic alcohols, fatty acids, and hydrocarbons [2-5]. Due to this similarity, lanoline is widely used in experiments related to skin absorption [6].

Lanoline, a natural substance derived from sheep wool, is commonly referred to as "wool wax" or "wool oil." It is highly valued in the cosmetics and pharmaceutical industries for its beneficial properties in treating skin and hair [4]. Its resemblance to the lipid matrix of the SC makes it an excellent candidate for use in skin-related applications.

Polylactic acid (PLA), a biodegradable and environmentally friendly polymer, has also gained attention for its role in tissue engineering. Derived from renewable resources such as corn starch, sugarcane, and yucca, PLA offers advantages such as non-toxicity, bioabsorbability, hemocompatibility, and biocompatibility, making it suitable for promoting tissue repair and regeneration [7-10]. A key technique for fabricating PLA-based scaffolds is electrospinning, which produces fibres with diameters ranging from micrometres to nanometres. These fibres exhibit unique characteristics, including surface flexibility, high

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porosity, interconnected pores, and strong mechanical performance [11]. During electrospinning, a polymer solution is stretched through high electric fields, creating a jet that solidifies as the solvent evaporates, forming randomly deposited fibres [11].

The antibacterial properties of scaffolds are critical in preventing infections during wound healing. Antibacterial substances inhibit microbial growth by disrupting cell wall synthesis, membrane integrity, nucleic acid replication, or protein production [12]. Essential oils, with their lipophilic properties, are effective antibacterial agents. Cinnamon essential oil (Cinnamomum zeylanicum) has been widely studied for its antibacterial activity, attributed primarily to its cinnamaldehyde content (60–75%) and other volatile compounds, including cinnamyl acetate, 2-methoxy-cinnamaldehyde, cinnamyl alcohol, and coumarin [13]. However, its volatility makes it sensitive to light and heat, leading to rapid degradation [14].

Cinnamon essential oil (CEO) was chosen due to its strong antibacterial activity compared to other essential oils [13]. By combining it with PLA and lanoline emulsions, it is possible to develop scaffolds with enhanced properties for skin graft applications.

Therefore, the aim of this study is to prepare and characterize scaffolds with PLA nanofibers incorporating lanoline emulsions and CEO, evaluating their antibacterial potential as a possible graft material for future medical applications.

2. EXPERIMENTAL PROCEDURE

2.1. Materials

Polylactic acid (PLA) as polymer and Hexafluoro isopropanol (HFIP) as solvent, both from Sigma-Aldrich, were used in the membrane formation.

Cinnamon essential oil (CEO) (Nature Cosmetics, UK), Lanoline, Tween 20, Span 60 and SDS (Merck, Germany) were used to prepare the scaffold.

For antibacterial tests two specimens were used: *Escherichia coli* (negative-gram) and *Staphylococcus aureus* (gram-positive) from Essays Laboratory (Barcelona, Spain)

2.2. Membrane Formation

Nonwovens-fabrics were prepared using an electrospinning prototypal device with biocompatible polymers, a machine designed and built by INTEXTER-UPC (Terrassa, Spain) It makes possible to change process parameters as required since the concept of the machine is totally open. The new contribution to the structure has been a cylindrical rotor as a collection electrode, which will allow the desired veils to be obtained directly. The basic assembly consists of a capillary through which the polymeric solution is expelled, a high-voltage source that possesses two electrodes that connect one by where the solution goes out and another at the collector plate where the fibres will be placed [11]. It can be developed in different forms, in our case the collector will be placed on top of the capillary, avoiding possible drops of solution dropping and damaging the membrane (Figure **1**).

Figure 1: Electrospinning machine from INTEXTER- UPC.

Polymeric solution of PLA and HFIP is detailed at Table **1**. A magnetic mixer at 300rpm for at least 19 hours was used.

Table 1: Quantity of PLA and Solvent used for the Preparation of the Polymeric Solution

Substance	Order of Measure	Quantity
PLA	1	1,58g
HFIP	2	10mL

Once the solution is prepared, we have to make the veils with the electrospinning machine. In order to decide the ideal parameters two different options were carried out. The different variables can be seen in Table **2**.

