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Research Article

Production of Electrospun Fast-Dissolving Drug Delivery Systems with Therapeutic Eutectic Systems Encapsulated in Gelatin

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Abstract. Fast-dissolving delivery systems (FDDS) have received increasing attention in the last years. Oral drug delivery is still the preferred route for the administration of pharmaceutical ingredients. Nevertheless, some patients, *e.g.* children or elderly people, have difficulties in swallowing solid tablets. In this work, gelatin membranes were produced by electrospinning, containing an encapsulated therapeutic deep-eutectic solvent (THEDES) composed by choline chloride/mandelic acid, in a 1:2 molar ratio. A gelatin solution (30% *w*/*v*) with 2% (*v*/*v*) of THEDES was used to produce electrospun fibers and the experimental parameters were optimized. Due to the high surface area of polymer fibers, this type of construct has wide applicability. With no cytotoxicity effect, and showing a fast-dissolving release profile in PBS, the gelatin fibers with encapsulated THEDES seem to have promising applications in the development of new drug delivery systems.

KEY WORDS: anti-bacterial studies; fast-dissolving drug delivery systems; gelatin; mandelic acid; therapeutic deep-eutectic solvents.

INTRODUCTION

The development of new drugs is time-consuming and expensive. Some of the problems associated to the processing, and administration of conventional drugs, are associated to the compound concentration needed and the possibility of developing of toxic metabolites (1).

The common use of oral capsules administered with water can be impractical for children and elder people due to their difficulties in swallowing. In an attempt to improve drug safety and efficacy, alternative methods have been implemented. Controlled and slow-release systems, or systems that target specific areas, are some of the drug administration improvements (1,2). Nevertheless, in order to simplify oral drug administration, fast-dissolving intraoral drugs offer some advantages (3). The possibility of using fast-dissolving tablets not only allows for an extremely quick drug administration but also avoids physical obstruction (3,4). This type of tablets is also convenient for patients with low access to water, disabled or bedridden. Fast-dissolving delivery systems (FDDS) enable a significant increase in drug bioavailability when compared to common oral administration systems and allow for a more accurate dosage when compared to orally administered liquids (3). When in contact with the saliva, the drug in the FDDS can be absorbed in the mouth, or can be taken down to the pharynx, and esophagus, by saliva that passes down to the stomach (5). For the application of an FDDS system, the excipient or the carrier material used must exhibit certain chemical, physical and biological properties (1). Furthermore, this system should present a degradation time adequate to its function, so that it does not induce an inflammatory response (6). Its degradation products cannot be toxic and should be readily reabsorbed, or excreted, by the organism (6). On the other hand, the drug used should not influence the carrier matrix, by impacting negatively on its mechanical resistance or by promoting matrix disintegration.

The drugs used for FDDS systems belong, mostly, to the class II of the biopharmaceutics classification system, due to their poor water solubility and high tissue permeability (5).

A wide range of natural and synthetic biodegradable polymers have been studied (7) and are reported in literature,

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e.g. chitosan (8), chitin (9), starch (10), poly-ε-caprolactone (11), among others (8,12). The application of these biomaterials are wide and goes from surgical sutures and implants to tissue engineering and drug delivery (7), making a significant impact on modern biology and medicine.

Ideally, the biomaterials used for FDDS development should be from natural sources, not cause any damage or inflammatory response in the host, and be metabolized, excreted, or even reabsorbed by the human body (6,12).

Being obtained by the hydrolysis of collagen, gelatin is widely used in clinical processes (13,14). It is non-antigenic, with a favorable absorbability and relative low cost, when compared to other compounds (15). Furthermore, gelatin presents some inherent biological features, since it can accelerate wound healing and tissue regeneration (15). Furthermore, it is also used as an ingredient in drug formulations (16), sealant in prostheses and in several biomedical and medical applications (17).

Deep-eutectic solvents (DES) are a new class of solvents, with straightforward synthesis and useful properties, making them interesting alternative solvents for green applications. DES are a combination of two (or more) compounds that, in certain molar ratio, suffer a high decrease in the melting point when compared with its pure constituents (18).

