Digest Journal of Nanomaterials and Biostructures Vol. 15, No. 4, October-December 2020, p. 1165-1173

ELECTROSPUN POLYCAPROLACTONE/SILK FIBROIN NANOFIBERS LOADED WITH CURCUMIN FOR WOUND DRESSING APPLICATIONS

B. CAGLAYAN, G. BASAL*
Department of Textile Engineering, Faculty of Engineering, Ege University, Izmir, Turkey

This study aims to develop an active wound dressing material from polycaprolactone /silk fibroin (PCL/SF) blend nanofibers and curcumin (CU), as a therapeutic agent. PCL is a widely used polymer in biomedical application, but its hydrophobic characteristic restricts its use in wound dressings. SF was blended with PCL in order to overcome this limitation. Results showed that SF improved the water take up capacity and hydrophilicity of PCL nanofibers, but also decreased their strength. When CU was added to spinning solution of PCL/SF nanofibers average fiber diameter increased dramatically. Nanofibers became more hydrophilic, but also weaker. In-vitro release study revealed that a burst release of CU from nanofibers within first 3 hours followed by a sustained slow release up to 240 hours.

(Received September 29, 2020; Accepted November 20, 2020)

Keywords: Polycaprolactone, Silk fibroin, Curcumin, Nanofiber, Wound dressing

1. Introduction

Skin is the largest organ in the body. It provides many essential functions, such as protecting the body against harmful foreign agents, regulating body temperature and preventing water loss from the body [1]. When the integrity of skin is damaged through acute or chronic injuries, a complex and dynamic healing process with four overlapping phases: hemostasis, inflammation, proliferation and remodeling starts. Hemostasis involves forming a clot to stop blood loss from a damaged vessel. During the Inflammation phase damaged cells, pathogens, and bacteria are removed. Proliferation phase includes rebuilding of new collagen tissue and extracellular matrix. Finally, remodeling phase consists of remodeling of collagen from type III to type I, removal of cells that are no longer needed by programmed cell death and the fully closure of wound [2]. Wound dressing materials can play an important role during these phases. They can provide better healing conditions by protecting the wound from bacterial infection, minimizing inflammation and helping the reconstruction of damaged tissue [3]. In order to achieve these functions an ideal dressing should have appropriate porosity and oxygen permeability and its structure should resemble skin's extracellular matrix (ECM). Electrospinning is a relatively simple and cheap method of producing substrates with controllable porosity and a fibrillar structure similar to extracellular matrix (ECM) [4, 5]. Both natural and synthetic polymers can be used to produce nanofiber webs by electrospinning method [6]. Polycaprolactone (PCL) is one of the most widely used synthetic polymers for biomedical applications. It is a semi crystalline homopolymer of ε-caprolactone with a glass transition temperature of -60 ° C and a melting point of around 60 °C. PCL has good biocompatibility, biodegradability, mechanical strength, flexibility and solubility[7]. However, the high hydrophobicity of PCL restricts its use as a wound dressing material [8]. Adding a hydrophilic and biocompatible material such as silk fibroin can solve this problem [9,10]. Silk fibroin (SF) is a natural protein produced by the domestic Bombyx mori or wild silkworm. It has excellent biocompatibility and biodegradability [11]. Unfortunately, although natural silk fibers possess excellent mechanical properties, regenerated SF fibers are known to have poor mechanical properties, mainly due to the degradation of fibroin molecule and the destruction of crystal structure after regeneration process [12, 13, 14]. Polymer blending is one of the most effective methods for enhancement of mechanical properties of silk fibroin. Thus, a wound dressing material with both sufficient hydrophilicity and mechanical strength can be

* Corresponding author: guldemet.basal@ege.edu.tr

produced by blending polycaprolactone and silk fibroin. This wound dressing material can be further enhanced by incorporating a therapeutic agent into the structure. Curcumin, a naturally-occurring polyphenol found in Curcuma longa L. (Zingiberaceae), commonly known as turmeric, is one of such promising therapeutic agent. In fact, turmeric has been traditionally widely used as medical herb in Asian countries. Curcumin is best known for its antimicrobial [15], antioxidant [16,17], anti-inflammatory [18], and anticancer [19] properties. Particularly, anti-inflammatory, anti-infectious and anti-oxidant properties of curcumin makes it an ideal wound healing agent. Even though inflammation is a crucial phase during wound healing persistent and uncontrolled inflammation slows down the wound healing by delaying the proliferation and remodeling phases [20]. Previous studies showed that curcumin reduces inflammatory response. Curcumin also reduces oxidation which is a major cause of inflammation during wound healing by blocking the free radicals. The prolonged presence of free radicals at high concentrations prevents tissue remodeling [21, 22]. In addition, curcumin supports formation and deposition of collagen in a wound site, it increases the rate of programmed cell death and wound contraction [21]. Despite all these desirable properties, curcumin has poor water solubility, poor absorption, rapid metabolism, and rapid systemic elimination resulting in poor bioavailability. Because of its poor bioavailability topical application of curcumin provides greater accessibility of the therapeutic agent at the wound site compared to oral applications [22].