Table 2: Comparison between the Preparations of the Different Membranes (Veils)

Code	Time (min)	Voltage (kV)	Flow (mL/h)	Height capilar-collector plate (cm)
V1	1	6	1,4	8
V2	2	6	1,4	8

2.3. Essential Oil Formulations: Lanolin Emulsion

The essential oil cannot be applied onto the polymer chains of the scaffold directly. Not all the components of the formulae of EO behave equal in front of the interactions of the matrix base. In order to ensure the uniformity of composition, and the size of the colloidal particles, an emulsification procedure will be applied. Emulsion will allow to keep the oil on the veils, being recovered by lanoline (LAT). It was prepared using Ultraturrax 75 (IKA, Germany) at 10,000 rpm during 5 minutes, at environmental temperature (25ºC), and avoiding its direct contact with the fibres and protecting the membrane from its rupture. The composition of the emulsion can be seen in Table **3**.

In this case, the droplet touched the membrane and was distributed quickly by all the surface (see Figure **3**).

2.4. Scaffold Preparation

Different scaffolds were prepared during all the study due to the optimization of the structure composed by nanofibres veils and essential oil emulsion layers. The preparation consists of layering the chosen formulation onto the veils in a sandwich-like arrangement. The order is Nanofibers-Emulsion-Nanofibres- Emulsion-Nanofibers. That makes possible to control the hydrophilicity of the external surface and regulate the permeability of the sandwich structures using the amount and chemical character of the emulsified Lanolin.

2.5. Scaffold Characterization

2.5.1. FT-IR

FT-IR Spectrophotometric measurements were done using *FTIR-8300* (Shimadzu, Japan) in the spectral region of 4000 to 500 cm^{-1} to verify the interactions of the polymers of scaffolds and lanoline.

2.5.2. SEM

Superficial morphology and topography analysis of the scaffolds were carried out with scanning electron microscope JEOL (Japan), JS-5610, and the gold overlay made in the SCD 005, Bal-Tec equipment.

2.5.3. Surface Zeta Potential

To determine the zeta potential and how it changes when adding different layers to the scaffolds the samples were analysed with the EKA (electrokinetic analyser, Anton Paar USA). The electrokinetic cell working at variable electrical voltage, with a solution of 0,01 M KCl as the basis electrolyte. The exposed area, 2cm diameter tissue discs with variable flux of the electrolyte (right to left and left to right).

To protect the membranes and avoiding their rupture because of the water jet, two cotton layers (fabrics) of 1 mm width, were used, during the measurements of the sandwich scaffolds and they were also added during the rest of the tests to act as the blank reference.

2.5.4. Antibacterial Test

Experiments were designed based on the AATCC protocol 147" Antibacterial Assessment of textile materials" [16]. The main objective of this analysis is to check the bacterial activity of each tissue sample, at static conditions and using the decrease of the number of bacterial colonies as a level of antibacterial activity. It is based on the growth of the organism when it comes in close contact with the impregnated sample, keeping the tissue only submitted to the diffusion and transport mechanisms promoted by its own structure.

Table 3: Percentages from the Emulsion LAT

	CEO	Lanoline	Tween20	Span60	SDS	Water
%	0'8	2'7	3'9	0'1	5'3	87'2

Following the instructions by AATCC [16], samples were incubated during 24 hours at 37˚C to see whether tissue samples present, or not, antibacterial properties. The study was carried out for the two types of bacteria, described before, *Escherichia coli* and *Staphylococcus aureus.*

2.6. Drug Delivery

The retention of the system emulsion-nanofibers, in an aqueous media, should control the antibacterial effect of the essential oil as well as the migration of Lanoline inside the scaffold. The cinnamon oil, inside the micelle formed by lanolin and the mixture of surfactants, will be delivered in two different mechanisms: firstly, the oil will diffuse trough the layer of the micelle and will be retained inside the polymeric matrix, from where it will diffuse towards the surface of the scaffold and transported to the skin to avoid the growth of bacteria.

These two mechanisms will be developed simultaneously as it happens in a Serie of consecutive mass transport mechanisms. The concentration gradient in each phase will control the rate of the apparent diffusion from the inner structure to the surface.

The delivery is catalysed by water that will enter, very quickly inside the scaffold, displacing the essential oil towards the external region. Once the essential oil is on the bath, and due to the organic character of its components, it will be absorbed by the substrate and then there will be an equilibrium situation that should be quantified using the approach of Peppas-Sahlin [15, 17].

3. RESULTS AND DISCUSSION

3.1. Scaffold Preparation

One PLA non-woven veil, as detailed at experimental part, was prepared, M2. The most notable characteristic is the transparency, as the M2 that has been 20 minutes. The elasticity of M2 is similar to human skin and so M2 was chosen to build up the sandwich structure as shown in Figure **2** to human skin.