The need of increasing drug bioavailability and developing safer excipients has led the investigation on DES for the development of eutectic solvents composed by one or more active pharmaceutical ingredient (API). This class of DES was named therapeutic deep-eutectic solvents (THEDES) (19,20). API in the form of THEDES presents higher permeability and dissolution rate than its pure form (21). In this work, it was used as gelatin fibers and mandelic acid was used as API. According to the ATC code, mandelic acid is an antibacterial compound for systemic use (ATC code J01XX06) and it also presents high solubility in water.

Electrospinning is a multicomponent technique for the production of different nanostructures. Besides typical fibers from what is commonly known, electrospinning also enables the production of structures such as core-shell by coaxial electrospinning, Janus fibers through side-by-side electrospinning and three layers fibers by using a triaxial electrospinning setup (22–24). Being a versatile and common technique in the biopolymer manipulation, mainly in the bioengineering area (7), electrospinning was used in this work for the production of gelatin fibers. It allows the production of thin fibers, with smooth surfaces and high superficial area, and in some cases fibers with high porosity (25,26).

Therefore, the combination of a THEDES with a biopolymer fiber structure creates a viable alternative for the design of biocompatible, biodegradable, drug delivery systems.

Herein, it is reported, as proof-of-concept, the possibility of producing biocompatible fibers with THEDES. The fibers were morphologically characterized and their biological properties were also evaluated in terms of cytotoxicity and antimicrobial assays.

EXPERIMENTAL SECTION

Chemicals

Gelatin was from Panreac (CULTIMED, gelatin for bacteriological culture, EINECS: 232–554-6). R-(-)-mandelic acid was from Alfa Aesar (MandAc; 98%) and choline chloride was from Sigma-Aldrich (ChCl; ≥98%). All reagents were used without further purification.

Sample Preparation

The choline chloride/mandelic acid (ChCl:MandAc) THEDES was synthesized in a 1:2 molar ratio. Solid mandelic acid (melting point 130–134°C) and solid choline chloride (melting point 302–305°C) were weighed and mixed. The mixture was kept under stirring at 70°C, until a clear solution was obtained.

The gelatin solution, in a 30% (*w*/*v*) concentration, was prepared by mixing gelatin in preheated water (40°C), containing 2% (*v*/*v*) of ChCl:MandAc. The mixture was stirred until dissolution was complete, and a clear and homogenous solution obtained.

Electrospinning Process

For the electrospinning, a 1-mL syringe fitted with a 23-gauge needle (internal diameter = 0.41 mm) was used. The syringe was connected to a syringe pump (KDS100) to control the feed rate. The needle was electrically linked to a conducting ring, with a diameter of 15 cm, and both were directly connected to a high voltage supply (Glassman EL 30 kV). Fiber collection was performed on a fixed collector coated with aluminum foil. All the equipment was placed inside an acrylic chamber, for a better control of the operating parameters, namely temperature and humidity. In order to ensure the liquid state of the gelatin solution, an electric air heater was used for heating the metallic needle. The heater was placed close to the needle while also heating and homogenizing the temperature inside the acrylic box. Because of the position of the heater, it was possible to control the temperature inside the box while assuring the needle temperature was always above 40°C.

The optimization process was made by fixing all the parameters but one and assessing the influence of the latter in fiber morphology. The fiber morphology was first evaluated using an optical microscope, and the fibers which presented smooth and uniform shape were further studied by SEM.

Fiber Characterization: Morphology and Internal Composition

Scanning electron microscopy (SEM) was used to study fiber morphology. The fibers were sputter-coated with Au, for 15 s, with a Polaron SC502 sputter coater. The microscope used was DSM 962, from Zeiss.

Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) were used to evaluate the fibers' internal composition. TEM results were obtained at MicroLab (IST/Universidade de Lisboa) using a Hitachi 8100, with ThermoNoran light elements EDS detector. For FITR results, a Tensor 27 FTIR Spectrometer, from Bruker Optic GmbH, with the help of appropriate software (OPUS 6.0) was used.