In the current study, PCL/SF nanofibers loaded with curcumin was developed for wound dressing applications. The morphology, chemical and structural properties, mechanical properties, hydrophilicity and release profile of resultant nanofibers were investigated.

2. Experimental

2.1. Materials

Polycaprolactone (PCL) with an average molecular weight of 80,000 g/mol was purchased from Sigma-Aldrich. Raw Bombyx mori silk was obtained from Bursa Institute for Silkworm Research (Bursa, Turkey). Sodium carbonate, calcium chloride, formic acid and ethanol were purchased from Sigma-Aldrich(Germany). Curcumin was kindly provided from NPRO (Izmir, Turkey).

2.2. Preparation of PCL and PCL/SF nanofibers

Extraction of silk fibroin was carried out following the method used in the previous study [23]. Bombyx mori cocoons was treated with boiling aqueous solution of 0.05% sodium carbonate (50 times v/w) for 30 min for degumming process. This treatment was repeated three times. The degummed silk rinsed 4–5 times with distilled water and kept overnight for drying at room temperature. Aqueous SF solution was obtained by dissolving 1.2 g SF in 20 (v/w) CaCl2/distilled water/ethanol (molar ratio 1:8:2) by stirring at 150 rpm and 78C for 3 h and then dialyzed at +4C for 3 days to remove the ions and other impurities. Dialyzed solution was put in Petri dishes and dried under vacuum to obtain SF films which was used in the preparation of electrospinning solution.

In order to find out the optimum solution concentration for PCL, PCL solutions with concentrations of 10 and 15 % (w/v) were prepared by dissolving PCL in formic acid. After morphological analysis 15 % (w/v) PCL solution was chosen to prepare PCL/SF blend nanofibers. First, 15 % (w/v) PCL and SF solutions were prepared in formic acid separately and then mixed together in a 1:1 ratio. Curcumin loaded nanofibers were prepared by adding 3% /w/w) curcumin into the PCL/SF solution and stirring for an hour to obtain homogeneity. Viscosity and conductivity of solutions was measured by JP Selecta Rotary Viscometer (Barcelona, Spain) and JP Selecta Conductivity meter (Barcelona, Spain), respectively.

For electrospinning, the solution was transferred into a 10 ml syringe with the 21G (0.8mm) needle connected to a positive high voltage source. A plate collector was used to collect nanofibers. The distance between the needle tip to collector was set to15 cm. Electrospinning was performed at flow rates of 1, 2 and 3ml/hr. and voltages of 15, 20 and 25 kVs.

2.3. Morphological properties

The morphology of nanofibers was observed using a scanning electron microscope (SEM; Carl Zeiss 300VP, Germany). The mean fiber diameter and diameter distribution of the electrospun nanofibers were calculated from SEM images by Image-J software.

2.4. Chemical/structural properties

The structural changes occurred during electrospinning was examined by FTIR spectrum analysis using Nicolet iS50 FTIR Spectrometer (Thermo Scientific, USA).

2.5. Mechanical properties

Breaking load of nanofibers were determined using Zwick /Roell Z010 Tensile testing machine with a 10 N load cell at 20 °C and 65 RH%. Sample size was 40x 10 mm. The crosshead speed was 10 mm/min. Each sample were tested three times and the results were averaged. Thickness of the samples were measured at five different points of nanofibers by a micrometer screw gauge and the mean thickness values were used to calculate tensile strength.

2.6. Water uptake

The water up take capacity of the nanofibers was determined using the following equation

$$\text{Water Uptake (\%)} = (Ww - Wd)/Wd \times 100 \quad \text{Eq.} \quad (1)$$

where Wd is the weight of dry sample and Ww is the weight of wet sample. The wet samples were prepared by immersing dry samples in distilled water at room temperature for 24 h and then drying their surface with a filter paper. This test was performed in triplicate for each sample and average value was calculated.

2.7. Contact Angle Measurements

The wetting behavior of electro spun fibers was evaluated by contact angle, measured with the optical tensiometer (Theta, Attension, Finland). A 5 μL water droplet of deionized water was pipetted and contact angle was measured according to the sessile drop technique. This procedure was repeated at three different places for each sample and average was taken from these results.

2.8. Release study

As release medium 140 ml ethanol and 60 ml distilled water mixed together and one PBS tablet was added to this mixture. Weighted samples of nanofibers (1x12 cm in size) were placed into 10ml release medium in a beaker and stirred at 150 rpm. At specific time intervals (1, 2, 3, 4, 5 hours, 1 week and 2 weeks), the sample solutions were taken and analyzed at wavelength of 429 nm by a UV spectrophotometer Each time same amount of fresh media was added to beaker. Accumulative curcumin release percent was calculated using a calibration curve and it was plotted versus time. All measurements were made in triplicate and averaged.

