Basar, AO.; Castro, S.; Torres-Giner, S.; Lagaron, JM.; Turkoglu Sasmazel, H. (2017). Novel poly(e-caprolactone)/gelatin wound dressings prepared by emulsion electrospinning with controlled release capacity of Ketoprofen anti-inflammatory drug. Materials Science and Engineering C: Biomimetic materials, sensors and systems (Online). 81:459-468. https://doi.org/10.1016/j.msec.2017.08.025

Novel poly(ε-caprolactone)/gelatin wound dressings prepared by emulsion electrospinning with controlled release capacity of Ketoprofen antiinflammatory drug

A.O. Basar[a], S. Castro[b], S. Torres-Giner[b], J.M. Lagaron[b,*], H. Turkoglu Sasmazel[a,*]

[a] Department of Metallurgical and Materials Engineering, Atilim University, Incek, Golbasi, 06836 Ankara, Turkey

[b] Novel Materials and Nanotechnology Group, Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Calle Catedrático Agustin Escardino Benlloch 7, Paterna 46980, Spain

* Corresponding authors.

E-mail addresses: lagaron@iata.csic.es (J.M. Lagaron), hilal.sasmazel@atilim.edu.tr (H. Turkoglu Sasmazel).

ABSTRACT

In the present study, a single and binary Ketoprofen-loaded mats of ultrathin fibers were developed by electrospinning and their physical properties and drug release capacity was analyzed. The single mat was prepared by solution electrospinning of poly(ε-caprolactone) (PCL) with Ketoprofen at a weight ratio of 5 wt%. This Ketoprofen-containing PCL solution was also used as the oil phase in a 7:3 (wt/wt) emulsion with gelatin dissolved in acidified water. The resultant stable oil-in-water (O/W) emulsion of PCL-in-gelatin, also containing Ketoprofen at 5 wt%, was electrospun to produce the binary mat. Cross-linking process was performed by means of glutaraldehyde vapor on the electrospun binary mat to prevent dissolution of the hydrophilic gelatin phase. The performed characterization indicated that Ketoprofen was successfully embedded in the single and binary electrospun mats, i.e. PCL and PCL/gelatin, and both mats showed high hydrophobicity but poor thermal resistance. In vitro release studies interestingly revealed that, in comparison to the single PCL electrospun mat, the binary PCL/gelatin mat significantly hindered Ketoprofen burst release and exhibited a sustained release capacity of the drug for up to 4 days. In addition, the electrospun Ketoprofen-loaded mats showed enhanced attachment and proliferation of L929 mouse fibroblast cells, presenting the binary mat the highest cell growth yield due to its improved porosity. The here-developed electrospun materials clearly show a great deal of potential as novel wound dressings with an outstanding controlled capacity to release drugs.

1. Introduction

In clinical treatments, drugs are usually administered to patients who suffer from different pains and diseases. Drug intake could be either oral or by injection targeting specific areas. However, the initial amount of drug dose decreases over time due to spreading to healthy areas through the blood circulatory system. This leads patients to take an extra amount of drug, which may result in undesired side effects. Therefore, minimizing the drug intake by local delivery on the disease or infected site is of high importance from a pharmaceutical and biomedical point of view. Today, locally controlled release vehicles are becoming prominent against conventional dosage forms by its improved therapeutic effect, reduced frequency of drug intake, lower level of toxicity, and more convenience [1–5]. Large, i.e. tens to hundreds of microns' size, controlled release systems developed using techniques such as spray-drying and solvent-evaporation have been recently studied due to their simplicity and availability at industrial scale.

However, these methods typically exhibit several drawbacks such as low encapsulation efficiency and leakage of hydrophilic drugs [6]. Interestingly, decreasing the size of the drug-loaded material leads to an increased surface area, which can result in a more controlled drug release timescale (e.g. from hours to days). Hence, polymer-based drug delivery vehicles, such as polymer fibers mats, hydrogels or micelles, both in the micro-, submicro-, and nano-range, can certainly play an important role in designing novel pharmaceutical applications [7–10].

Electrospinning is a well-known processing technology that generates ultrathin polymer fibers with diameters ranging from below 100 nm to above several microns [11,12]. Interestingly, nanofibers and other nanostructures with bioactive properties can be smartly generated by electrospinning of mixture solutions that consist of a polymer or polymer blend, a solvent

or combination of solvents, and a given bioactive compound (e.g. drugs and nutraceuticals) [7,13,14]. Electrospun drug-loaded nanofibers have recently received a great attention due to their optimal high drug-encapsulation efficiency, high surface area-to-volume ratio, suitability as wound dressings with high cellularadhesion capacity, enhanced mechanical resistance, and good thermal stability [6,7,15]. However, the use of solution electrospinning presents some drawbacks in terms of severe burst release of the loaded drugs, which certainly makes the resultant electrospun mats unable to provide the drug delivery profile required for certain bioactive applications [7,16].

Compared to classical electrospinning methods, traditionally based on the above-described polymer solutions, the application of emulsion electrospinning is a promising alternative. This is related to the fact that this technology allows the encapsulation of lipophilic compounds by means of more easy-to-handle hydrophilic polymers, which cannot only avoid burst release but it also fully or partially enables the use of waterbased solvents [17]. In addition to offer a more sustained release capacity, the application of drug-loaded electrospun mats obtained from polymer emulsions has been reported to provide good bioactivity, which can certainly increase effectiveness of the encapsulated drugs after their release and then simplify the metabolism, proliferation, and differentiation of cells [18–21].

Based on the spatial organization of the oil and water phases, emulsion systems can be conveniently categorized into oil-in-water (O/ W) and water-in-oil (W/O) emulsions. In an O/W emulsion, oil droplets are dispersed in a continuous water phase, whereas W/O emulsions are dispersions of aqueous droplets in an oil phase. Typically, the oil phase is prepared by dissolving the hydrophobic or low hydrophilic polymer and the hydrophobic drug in an organic solvent, whereas the water phase is composed of the hydrophilic polymer dissolved in a waterbased solvent. Due to the high electrostatic forces that take places during electrospinning, the emulsion is efficiently converted into ultrathin fibers upon solvent evaporation and polymer entanglement. Resultant electrospun mats of drug-containing nanofibers are becoming a promising new class of material with controlled release capacity of drugs that can be of high interest in biomedical uses requiring local treatments (e.g. wound dressings) [7,26].