Table I. Electrospinning Conditions (Environmental and Process Parameters) when Using an Aqueous Solution with 2% (*v*/*v*) ChCl:MandAc and 30% (*w*/*v*) Gelatin

Temperature (°C)	Humidity (%)	Flow rate (mL/h)	Distance (cm)	Voltage (kV)	Electrospinning result
39–40	20	0.1	18	20	Impossible to produce fibers
30–32	35–40	0.2	16	15	Misaligned and very coiled fibers
			20		Some coils and some misaligned fibers
			15	17.5	Some coils and some misaligned fibers
			18		Some coiled fibers
			16	20	Some coiled fibers
			18		Uniform and smooth fibers

In Vitro Biological Studies

Cytotoxicity

The cytotoxicity of the fiber membranes impregnated with mandelic acid was evaluated through a standard viability test according to the ISO/EN 10993 norm (MTS assay). An immortalized mouse lung fibroblasts cell line (L929), from the European Collection of Cell Cultures, UK, was maintained in basal culture medium DMEM (Dulbecco's modified Eagle's medium; Sigma-Aldrich, Germany), plus 10% FBS (heat-inactivated fetal bovine serum, Biochrom AG, Germany) and 1% A/B (antibiotic–antimycotic solution, Gibco, UK). Cells were cultured in a humidified incubator at 37°C in a 5% CO_2 atmosphere. Confluent L929 cells were harvested, were seeded at a concentration of 1.5×10^4 cells/mL in a 96-well cell culture plate (BD Biosciences, USA) and were cultured for 24 h, as described before. Extracts of the fibers were prepared by immersion of 6 cm^2 of a fiber membrane in 1 mL of DMEM medium for 24 h at 37°C and 60 rpm. After 24 h, the culture medium was replaced by the extract prepared, which was statically cultured under the culture conditions previously described. Cell metabolic activity after 72 h was determined using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega, USA), according to the instructions of the manufacturer. Absorbance was measured at 490 nm using a microplate reader (Synergie HT, Bio-Tek, USA). Optical density was determined for sample and compared to polystyrene tissue culture plate, used as a positive control. All cytotoxicity screening tests were performed in triplicate.

Determination of Antimicrobial Activity

The capacity to inhibit microbial growth of the gelatin fiber membranes with encapsulated THEDES was assessed by determination of the minimum inhibitory concentration (MIC). MIC is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation (27). To determine the MIC, the broth macrodilution method was used with the reference strains *Escherichia coli* K12 DSM498 and *Staphylococcus aureus* NCTC8325. A twofold serial dilution of the API and THEDES was prepared in sterile LB medium to achieve decreasing concentrations in six sterile tubes (for each concentration a replica was prepared). The initial concentration was mandelic acid (20 mg/mL), ChCl:MandAc (30 mg/mL) and gelatin with THEDES (75 mg/mL of gelatin and 30 mg/mL of THEDES). In all cases, irrespective of the form in which mandelic acid was tested (pure form, as THEDES, as gelatin encapsulated THEDES), the initial concentration of mandelic acid was 20 mg/mL. The tubes were inoculated and incubated for 24 h at 37°C, at 120 rpm.

Quantification of Mandelic Acid in Gelatin Membranes

The amount of mandelic acid impregnated in the fibers was determined by UV spectroscopy, after immersion of 4 cm^2 of fiber membrane in 10 mL of PBS solution (pH 7.4, 37°C). The concentration of mandelic acid was determined against a calibration curve prepared with standard concentrations of the active principle ($R^2 = 0.9924$), measured at 260 nm (the wavelength of maximum absorbance of mandelic acid). The results presented are an average of three measurements.

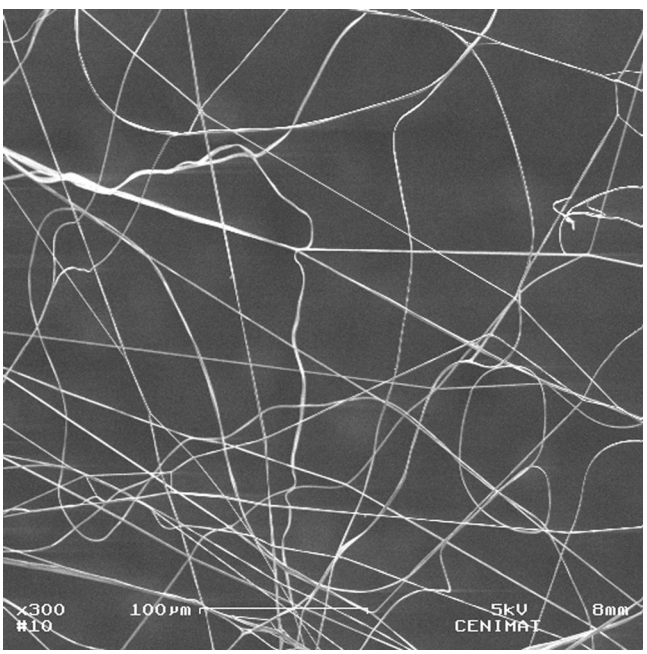

Fig. 1. SEM image of fibers obtained from aqueous solutions with 2% (*v*/*v*) ChCl:MandAc and 30% (*w*/*v*) gelatin. Image amplification ×300