To check previously what happens between CEO and PLA membranes, three different formulations were used to prepare first sandwich structures. The base would be the same for all of them, using five veils M2 with the formulation at the centre of the structure, as can be seen in Figure **2**.

The formulation 1 (F1) was a droplet of CEO in pure liquid form. In another structure, F2, one droplet of dissolution with micelles of CEO with a ratio of 1:1.5:1.5 of acacia gum and chitosan. The last sandwich had F3, a droplet of dissolution with micelles of CEO with a ratio of 1:0’5:1 of acacia gum, and chitosan (Table **4**).

Little fissures appeared in all the structures; this may be because one of the components of the CEO migrates faster than the rest and degrades the PLA (see Figure **3**).

Figure 2: Simplified structure of the sandwich scaffolds.

Table 4: Formulations of CEO Applied to form Sandwich scaffolds (MC: Microcapsules with Lanolin)

	Span 80	CEO	Acacia gum 1%	Chitosan 1%
F1	-	1 droplet	-	-
F2	20 ml of 0.4 g/L	1 droplet MC	30ml	30 ml
F3	20 ml of 0.7 g/L	1 droplet MC	20 ml	10 ml

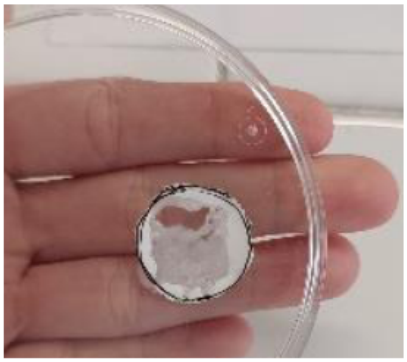

Figure 3: Photo of the results for the first trials with CEO directly applied on veils.

Given the inability to attach the oil without degrading the polymer in the membranes as initially studied, alternative options were explored. A solution was prepared to allow the oil to remain on top of the veils, coated with lanolin to prevent direct contact with the fibres and protect the membrane from rupture. That means that it is necessary to increase the amount of PLA nanofibers between the possible formulations of CEO, and that biopolymers without any order or structure cannot retain the component responsible of the degradation of the PLA layer.

The next trial was using lanoline emulsion with CEO as formulation (LAT) applied between veils, see in Figure **4**.

The use of the emulsion facilitated scaffold formation, allowing for the evaluation of various properties.

3.2. Migration Tendency

The migration tendency of the emulsion into the scaffolds prepared was analysed through the EKA tests. When the emulsion migrates in front of a flux of ionic solutions, that means that the content of lanolin-oil emulsion is being uniform inside the scaffold. Meanwhile, if the emulsion stays static, there should be differences between the flux in one sense related with the flux in the contrary sense. In this case, two nude membranes, one scaffold of 2 layers and another one of 4 layers were analysed. The blank sample, as mentioned before, was two layers of cotton, they were also added during the rest of the tests protecting the membranes and avoiding their rupture because of the water jet.

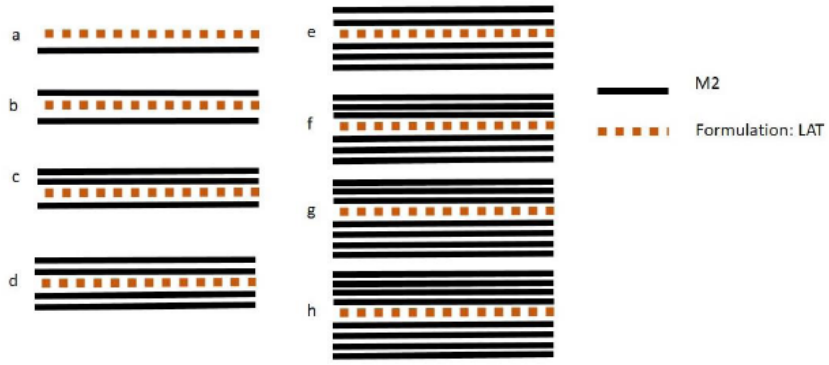

Figure 4: Different scaffold structures **a**) 1 layer, **b**) 2 layers **c**) 3 layers, **d**) 4 layers, **e**) 5 layers, **f**) 6 layers, **g**) 7 layers and **h**) 8 layers

Table 5: Results Obtained at the EKA Tests. (% LAT over Total Amount of the Structure)

Sample	%LAT	Flux Direction	Zeta Potential (mV)
Cotton blank	0,0	Left-Right	-0,1774
		Right-Left	-42,58
2 nude membranes	0,0	Left-Right	-0,1117
		Right-Left	-0,7981
Scaffold 2 layers	79,1	Left-Right	-0,0372
		Right-Left	-0,5796
Scaffold 4 layers	87,9	Left-Right	-0,0159
		Right-Left	-19,99

The charge and the potential vary if the sample's structures are modified, changing its properties. Any migration of LAT can be seen in Table **5** with the zeta potential values obtained.