As widely discussed in the literature, in addition to the typical processing conditions affecting electrospinning [22–24], there are three essential parameters exerting a high influence on the emulsion systems: The surfactants, also referred as emulsifiers, type of oil phase, and type of water phase [27]. Therefore, it can be considered that the release rate of the loaded drug from the electrospun mats can be efficiently controlled by manipulating the above parameters. In the present study, two biopolymers, namely poly(ε-caprolactone) (PCL) and gelatin, were used. PCL is a semi-crystalline synthetic biopolymer that has been electrospun for both food [28] and biomedical applications [29,30] due to its extremely low toxicity and good biocompatibility, respectively. However, current uses of electrospun neat PCL nanofibers are very limited by their limited bioregulatory activity and high hydrophobicity, which handicap cell adhesion and proliferation [31]. Gelatin, as opposite, is a biopolymer derived from the collagen protein, which is known to highly promote the proliferation of a wide variety of cells [32,33], particularly in the form of electrospun nanofibers [34,35]. As the model drug to investigate the release profile from the electrospun biopolymer mats, Ketoprofen was selected. This is a non-steroidal antiinflammatory drug (NSAID) that has been used as an effective treatment to reduce inflammation, pain, and rheumatism, showing poor water solubility (0.5 μg/mL) and a relatively short biologic half-life (1.5–2 h) [36]. Ketoprofen is commercially available in a various number of formulations, including Ketoconazole and Ketolac. Common dosage of Ketoprofen is 50–100 mg twice per day [37]. Therefore, the development of electrospun mats based on biocompatible ultrathin fibers with controlled release capacity of Ketoprofen would be of high interest in the design of novel dressings for wound healing applications.

2. Materials and methods

2.1. Materials

Linear PCL grade 440,744 with a weight-average molecular weight (M_W) of 80 kDa, gelatin from bovine skin grade G9391, and Ketoprofen grade K1751 were purchased from Sigma-Aldrich S.A. (Madrid, Spain). Glutaraldehyde grade 340,855 with 50% of purity, Span80 grade S6760, glacial acetic acid, chloroform, methanol, and phosphate buffer saline (PBS) were obtained from Panreac S.A. (Barcelona, Spain). For cell cultivation, Dulbecco's Modified Eagle Medium (DMEM/F12), penicillin/streptomycin, L-glutamine, fetal bovine serum (FBS), ethanol with 96% (vol/vol), acridine orange base (AO), propidium iodide (PI), and hexamethyldisilazane (HMDS) with ≥99% were purchased from Sigma-Aldrich Co. (St. Louis, USA).

2.2. Electrospinning process

A PCL solution was prepared by dissolving 8% (wt/vol) of PCL in a chloroform/methanol 4:1 (vol/vol) mixture at 30 °C.

Ketoprofen was incorporated into the solution at 5% in weight (wt%) in relation to PCL. This solution was used to prepare the single mat. In the case of the emulsion, the same PCL solution was used adding 1 wt% of Span80 surfactant [3]. To prepare the gelatin solution, 32.5% (wt/vol) gelatin was dissolved in a 25 wt% acetic acid aqueous solution at room temperature [4]. Then, the PCL solution containing Ketoprofen was added into the gelatin solution a ratio 3:7 (wt/wt), readjusting the final drug content in the biopolymers to 5 wt%. The resultant mixture was then stirred using a high-speed stirrer T-25 digital ULTRA-TURRAX® from IKA-Werke GmbH & Co. KG (Staufen, Germany) to generate an emulsion. As the control, PCL and PCL/gelatin solutions without Ketoprofen were prepared in identical conditions.

The electrospinning apparatus was a Fluidnatek® LE500 pilot line from Bioinicia S.L. (Valencia, Spain). This was used in its laboratory mode using a single emitter with a collecting plate. For the electrospinning of PCL, an applied voltage of 13 kV, a flow-rate of 13 μL/min, and a tip-to-collector distance of 20 cm were used. In the case of the PCL/gelatin emulsions, processing conditions were respectively set at 18 kV, 18 μL/min, and 13 cm.

2.3. Cross-linking treatment

To prevent dissolution of the gelatin phase in water-based media during the drug release tests, cross-linking was performed by placing 0.5 g of the gelatin/PCL-Ketoprofen mat in contact with the gas phase of a 25 wt% glutaraldehyde solution in water for 1 h [25].

2.4. Characterization

2.4.1. Contact angle measurements

Water contact angle of the electrospun mats was measured by a contact angle goniometry Phoenix 300 equipment from Surface Electro Optics (Suwon, South Korea). The contact angle values were determined using the images of the water drops on the surface of the electrospun mats, which were taken 2 min after the droplet-mat contact. Each sample was measured at three different points at room temperature.

2.4.2. Scanning electron microscopy

The morphology of the electrospun mats was examined by scanning electron microscopy (SEM). The SEM micrographs were taken using a Hitachi S-4100 electron microscope (Tokyo, Japan) at an accelerating voltage of 5 kV and a working distance of 8–10 mm. The mats were previously sputtered with a gold-palladium mixture for 3 min under vacuum. The average fiber diameter and porosity was determined via ImageJ Launcher software program using at least 50 original SEM micrographs.

2.4.3. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was carried out in order to characterize the surface composition of the samples. The measurements were performed using an X-ray photoelectron spectroscopy analysis system with an Al Kα Monochromatic source at 600 W and a PHI 5000 VersaProbe from Physical Electronics (Chanhassen MN, USA). The surface compositions and the functional carbon, oxygen, and nitrogen groups were determined from the high-resolution scans.

2.4.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) curves of the mats were obtained with a DSC Diamond device from PerkinElmer (Waltham, MA, USA). Approximately 4 mg of each sample was placed in standard aluminum pans and heated from 0 to 200 °C, cooled down to 0 °C, and heated back to 200 °C at a rate of 10 °C/min, using a nitrogen flow of 20 mL/min as the sweeping gas. Crystallinity values were determined using following Eq. (1):

$$Xc(\%) = \left(\frac{\Delta H_m - \Delta H_{cc}^{o}}{\Delta H_m^{o} \times (1 - w)} \right) \times 100 \quad (1)$$

where ΔH_m and ΔH_{cc}^{o} correspond to the normalized melting and cold crystallization enthalpies of the PCL-based electrospun materials, respectively, w refers to the weight fraction of non-PCL material, and ΔH_m^{o} corresponds to theoretical melting enthalpy of a fully crystalline PCL material, i.e. 139 J/g [38].

2.4.5. Drug release

The studies of drug release were carried out in aqueous phosphate buffer solution (PBS). This medium was prepared by dissolving 1 tablet of sodium phosphate dibasic (Na_2HPO_4) in 100 mL of deionized water, in which the pH was adjusted to 7.4 using 0.1 M sodium hydroxide and 0.1 M hydrochloric acid solutions. The release rate of Ketoprofen from the electrospun mats was determined by ultraviolet–visible (UV–Vis) spectrophotometer in a NanoDrop ND-1000 from Isogen Life Sciences (Utrecht, Netherlands). For this, 3.4 mg of the electrospun mat was weighed and placed into 0.5 mL of the prepared PBS solution at 37 °C. For each measurement, 2 μL of the resultant solution was used. The concentration of released Ketoprofen into the medium was monitored as a function of time measuring the absorbance band at 260 nm (λ_{max}), which was based on a previously prepared calibration curve [2]. The release experiments were carried out in triplicate

and the release kinetics of the drug were adjusted to the Krosmeyer-Peppas equation:

$$\frac{M_t}{M_0} = Kt^n \quad (2)$$

where t is the experimental time, ^{Mt} is the fraction of drug released, k is

^{Mo}

the kinetic constant, and n is the diffusional exponent. This equation can be used for $0 < M_t/M_0 < 0.6$ of released drug [39]. Furthermore, the apparent diffusion coefficient (D_{app}) of a polymer matrix having a diffusing agent was calculated in terms of 1 − D unsteady-state form of the Fick's second law of diffusion [40]:

$$\frac{M_t}{M_\infty} = \left(\frac{16D_{app}t}{\pi H^2}\right)^{0.5} \quad (3)$$

where ^{Mt} is the fraction of drug released and H is the mean thickness of

^{Mo}

the electrospun fibers.