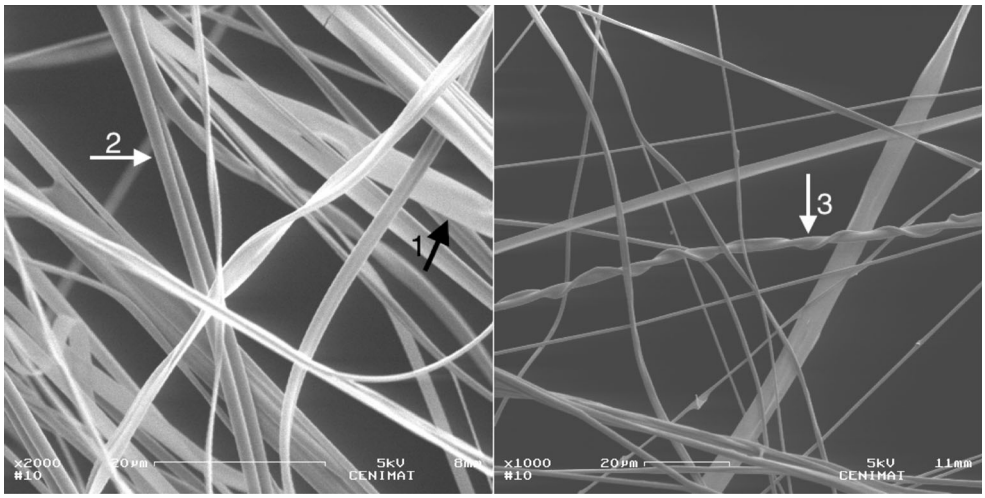

Fig. 2. SEM images of fibers obtained from aqueous solutions with 2% (*v*/*v*) ChCl:MandAc and 30% (*w*/*v*) gelatin showing different conformations, such as flat ribbon (*1*), ribbon with two tubes (*2*) and helix (*3*). Image amplification ×2000 (*left*) and ×1000 (*right*)

RESULTS AND DISCUSSION

Electrospinning Optimization

As expected, the viscosity of the solution increases with the concentration of gelatin, promoting faster jellification rates. Previous studies on the production of electrospun gelatin fibers were used as reference. Zhang *et al.* reported that gelatin produces good fibers in a concentration between 30 and 50% (*w*/*v*) (28), while Santos *et al.* concluded that a concentration of 30% (*w*/*v*) was adequate for fiber formation (29). In line with these results, in this present study, uniform and smooth fibers were obtained with both 30 and 35% (*w*/*v*) gelatin. Nevertheless, at 35% (*w*/*v*), due to the higher surface tension of the solution, a more rigorous control of experimental conditions was necessary.

Environmental and process parameters in the electrospinning were adjusted for the DES/gelatin solution, as indicated in Table I. For humidity values lower than 30%, jellification occurred at the needle tip. On the other hand, above 40%, the solution started to form droplets at the end of the needle, preventing the formation of a jet stream.

Temperature was also a conditioning factor, since it was needed to use temperatures above 30°C to ensure that gelatin did not became solid inside the syringe. However, at temperatures higher than 35°C, no fibers were obtained.

Distance and voltage are parameters that influence fiber morphology. For lower distances and voltages, only misaligned and coiled fibers were obtained. By increasing simultaneously distance and voltage to 18 cm and 20 kV, respectively, it was possible to obtain smooth and uniform fibers. Flow rates in the range 0.1–0.2 mL/h were tested and found that this parameter does not have a marked influence on the fiber morphology.

Fiber Characterization

Gelatin fibers when submitted to stress conditions assumed a straight configuration (29). However, when the stress is removed, fibers assume a more disorganized configuration (Fig. 1).