As can be perceived, the change at the 2 nude membranes is small, just as in the scaffold of 2 layers case. In the scaffold test what happened is that under the critical micelle concentration (CMC) and water shows not affinity for the LAT, also, the system still has not reached the critical potential, so it is not possible to see a noticeable difference. However, a huge difference is observed when comparing the results from the scaffold of 4 layers. In this case, we can see that LAT migrated satisfactorily.

3.3. FT-IR Spectroscopy

Another way to detect the migration is using the FT-IR in ATR device. In this case, we compared the spectrum obtained from 2 nude membranes (Figure **5**) with the spectrums obtained from scaffolds of 2 and 4 layers before and after the drug delivery test (Figure **6**).

We can conclude that molecular interaction has happened because there are no the same functional groups in the spectrums. Between 3500 and 3200 cm^{-1} we can see a peak in the spectrums from the scaffolds before doing the drug delivery that disappears after this test. This peak is from the bound O-H of LAT. Another peak that appears in the spectrums of the 2 nude membranes and both scaffolds after drug delivery is the one from the bound C-H between 3000 and 2800 cm^{-1}.

3.4. Drug Delivery

The drug delivery test was done on scaffolds from 1 to 8 layers to see how the CEO migration changes when adding membranes. To see the migration tendency, it was decided to plot the experimental results next to the data gathered with equation 1, the Peppas-Sahlin equation [15].

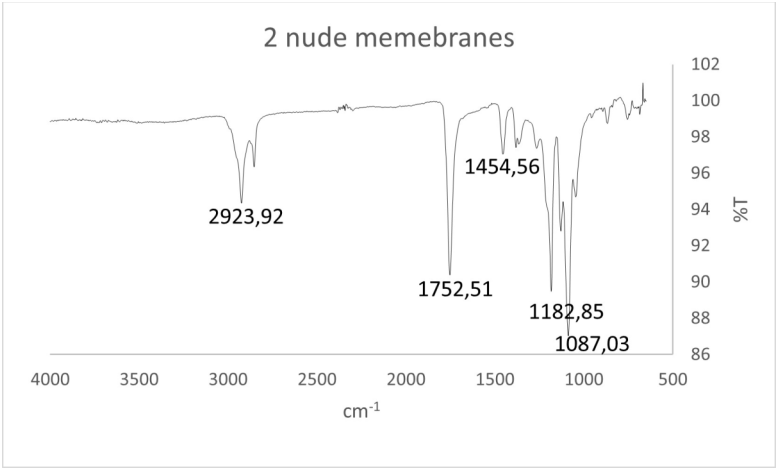

Figure 5: FT-IR Spectrum from 2 nude membranes.

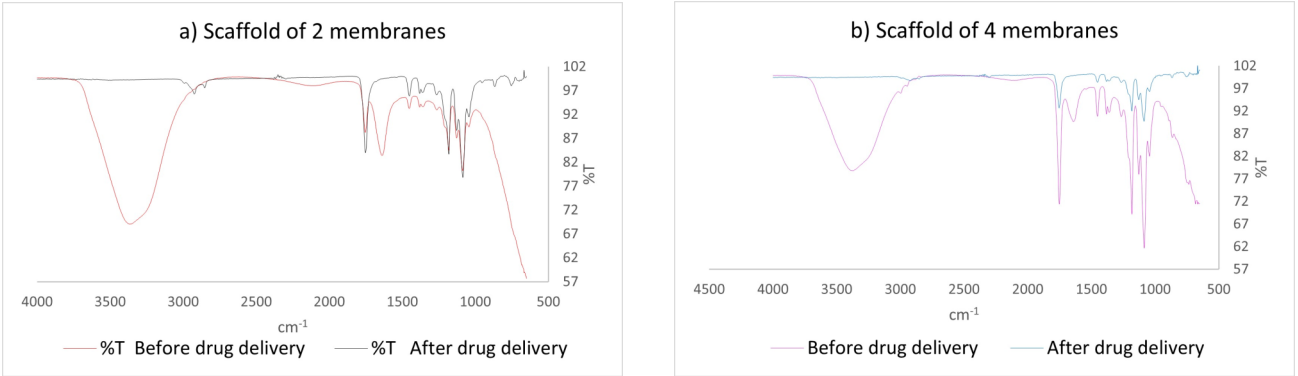

Figure 6: FT-IR Spectrum from (**a**) scaffold of 2 membranes where red is before and black after drug delivery and (**b**) scaffold of 4 membranes where pink is before and blue after drug delivery.