2.4.6. Cell culture

Cell proliferation on the electrospun mats was examined by culturing L929 ATCC CCL-1 mouse fibroblast cells. Electrospun mats of 1 × 1 cm^2 were placed in 24 well-plate petri dishes. To ensure sterilization, these were initially exposed to ultraviolet (UV) radiation for 1 h in a Biostar cabinet from Telstar S.A. (Madrid, Spain). Studies of cell viability were conducted for 7 days with a cell seeding concentration of 5 × 10^4 cell/mL in a culture medium that consisted of: DMEM/ F12 + 10% in volume (vol%) of FBS + 1 vol% penicillin + streptomycin (100 units/mL penicillin, 100 µg/mL streptomycin) + 1 vol% L-glutamine. The petri dishes containing the cultured cells on the electrospun mats were then kept in an incubator at 5% CO_2 and 37 °C. The performance of the electrospun mats was compared to a standard tissue culture polystyrene (TCPS) plate.

2.4.6.1. Cell attachment. Cell attachment was studied by the haemocytometric counting technique for 3 h at following selected intervals: 30, 60, 90, 120, 150, and 180 min. For this, initially, unattached cells were removed by discarding the medium from the each well. Then, after incubating the mats for 15 min at 37 °C in a Trypsin/EDTA solution at 0.1% (wt/vol), the attached cells were harvested. Thereafter, the remaining cells were counted for each interval by the trypan blue dye exclusion technique. The attachment results were expressed as the percentage of viable cells in relation to the initial seeded cells.

2.4.6.2. Cell viability. The electrospun mats were placed in 24-well plates and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay as carried out for a period of 7 days, every 48 h, starting 24 h after seeding. Every 24 h, the medium was discarded and the samples were washed with PBS. This was followed by the addition of 600 µL of fresh medium and 60 µL of MTT solution, after which samples were incubated for 3 h. The medium was then removed and replaced with 1 mL of dimethylsulfoxide (DMSO) and placed into the incubator for another hour. Finally, for each solution, 200 µL were taken and placed into 96-well plates. Cell viability, in terms of absorbance, was determined by UV–Vis spectroscopy with a microplate reader at 540 nm. Each measurement was carried out in triplicate.

2.4.6.3. Cell proliferation. Fluorescence images were taken on the 3rd and 7th day of cell cultivation using a fluorescence microscope AMG EVOS-FL from Thermo Fisher Scientific (Mill Creek, USA). The cultured mats were previously stained with AO (25 µg/mL) and PI (25 µL/mL). In brief, these were taken out from the incubator and each well plate medium was removed and washed with PBS. The fixation was done with 2.5 vol% glutaraldehyde for 30 min and stained with an AO/PI 1:1 (vol/vol) solution in the dark for 10 min at room temperature.

SEM images were taken on the 7th day of cell cultivation. For this, after removing the mats from the incubator, each well medium was discarded followed by triple PBS rinsing. The mats were fixed with 2.5 vol% glutaraldehyde for 30 min at room temperature. Thereafter,
the mats were dehydrated with different concentration of ethanol, i.e. 30, 40, 50, 60, 70, 80, 90, and 100 wt%, for 2 min each, and then
immersed in pure HMDS for 5 min and air-dried.

3. Results and discussion

3.1. Morphology

Fig. 1 shows the SEM images of the electrospun mats for the neat and drug-loaded PCL-based fibers. As it can be observed in Fig. 1a, the neat PCL solution resulted in ultrathin fibers with an average diameter of 331 nm. These fibers exhibited a similar morphology than the drugloaded PCL fibers, which can be seen in Fig. 1b. It is also interesting to note that the presence of Ketoprofen crystals was not observed on the surface of PCL ultrathin fibers, which preliminary indicates that the drug was efficiently embedded in the fiber structure. Fig. 1c shows the electrospun mat of the drug-loaded PCL/gelatin fibers prior to crosslinking process. Whereas the drug-loaded neat PCL fibers, shown in Fig. 1b, exhibited a uniform fiber morphology, the untreated drugloaded PCL/gelatin fibers, in Fig. 1c, were relatively heterogeneous and displayed a ribbon-like shape with the presence of some beaded regions. Mean fiber diameters were 345 nm (Fig. 1b) and 272 nm (Fig. 1c) for the PCL and PCL/gelatin fibers containing Ketoprofen, respectively. The here-observed changes in the electrospun PCL/gelatin mat can be related to the electrospinnability of gelatin from the acetic acid solution, which is known to present certain difficulties due to its strong dependency on various solution properties (e.g. viscosity, surface tension, and conductivity) and processing parameters including polymer degradation [6]. As it can be seen in Fig. 1d, as a result of the crosslinking process, the PCL/gelatin mat containing Ketoprofen partially lost its fiber-based morphology but, interestingly, it resulted in a more continuous structure showing a very high porosity.

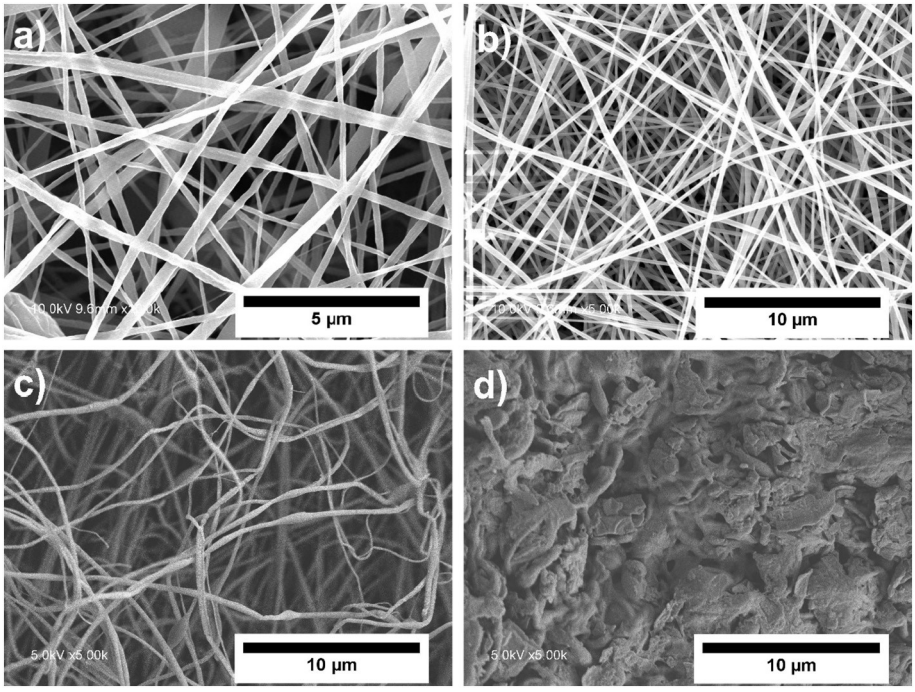

Fig. 1. Scanning electron microscopy (SEM) images of the electrospun mats of: a) Neat poly(ε-caprolactone) (PCL) ultrathin fibers. Scale marker of 5 µm. b) Ketoprofen-containing PCL ultrathin fibers; c) Ketoprofen-containing PCL/ gelatin untreated fibers; d) Ketoprofen-containing PCL/gelatin cross-linked fibers. Scale markers of 10 µm.