Gelatin is an elastomer and produces fibers with different conformations, being the helix the most typical shape observed (29–32). However, variations in electrospinning parameters are responsible for other conformations, *e.g.* helical shape, flat ribbon or a ribbon with two tubes (33). These morphologies were studied through SEM, and the results can be seen in Fig. 2.

Figure 3 shows a TEM image of the fibers. By the interaction between the electron beam and the sample, it is possible to create an image contrast, where a region appears darker, or lighter, according to its density.

Darker spots can be seen inside the tubes, which are attributed to solid ChCl:MandAc.

From Fig. 4, it possible to see the FTIR spectra from the fibers, and its individual components, gelatin and THEDES. Gelatin shows typical absorption bands associated to C=O stretching ($\nu = 1690–1630$ cm^{-1}), and N–H stretching ($\nu = 3325–3330$ cm^{-1}) (34).

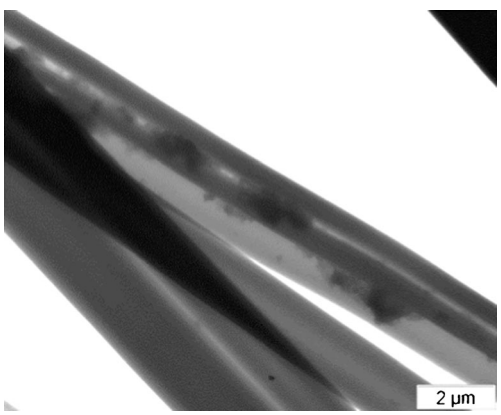

Fig. 3. TEM image of fibers obtained from aqueous solutions with 2% (*v*/*v*) ChCl:MandAc and 30% (*w*/*v*) gelatin

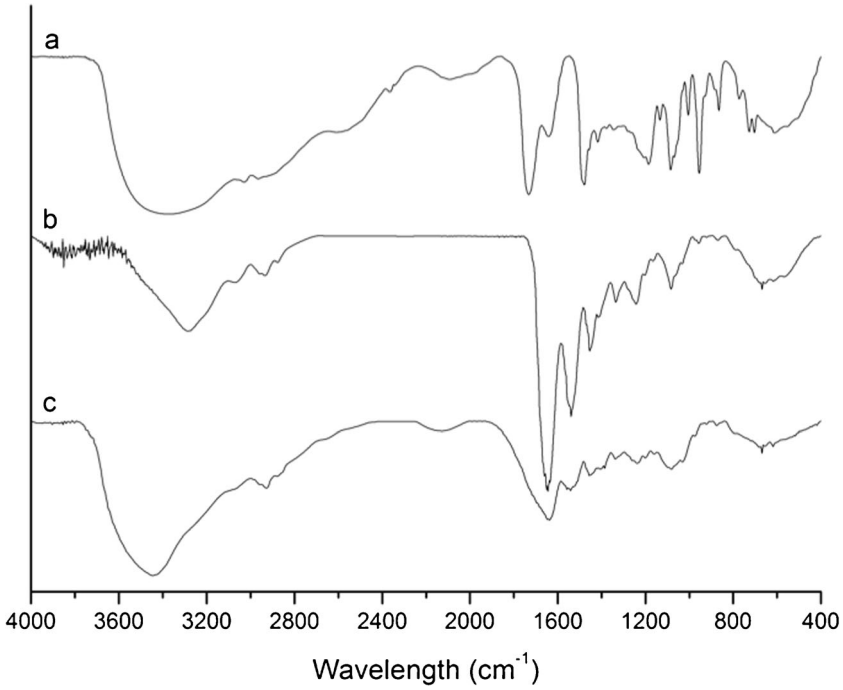

Fig. 4. FTIR spectra of *a* ChCl:MAndAc, *b* electrospun fibers obtained from aqueous solutions with 2% (*v*/*v*) ChCl:MandAc and 30% (*w*/*v*) gelatin and *c* gelatin

In the fiber spectra, it is also possible to see these two bands. However, both C=O and N–H bands are shifted to lower frequencies (1650–1515 and 3350–3180 cm^{-1}, respectively). This could be due to hydrogen bonding between gelatin and THEDES (35).