$$\frac{C_t}{C_\infty} \cong \frac{C_t}{C_{max}} = k_1 \cdot t^n + k_2 \cdot t^{2 \cdot n} \quad \textbf{Eq. 1}$$

Two random dots of C_t/C_{max} were chosen, specifically the corresponding to minutes 2 and 20 of each scaffold. With these values and changing n, always around 0,5, we could obtain k1 and k_2. Once we obtained all the variables of the equation, we plotted all graphics of the different tendencies (Figure **7**).

The different values of k1, k2 and n can be seen in Table **6**.

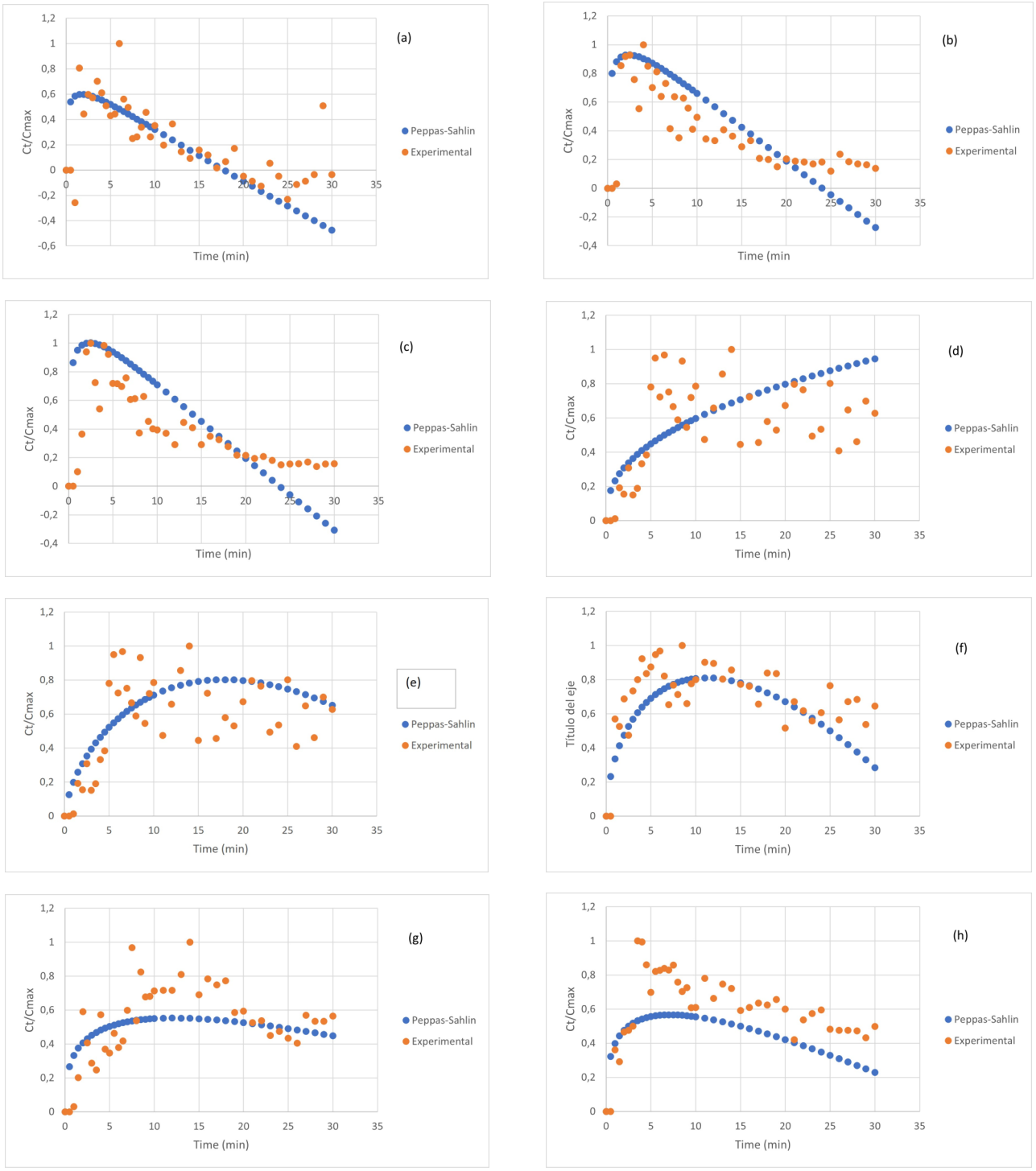

Figure 7: Comparison between C_t/C_{max} experimental and theoretical through Peppas-Shalin equation from scaffolds of (**a**) 1 layer, (**b**) 2 layers, (**c**) 3 layers, (**d**) 4 layers, (**e**) 5 layers, (**f**) 6 layers, (**g**) 7 layers and (**h**) 8 layers.

Table 6: Values of k1, k2 and n from the Equation Peppas-Sahlin of each Scaffold

	1 layer	2 layers	3 layers	4 layers	5 layers	6 layers	7 layers	8 layers
k_1	0,771	1,432	1,548	0,229	0,213	0,380	0,407	0,515
k_2	-0,241	-0,552	-0,598	0,004	-0,015	-0,045	-0,075	-0,117
n	0,4	0,3	0,3	0,4	0,7	0,6	0,4	0,4

The most similar to the theoretical ideal is the scaffold of 7 layers, as the second part of its curve is the least pronounced. In his case, diffusion and absorption are in equilibrium, not as in the scaffolds of an even number of layers.

3.5. Interphases

The different values we obtained realizing this analysis are found in Table **7**.

Table 7: Results from the Contact Angle Analysis