3.2. Water resistance

Contact angle values of the prepared electrospun mats are shown in Table 1. As it can be seen in the table, neat PCL electrospun mats presented a relatively high hydrophobic character with a contact angle over 100°. In the case of the untreated PCL/gelatin binary mat, this value was extremely low (< 0.1°), which indicates that the material is hydrophilic and then it can highly affected by water. Interestingly, the cross-linked electrospun PCL/gelatin fibers mat exhibited a moderate hydrophilicity, i.e. ~76°. These results clearly indicate that the wettability of the electrospun binary mats evolved significantly after the cross-linking process [41]. This water resistance increase is a positive result for biomedical applications since it has been previously reported that hydrophobic or low hydrophilic structures support cell adhesion, migration, and growth in a higher extend than hydrophilic structures [31,44].

Table 1
Contact angle values for the electrospun mats of neat poly(ε-caprolactone) (PCL) ultrathin fibers, PCL/gelatin untreated fibers, and PCL/gelatin cross-linked fibers.

Electrospun mat	Contact angle (°)
PCL ultrathin fibers	116.7 ± 5.6
PCL/gelatin untreated fibers	< 0.1
PCL/gelatin cross-linked fibers	76.5 ± 4.7

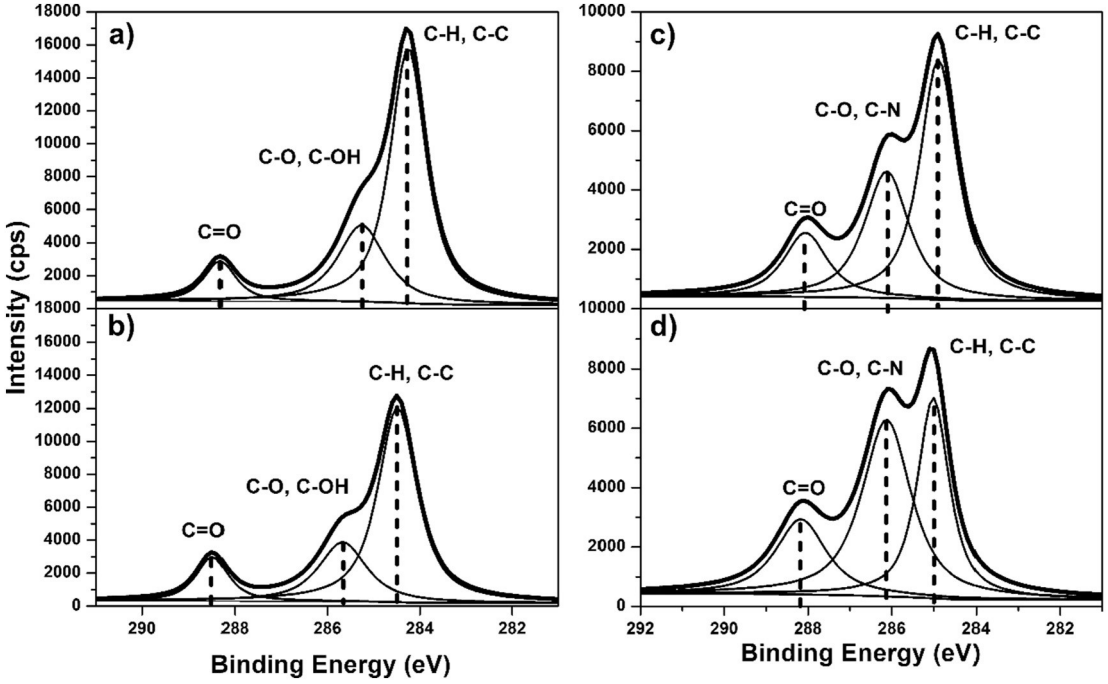

Fig. 2. High-resolution carbon (C_{1s}) spectra obtained by X-ray photoelectron spectroscopy (XPS) for the electrospun mats of: a) poly(ε-caprolactone) (PCL) ultrathin fibers; b) Ketoprofencontaining PCL ultrathin fibers; c) PCL/gelatin cross-linked fibers; d) Ketoprofen-containing PCL/gelation cross-linked fibers.

Table 2

Surface chemical compositions obtained by X-ray photoelectron spectroscopy (XPS) of electrospun mats of unloaded and Ketoprofen-loaded poly(ε-caprolactone) (PCL) ultrathin fibers and PCL/gelatin cross-linked fibers.

Electrospun mats	C_{1s} (%)	O_{1s} (%)	N_{1s} (%)
PCL ultrathin fibers	82.4	17.6	0.0
Ketoprofen-loaded PCL ultrathin fibers	79.3	20.7	0.0
PCL/gelatin cross-linked fibers	69.1	19.4	11.5
Ketoprofen-loaded PCL/gelatin cross-linked fibers	67.1	21.1	11.8

3.3. Surface chemical composition

XPS analysis was carried out in the electrospun mats to determine their surface chemical composition. As it can be observed in Table 2, the XPS spectra of all electrospun mats based on PCL and PCL/gelatin revealed the presence of carbon and oxygen elements. In addition to that, nitrogen element was also present in the spectra of the binary electrospun mats, which arises from the nitrogen functional groups of both gelatin and glutaraldehyde cross-linker. In the particular case of the electrospun mats containing Ketoprofen, both PCL and PCL/gelatin, it can be seen a slight increase in the oxygen content due to the oxygen contribution of the drug. This confirms the drug encapsulation in the electrospun fibers.

The high-resolution carbon (C_{1s}) and oxygen (O_{1s}) spectra of the electrospun fibers mats are shown in Figs. 2 and 3, respectively. In the high-resolution C_{1s} spectra of both neat PCL and Ketoprofen-containing PCL electrospun mats, which can be respectively observed in Fig. 2a and b, three main characteristic peaks were identified. The peaks at 284.5, 285.5, and 288.5 eV correspond to the CeC, CeO, and C]O bonds, respectively [3]. Similar peaks can be observed for the electrospun PCL/gelatin and Ketoprofen-containing PCL/gelatin mats, shown respectively in Fig. 2c and d. In particular, it can be seen that the CeO and CeN peak is slightly displaced to ~286 eV while the C]O bond peak moved to 288 eV [46]. Comparison of Fig. 2a to c and b to d, shows a slight decrease in the single carbon signal. This observation is clearly related to the oxygen contribution of Ketoprofen [47], which further supports the drug encapsulation in the electrospun ultrathin fibers.