The bands near 1500 and 1400 cm^{-1} can be assigned to the skeletal vibration of the mandelic acid aromatic ring, specifically due to the C=C stretching within the ring (35). These bands are observed in THEDES and in the fiber spectra.

TEM, along with FTIR information, allows to conclude encapsulation of the THEDES within the fibers.

Fiber Dissolution and Mandelic Acid Quantification

A membrane of gelatin fibers with encapsulated THEDES was immersed in PBS solution. The membrane immediately dissolved in the buffer, confirming the possibility to use these gelatin membranes as FDDS. The content of mandelic acid in the gelatin fiber membranes was 3.4 ± 0.4% (*w*/*w*).

In Vitro Biological Studies

Cytotoxicity

Cytotoxicity assays are commonly used for testing compounds that could possibly compromise the integrity of the cellular membrane. Herein, the MTS assay for measuring the cytotoxicity of gelatin fiber and gelatin fiber with encapsulated THEDES is used (36). The results can be seen in Fig. 5. By comparing the results from the cells exposed to gelatin fiber membranes (a) and the ones not exposed (b), it can be concluded that the membranes with THEDES do not have any negative influence on the cell proliferation.

Bacteriological Study

To evaluate the anti-bacterial activity of mandelic acid in pure form, when part of a THEDES, and when part of a THEDES encapsulated in gelatin fibers, MIC values were determined for both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*), as previously described (21).

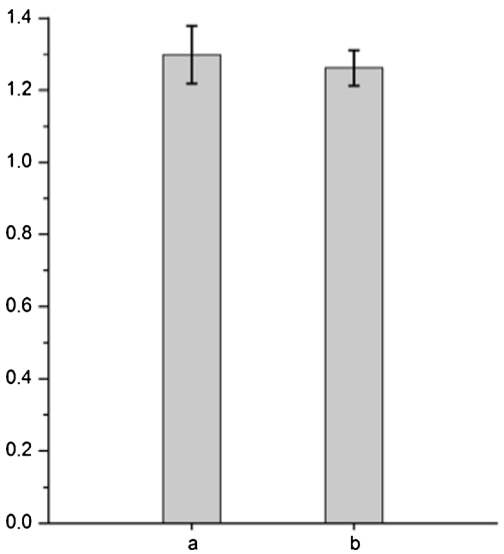

Fig. 5. MTS assay for gelatin fibers with THEDES, where *a* is the cell viability in gelatin fiber membranes with THEDES and *b* is the cell viability in the culture medium used as reference

Table II. Minimum Inhibitory Concentration (MIC—mg/mL) of Mandelic Acid in Pure Form, as Part of a THEDES and as Part of a THEDES Encapsulated in Gelatin Fibers

	MandAc	THEDES	Fiber-encapsulated THEDES
E. coli (Gram −)	2.5	5	5
S. aureus (Gram +)	2.5	5	5

THEDES therapeutic deep-eutectic solvent
The lowest concentration that completely inhibited the growth of microorganisms was examined after incubation for 24 h

Mandelic acid displayed a MIC of 2.5 mg/mL, which is in agreement with that reported for both bacteria (37). The data shown in Table II indicates that the antibacterial capacity of mandelic acid decreases slightly when it is part of the supramolecular THEDES structure with choline chloride. After the encapsulation of the THEDES in gelatin fibers, mandelic acid maintained its antibacterial activity.

CONCLUSION

The design of a fast-dissolving delivery system (FDDS) comprising electrospun gelatin fibers, with an encapsulated pharmaceutical compound (THEDES), was successfully achieved. A mixture of choline chloride and mandelic acid in a 1:2 molar ratio was used as model THEDES. The electrospinning parameters were optimized and the fibers characterized. They exhibit a smooth surface and can adopt various conformations. Among that, it was possible to determine the presence of mandelic acid inside the fibers. This was also confirmed by UV/Vis analysis, upon fiber membrane dissolution in PBS solution.

It was observed that gelatin fiber with THEDES were not cytotoxic. The antimicrobial effect against both Gram-positive and Gram-negative bacteria was also assessed. Although a slight decrease in antibacterial activity was observed, when compared with pure mandelic acid, results showed that the THEDES and the fibers with THEDES retained the antibacterial activity.

From this work, new opportunities in the pharmaceutical area can be openned, through the design of cheaper and biodegradable delivery mechanisms, using simple and common techniques.

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