Sample	% LAT	Contact angle (°)
2 nude membranes	0,0	84
Scaffold 2 layers	80,6	37
Scaffold 4 layers	78,2	<30

When analysing the 2 nude membranes we can perceive that the drop of water remains static on the fibres, barely absorbed by the structure (Figure **8**).

This is due to the polarities of the membranes' interphase and the water molecules. The zeta potential of the membranes is negative, so the water molecules are positioned in a way that the hydrogen atoms rest on the fibres and the oxygen ones are as far as possible (Figure **9**).

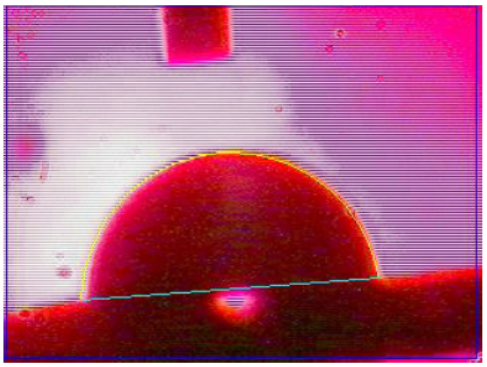

Figure 8: Image from the contact angle obtained for the 2 nude membranes.

In the scaffold made of 2 layers, we could obtain the contact angle, while in the one made of 4 layers, the drop was absorbed so fast that the corners were out of the image (Figure **10**). In this last case, we looked at the angle knowing that it was less than the obtained number, which is why in Table **6** is specified as less than 30.

The surfactants used to form the micelles reduce the interfacial tension, letting the water get into the membranes. In other words, this lets the membrane be hydrophilic so it can absorb easily the drop. When the micelles get in contact with water, they open themselves, letting the water get into the spaces the essential oil leaves.

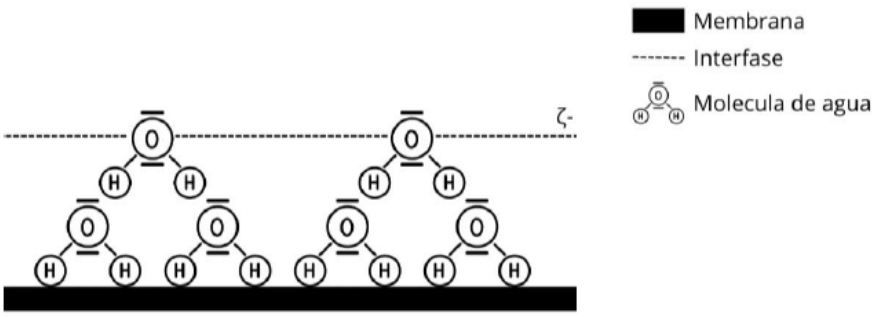

Figure 9: Model of how the water molecules are placed on the membranes without LAT.