In relation to the high-resolution O_{1s} spectra, shown in Fig. 3, two major peaks can be observed for the PCL-based electrospun mats. The peaks at ~532 and ~533 eV corresponds to CeO and C]O bonds, respectively [45]. Similar to the above C_{1s} spectra, a slight increase of oxygen signal was observed due to the presence of embedded Ketoprofen. These changes had low intensity, which can be ascribed to the relatively low amount of loaded drug in the PCL-based fibers, i.e. 5 wt %.

3.4. Thermal analysis

The thermal properties of the electrospun mats were also investigated by DSC and Fig. 4 shows the second heating endotherms of the two materials with and without Ketoprofen. For the neat electrospun PCL fibers, a single endothermic peak can be observed at 59.6 °C. This is ascribed to the melting point of the crystalline phase of PCL. In the case of the electrospun Ketoprofen-containing PCL fibers, this peak was displaced to 56.7 °C. This reduction in the melting point, of approximately 3 °C, indicates that Ketoprofen affects the crystallization process of the biopolymer leading either lower density crystals and/or crystals with more defects [48]. This is an interesting observation since it indicates certain chemical compatibility of the drug with the biopolymer. A similar observation was previously reported for Ketoprofenloaded polylactide (PLA) fibers [48].

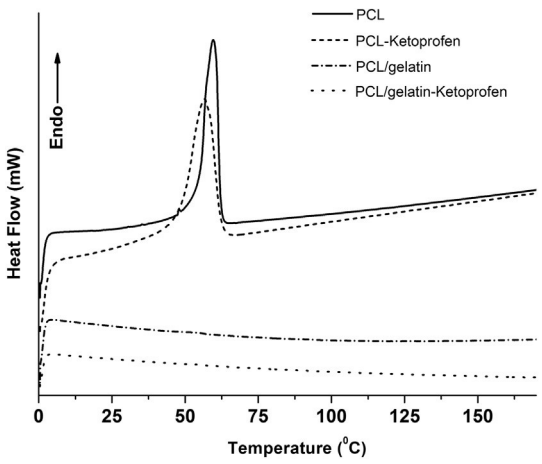

Fig. 4. Differential Scanning Calorimetry (DSC) heating thermograms for the electrospun poly(ε-caprolactone) (PCL) ultrathin fibers, Ketoprofen-containing PCL ultrathin fibers, PCL/gelatin cross-linked fibers, and Ketoprofen-containing PCL/gelatin cross-linked fibers.

For the electrospun PCL/gelatin and Ketoprofen-containing PCL/ gelatin electrospun mats, one broad peak was observed

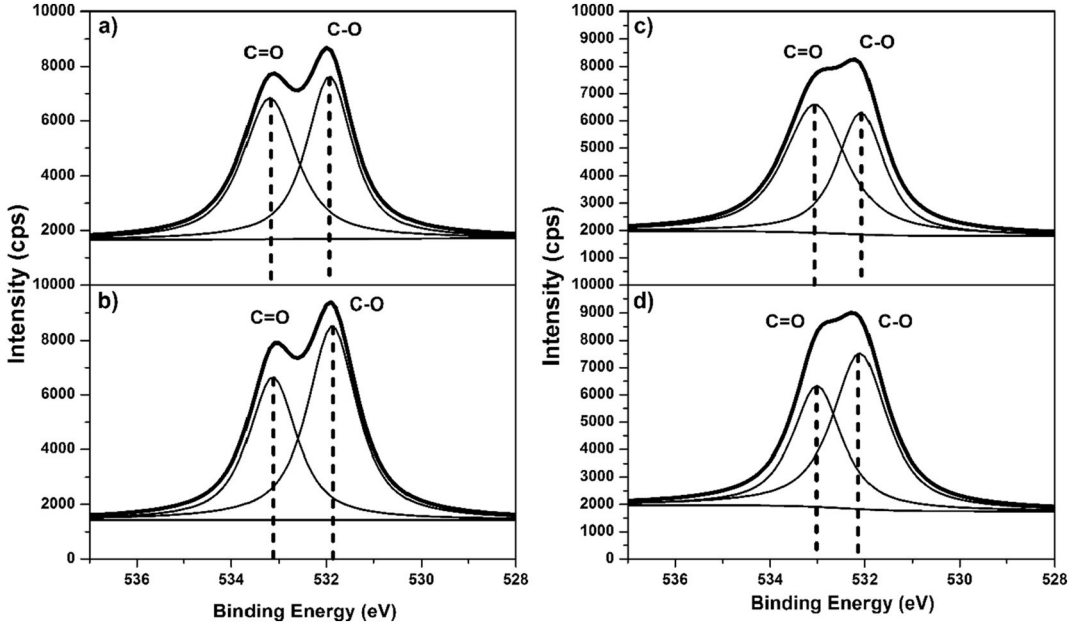

Fig. 3. High-resolution oxygen (O_{1s}) spectra obtained by X-ray photoelectron spectroscopy (XPS) for the electrospun mats of: a) poly(ε-caprolactone) (PCL) ultrathin fibers; b) Ketoprofencontaining PCL ultrathin fibers; c) PCL/gelatin cross-linked fibers; d) Ketoprofen-containing PCL/gelation cross-linked fibers.

during the first heating scan (not shown) in the range 40–140 °C, which is mostly ascribed to the evaporation of trapped water [49]. No endothermic peaks were unambiguously detected in the second heating scan of both PCL/ gelatin and Ketoprofen-containing PCL/gelatin electrospun mats since the PCL content was relatively low in the blend [50]. In relation to crystallinity, the electrospun mats of neat PCL and Ketoprofen-containing PCL ultrathin fibers showed values of about 49% and 53%, respectively. These results indicate that the addition of Ketoprofen in the semi-crystalline PCL could result in a

slight increase in the degree of crystallinity albeit the formed PCL crystals presented more defects or a lower lamellar thickness due to the reduction observed in the melting point [48].

3.5. Drug release profile

The amount of Ketoprofen released to the PBS medium was monitored by UV−Vis spectroscopy, according to the intensity of the 260-nm band. Additionally, in the UV−Vis spectra, the characteristic peaks of gelatin [42] and glutaraldehyde [43] were not observed, indicating that these did not interfere with the wavelength range of the measurements. This potentially suggests that both the cross-linking process was successful and the glutaraldehyde traces were successfully removed, as also supported by previous SEM evaluation. Fig. 5 shows the release profiles of Ketoprofen from the electrospun PCL and cross-linked PCL/gelatin mats. As one can observe in Fig. 5a, the ultrathin PCL fibers mat, i.e. the single mat, exhibited a burst release profile that reached a plateau after approximately only 12 min, time at which it released ~90% of the drug. This is also worthy to note that this phenomenon of burst release was observed even though the mat was immersed in PBS, for which PCL presents a low chemical affinity. In the case of the cross-linked PCL/ gelatin mat, interestingly, a more sustained released of the Ketoprofen drug can be observed in Fig. 5b. In particular, the binary electrospun mat extended the Ketoprofen release for about 4 days. This result clearly points out that this novel PCL/gelatin mat, prepared by emulsion electrospinning and subsequent cross-linking treatment, is much more efficient in retaining the drug and releases it in a controlled manner. In this sense, it can be considered that the loaded Ketoprofen was most likely confined within the PCL phase due to its higher chemical affinity with the synthetic polymer, from which it diffused out to the PBS medium. In this binary mat, the role of the gelatin phase was to provide functionality by means of cross-linking and thereafter acted as an efficient barrier against drug diffusion.

Drug release data obtained from above Fig. 5 was fitted to the Korsmeyer-Peppas equation. As shown in Eq. (2), the slope of the linear fitted log (M_t/M_0) vs. log (t) reflects the release exponent (n), which determines the release mechanism [39]. Furthermore, values of the apparent diffusion coefficient (D_{app}) of the PCL matrix were estimated from the slope of linear fitted data of M_t/M_0 vs. $t^{0.5}$ according to Eq. (3) [40]. The resultant values of release kinetics and diffusion coefficients are summarized in Table 3. As one can observe the estimated diffusion coefficient value of the Ketoprofen-containing PCL ultrathin fibers was about 500 times larger than the Ketoprofen-containing PCL/gelatin fibers.

3.6. Cell culture

Cell culture studies were performed by spreading L929 mouse fibroblast cells on the here-developed electrospun mats. The degree of cell attachment, expressed as the percentage of viable cells in relation to the seeded cells, is shown in Fig. 6. This reflects that, after 3 h, L929 fibroblast cells successfully attached onto both the electrospun mats and standard TCPS plate used as control. Despite the 3-D morphology of the electrospun PCL mat, based on ultrathin fibers, which is well reported to promote cellular adhesion [7,51], the control TCPS exhibited slightly higher cell-attachment performance, i.e. 72% vs. 60−65%. This is probably related to the wettability characteristics of the samples. In particular, highly hydrophobic polymers, which is the case of PCL, tend to exhibit relatively

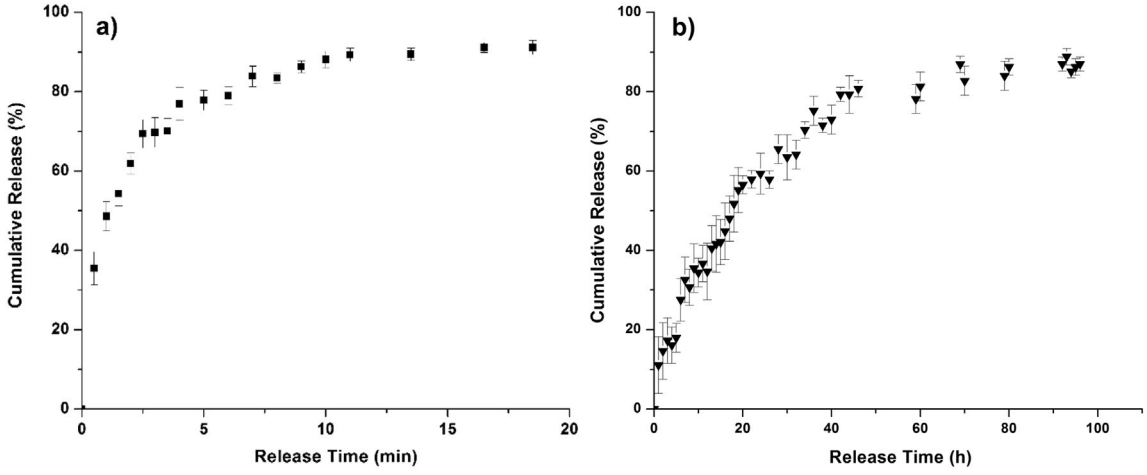

Fig. 5. Drug release profile of electrospun mats of: a) Ketoprofen-containing poly(ε-caprolactone) (PCL) ultrathin fibers; b) Ketoprofen-containing PCL/gelatin cross-linked fibers.

poor cell attachment performance [52]. Interestingly, the electrospun PCL/gelatin mat showed an improved attachment

Table 3

Drug release model parameters in terms of diffusional exponent (n) and linear regression (R^2), release mechanism, and apparent diffusion coefficient (D_{app}) for the electrospun mats of poly(ε-caprolactone) (PCL) ultrathin fibers and PCL/gelatin cross-linked fibers containing Ketoprofen.

Electrospun mat	Model parameters		Release mechanism	$D_{app} \times 10^{-15}$ (m^2/s)
	n	R^2		
PCL ultrathin fibers	0.395	0.988	Fickian diffusion	1220.00
PCL/gelatin cross-linked fibers	0.576	0.957	Non-Fickian diffusion	2.67

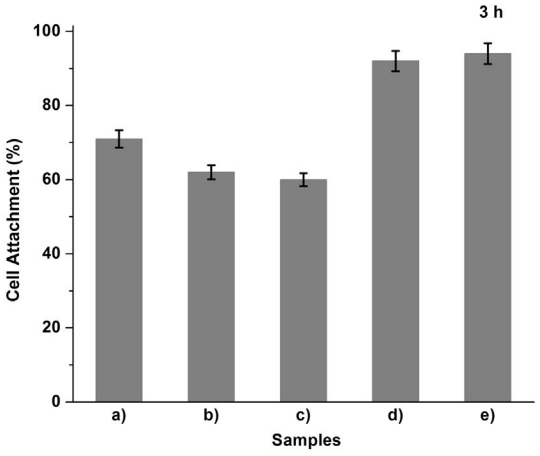

Fig. 6. Percentage of cell attachment on the a) standard tissue culture polystyrene (TCPS) plate and on the electrospun mats of: b) poly(ε-caprolactone) (PCL) ultrathin fibers; c) Ketoprofen-containing PCL ultrathin fibers; d) PCL/gelatin cross-linked fibers; e) Ketoprofen-containing PCL/gelatin cross-linked fibers.

performance, i.e. 90–95%, in comparison of both TCPS and the single PCL electrospun mat. This observation can be ascribed to the inherent hydrophilic nature of gelatin as well as the high porosity of the binary mat, which additionally presents improved water resistance as a result of the cross-linking process. As it was previously described by Gencer et al. [53], the high micro-porosity of the electrospun mats can serve as efficient points of attachment for the seeding cells while the presence of macro-sized pores allows cells to grow in a 3-D arrangement. Therefore, the observed increase in the cell-attachment performance, of about 34%, can be ascribed to the high porosity, i.e. 41.5% as determined in the SEM images, good wettability, and enhanced functionality of the PCL/gelatin mat. This in agreement with previous studies reported in literature [54,55].