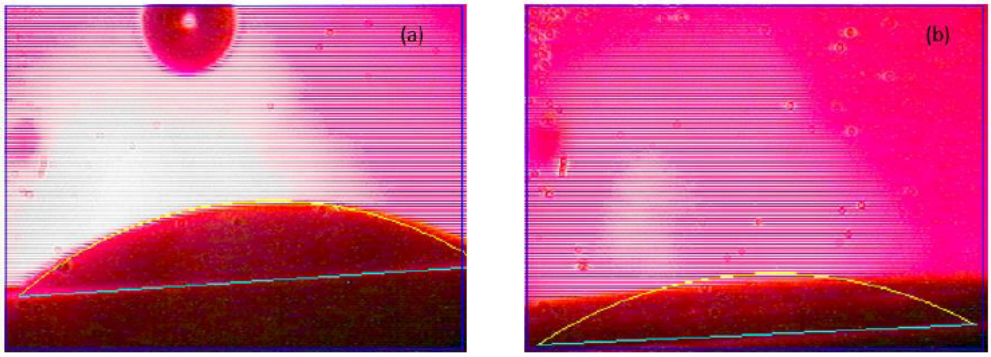

Figure 10: Images corresponding to the contact angles from the scaffold made of (**a**) 2 layers and (**b**) 4 layers.

3.6. Dissolution's Distribution

The scaffold analysed was formed by two membranes, under the microscope, the internal layer and the external layer were analysed.

In Figure **11** we can see what we obtained from the SEM when placing the nude membrane at 1000 magnifications. The interweaving of the different nanofibers and porous is perfectly observed. Little lumps are appreciated, this is known as the "pearl collar effect", which is very common at the electrospinning.

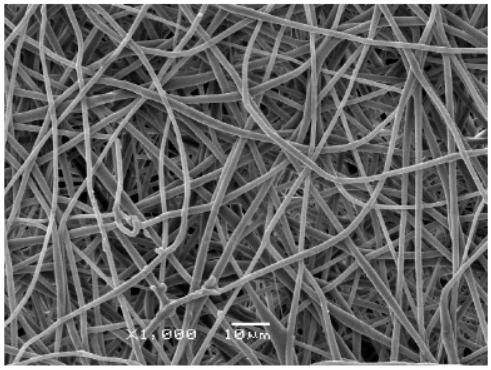

Figure 11: Obtained image from SEM of the nude membrane.

With the programme Image J the different diameters of the fibres were measured, which on average have a diameter of 1,677µm.

In Figure **12** the LAT distribution can be perceived and how it changes if we look at the internal or the external layer.

Analysing the obtained image of the internal layer we can see how LAT has been uniformly distributed at the porous. Looking carefully at the emulsion there can be seen little white dots which would correspond to the micelles formatted at the emulsion thanks to the surfactants. They can be seen better on the left side.

However, in the next image, some kind of lumps can be seen, with diameters between 13,154µm and 9,988µm. Those are aggregations of micelles. Looking with a critical eye we can also see some solitaire micelles stuck at the fibres, as in the right inferior corner.

3.7. Antibacterial Character

The percentages of LAT of the samples analysed can be seen in Table **8**.

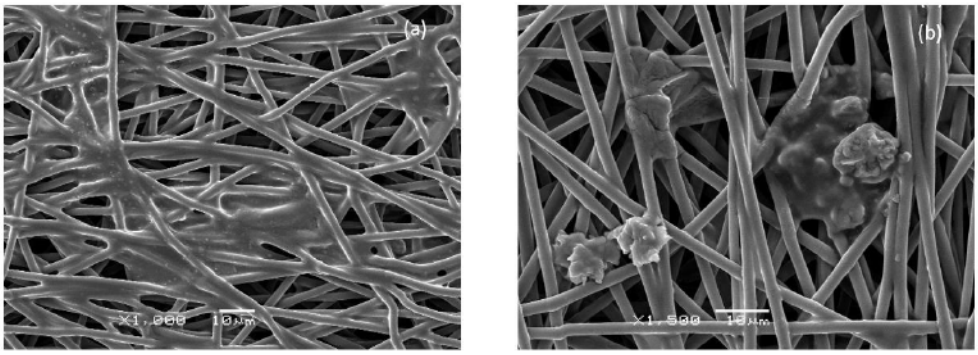

Figure 12: Obtained image from SEM layers (**a**) where LAT is located at 1000 magnifications and (**b**) external layer at 1500 magnifications.