The cytotoxicity and cell viability on the electrospun mats were determined by the MTT assay for 7 days at intervals of 48 h. As shown in Fig. 7a, the cell viability on the mats exhibited a steady increase, showing no indication of cytotoxicity. Similar to the cell attachment results, the control presented slightly higher absorbance values up to the 3rd day of study than the neat PCL electrospun mat, i.e. the single mat. This would be explained by the hydrophobic character of PCL that seems to be an undesirable feature for cell growth. Interestingly, from this day, cell growth on the TCPS plate revealed no absorbance increase in contrast to all electrospun mats. This indicates that, due to its 2-D morphology, cell cultivated on the control plate passed to a stationary phase [52]. Furthermore, the PCL and PCL/gelatin electrospun mats provided a continuous cell proliferation and, after the 5th day, their absorbance values were higher than those observed for the control. This supports the previous observations of Turkoglu Sasmazel et al. [56], who indicated that the 3-D structure of the electrospun fibers mats can effectively promote cell proliferation, in particular during the late stage of cell culture. Additionally, it can be seen that the highest value of absorbance was observed for the PCL/gelatin cross-linked mat, i.e. the binary mat, during the whole period of the experiment. This can be ascribed to, in addition to their 3-D morphology, the hydrophilic nature provided by gelatin and macro- and micro-porosity of these materials [54].

In relation to the electrospun mats containing Ketoprofen, similar values of cell growth were observed than in the mats without the embedded drug. This is an encouraging result for the potential application of the drug-loaded mats as wound dressings in tissue engineering applications to both promote healing and reduce inflammation. The main difference was observed between the electrospun mat of neat PCL ultrathin fibers and PCL/gelatin cross-linked fibers at the 7th day. This absorbance difference (~20%) is undoubtedly related to the hydrophilic characteristics of the gelatin phase [53]. Additionally, as shown in Fig. 7b, the cell growth yield at the end of the in vitro experiment for the binary electrospun mat was 1.93 and 3.07 times higher than that of the single mat and TCPS plate, respectively. Furthermore, interestingly, the presence of Ketoprofen had no influence on cell proliferation. This indicates that low amounts amount of the drug, i.e. 5 wt%, can be encapsulated in PCL by electrospinning without exerting any cellular toxic effect.

Resultant cell attachment and proliferation on the electrospun mats can be seen in the fluorescence microscope images gathered in Fig. 8, for which the materials were previously dye stained. One can observe that, during proliferation, cells preserved their typical fibroblast

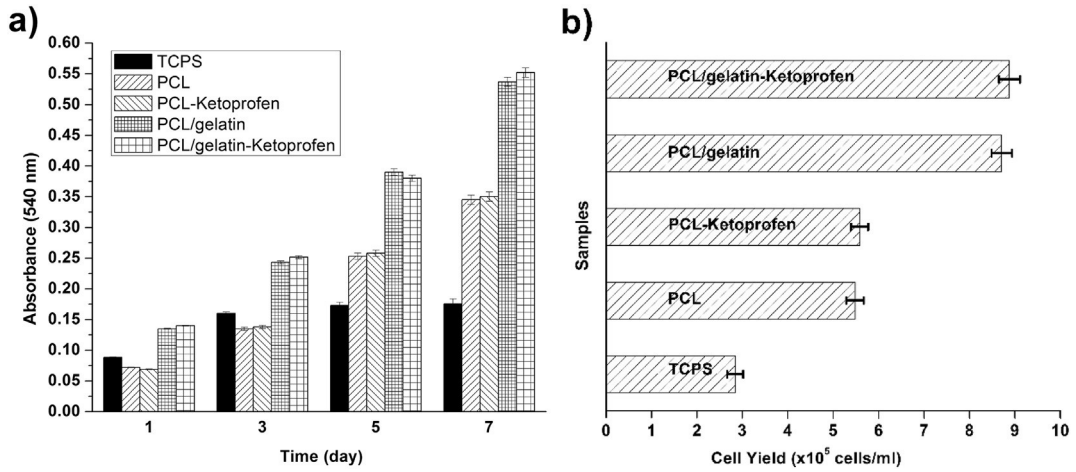

Fig. 7. a) Absorbance of the 540-nm band vs. time; b) cell growth yield for the standard tissue culture polystyrene (TCPS) plate and for the electrospun mats of poly(ε-caprolactone) (PCL) ultrathin fibers, Ketoprofen-containing PCL ultrathin fibers, PCL/gelatin cross-linked fibers, and Ketoprofen-containing PCL/gelatin cross-linked fibers.

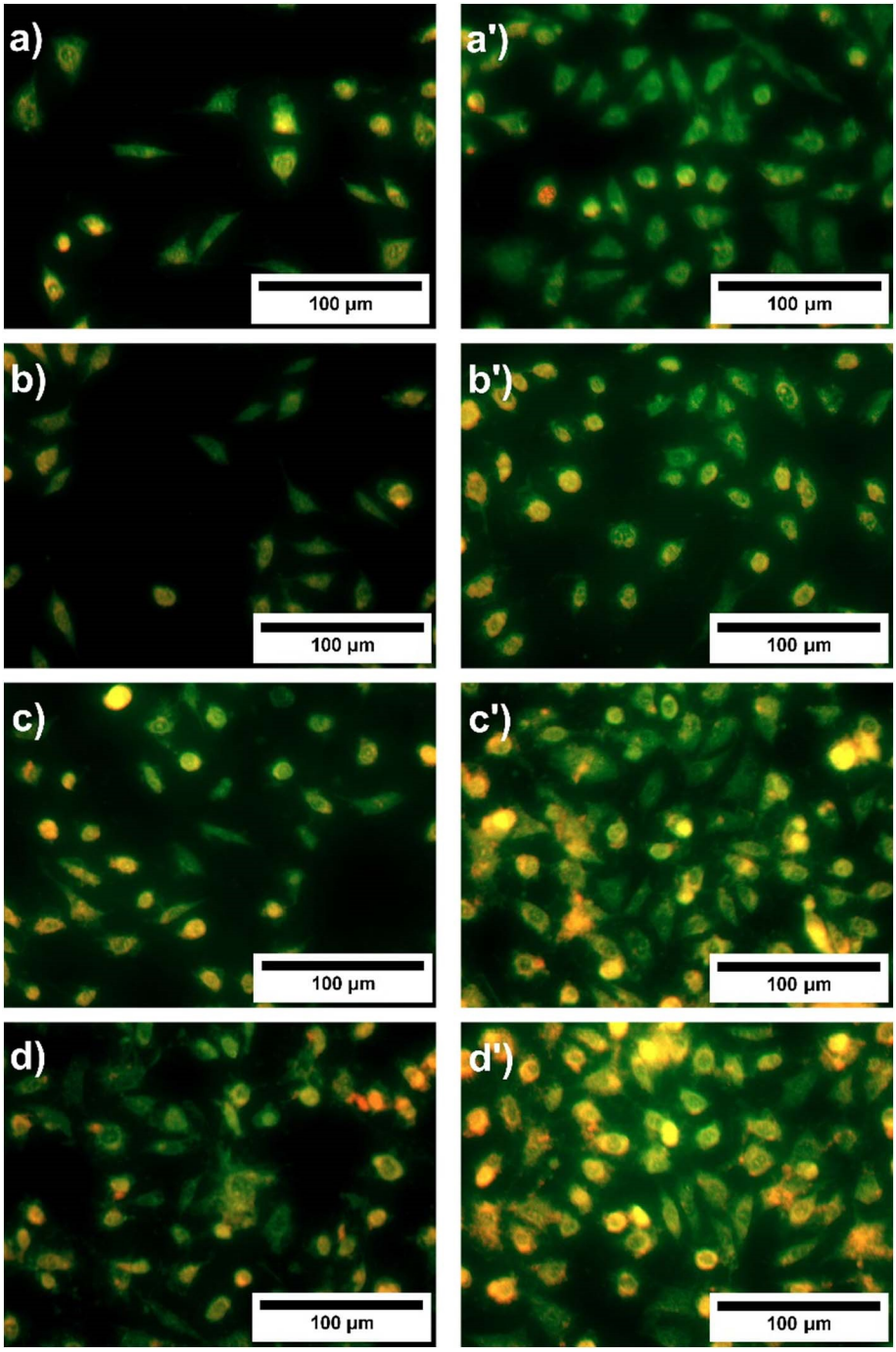

Fig. 8. Fluorescence images of the electrospun mats at the 3rd day (left) and 7th day (right) of cell growth for: a) poly (ε-caprolactone) (PCL) ultrathin fibers; b) Ketoprofen-containing PCL ultrathin fibers; c) PCL/gelatin cross-linked fibers; d) Ketoprofen-containing PCL/gelatin cross-linked fibers. Scale markers of 100 µm in all cases.

morphology. Comparison between the 3rd and 7th day also supported that the number of cells increased considerably. The images further confirmed that cell population was higher for the binary electrospun mat than for the single one and that the presence of Ketoprofen induced no effect on the cell proliferation.

Finally, on the 7th day of the cell growth, the cultured electrospun mats were also observed by SEM. As it can be in Fig. 9, cells highly proliferated on all electrospun mats. Furthermore, certain cell overgrowth or agglomeration can be noticed. In some images, it is even possible to observe some cells growing under the electrospun ultrathin fibers, supporting the above-described observation of 3-D proliferation through the whole porosity of the electrospun mat.

4. Conclusion

Ketoprofen-containing PCL (single) and PCL/gelatin (binary) electrospun fibers were prepared by solution and emulsion electrospinning, respectively. The morphology of the here-prepared electrospun mats was examined by SEM, showing that free-beaded and continuous ultrathin fibers were produced. Surface hydrophilicity was determined by the water contact angle method and it was observed to significantly improved for the binary PCL/gelatin mats after cross-linking with glutaraldehyde vapor. Surface chemical composition and thermal properties of the mats were characterized by means of XPS and DSC analysis, respectively. Obtained results indicated that the Ketoprofen drug was efficiently embedded in the PCL phase of the ultrathin fibers but the mats presented poor thermal stability. Release studies showed that, in comparison to the single PCL electrospun mat, the binary PCL/gelatin electrospun mat totally suppressed the burst release of Ketoprofen and it exhibited a continuous and sustained drug release for > 100 h (~4 days). In addition, the in vitro cytotoxicity studies performed with L929 mouse fibroblast cells revealed that the Ketoprofen-containing electrospun mats present no toxic effect on the cells. Biocompatibility was further evaluated by cell attachment and proliferation studies carried out by UV−Vis spectroscopy and MTT assay, which particularly indicated that cell growth was 1.93 and 3.07 times higher in the binary electrospun mat than in the single mat and TCPS plate, respectively. Finally, fluorescence microscopy images and SEM revealed that cells highly proliferated after seven days of culture and these were able to grow along the 3-D structure of the electrospun mats due to the ultrathin size of the fibers that highly contributes to the high porosity characteristics of these materials. According to these results, the heredeveloped Ketoprofen-loaded PCL/gelatin mats obtained by emulsion electrospinning can be considered as a very interesting candidate in the design of novel dressings for wound healing applications.

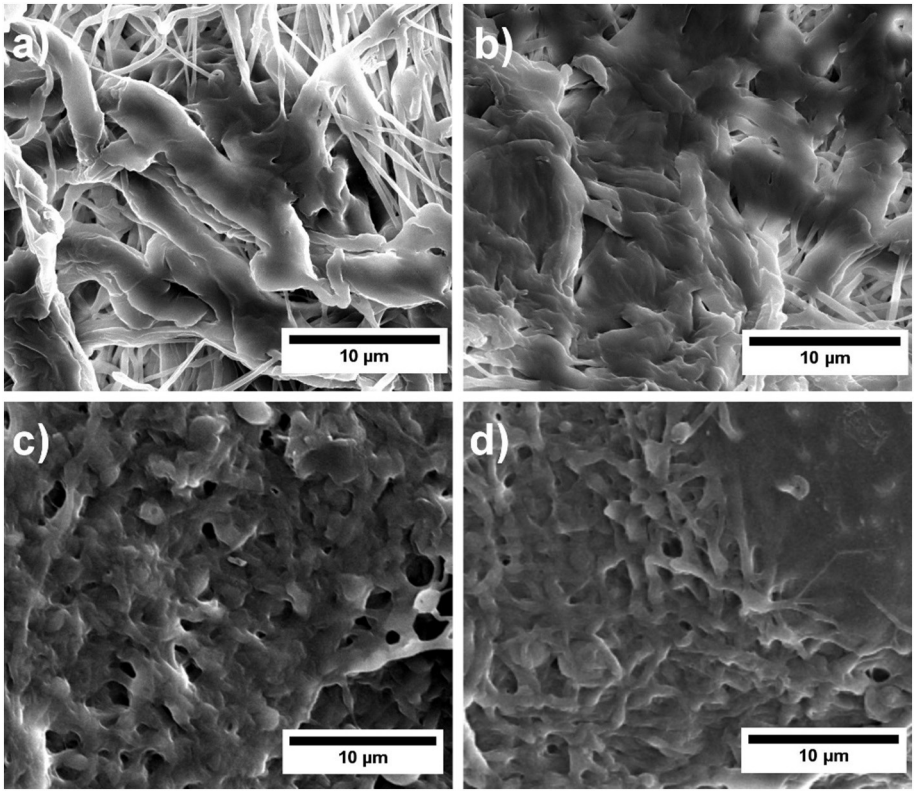

Fig. 9. Scanning electron microscopy (SEM) images of the cultured electrospun mats at the 7th day of cell growth of:
a) poly(ε-caprolactone) (PCL) ultrathin fibers; b) Ketoprofen-containing PCL ultrathin fibers; c) PCL/gelatin cross-linked fibers; d) Ketoprofen-containing PCL/gelatin cross-linked fibers. Scale markers of 10 μm in all cases.

Acknowledgements

The authors would like to acknowledge the Spanish Ministry of Economy and Competitiveness (MINECO) project AGL2015-63855-C21-R for financial support. The authors would also like to thank the European Cooperation Science & Technology (COST) Action MP1206 for their Short Term Scientific Mission (STSM) at IATA-CSIC, Valencia, and the Academic Writing Advisory Center (AWAC) of Atilim University for English language revision.

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