Table 8: Percentages of LAT in the Different Structures used at the Microbial Analysis

Sample	% LAT
Nude membrane I	0,0
Nude membrane II	0,0
Scaffold 2 layers I	81,9
Scaffold 2 layers II	79,1

The first structure was placed on the gram-negative bacterial strain, while the second structure was positioned on the gram-positive bacterial strain.

As can be seen in Figure **13** the scaffold presents an antibacterial character when analysing the *Escherichia coli* bacteria. If we compare the nude membrane with the layers that conformed the scaffolds, we can see that there is no bacterial growth.

However, it is perceived bacterial growth when observing the scaffold that was positioned on the *Staphylococcus aureus* bacteria. If we compare these layers with the nude membrane, we can see that the growth is less aggressive.

4. CONCLUSIONS

The principal aim of this project was to add to PLA membranes cinnamon essential oil so we could obtain antibacterial scaffolds.

Having analysed the gathered data from the different tests we can conclude that the oil is added satisfactorily and migrates just as it was expected. Moreover, the antibacterial character has been achieved too.

Among all the analytical methods used, the drug delivery test and the FT-IR test have been the most useful in order to determine the migration of the emulsion. While the EKA test and the contact angle test have been the most important ones to see the difference between the scaffolds and the nude membranes. With the microbial study, we have been able to conclude that the scaffolds are antibacterial for a certain bacterial type, as it was postulated in the hypothesis.

With the drug delivery test, we could also determine how the migration of LAT changes depending on the number of layers used to conform the scaffolds. The scaffold made of 7 layers, in this way, is the most similar to the theoretical, as the curve is less pronounced when descending.

5. ACKNOWLEDGMENTS

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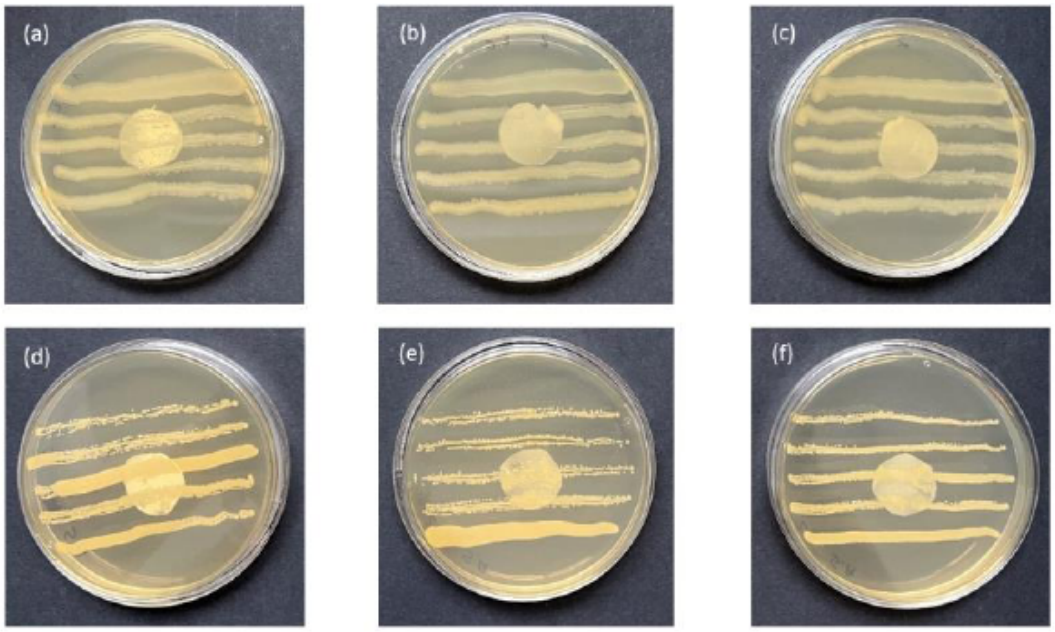

Figure 13: Results from the microbial study from **a**) nude membrane with *Escherichia coli*, **b**) interior layer with *Escherichia coli*, **c**) external layer with *Escherichia coli*, **d**) nude membrane with *Staphylococcus aureus*, **e**) internal layer with *Staphylococcus aureus* and **f**) external layer with *Staphylococcus aureus.*

CONFLICTS OF INTEREST

The author declared no conflicts of interest.

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