3. Results and discussion

3.1. Viscosity and conductivity of solutions

Viscosity and conductivity of the electrospinning solution affect electrospinnability and morphology of nanofibers. While low viscosity can cause bead formation due to insufficient chain entanglement between polymeric chains too high viscosity can cause blockage at the tip of the capillary. Similarly, polymer solution should have a certain conductivity otherwise it is impossible to electrospin. The fiber diameter decreases with increasing conductivity up to a certain point. After that the bead formation is observed [24]. Table 1 summarizes the viscosity and conductivity of electrospinning solutions. Pure PCL solution had the highest viscosity. Viscosity decreased with the addition of SF and curcumin. Conductivity, on the other hand, followed an opposite trend. Curcumin loaded PCL/SF solution had the highest conductivity.

Table 1. Conductivity and viscosity of electrospinning solutions.

Solutions	Viscosity (cP)	Conductivity(µS/cm)
PCL (15%)	2515.7	349
PCL/SF	1802.2	391
Curcumin loaded PCL/SF	955.4	411

3.2. Morphological properties

The morphology of nanofibers were observed by a scanning electron microscope (SEM). Solution concentration affects viscosity and therefore plays a critical role on fiber morphology in electrospinning [24]. Figure 1 shows the electrospinning of PCL nanofibers with 10% and 15 % solution concentrations. Mean fiber diameter increased with increasing solution concentration. As seen from Figure 1 even though both 10 and 15 % PCL solutions produced bead free nanofibers PCL nanofibers obtained from 15% PCL solution were more uniform. In case of 10 % PCL solution multiple neck formation was observed. In electrospinning uniform beadless nanofibers can be achieved at a specific solution concentration. Below that concentration beads or beaded nanofibers are formed due insufficient chain entanglement between the polymer chains. As concentration gets close to this critical value beads' shape change from a round shape to ellipse to smooth fibers [24]. This explains neck formation at 10 % PCL solution. Probably, these necks were elongated beads and as concentration increased further they disappeared completely. Therefore, for PCL/SF nanofiber solution concentration was set to 15%.

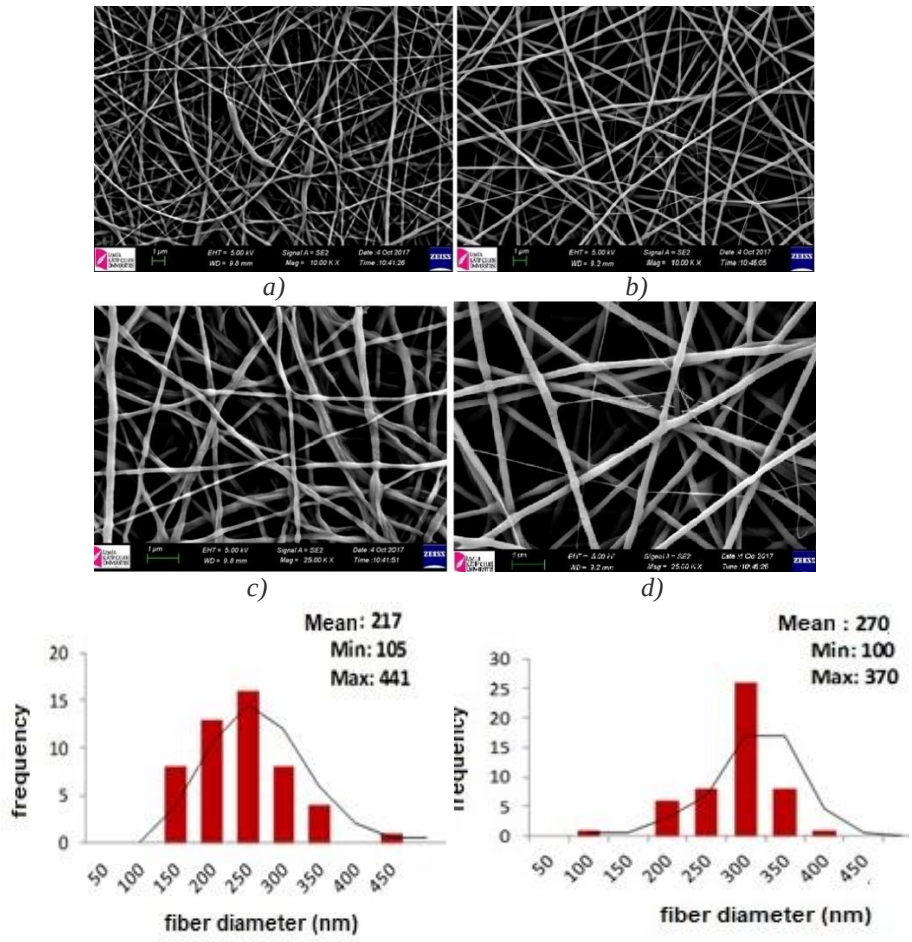

Fig. 1. Morphology and average fiber diameter of PCL nanofibers. 10 % PCL nanofibers flow rate: 2 ml/h Voltage:15 kV (a)10000 X (b)25000 X; 15% PCL nanofibers flow rate: 2 ml/h Voltage:20 kV (c)10000 X d)25000 X

Fig. 2 presents the morphology of PCL/SF nanofibers produced at 1 and 2 ml/hr flow rates and 15 and 20 kV . Mean fiber diameter slightly increased as flow rate increased. A similar effect was observed with increasing voltage.

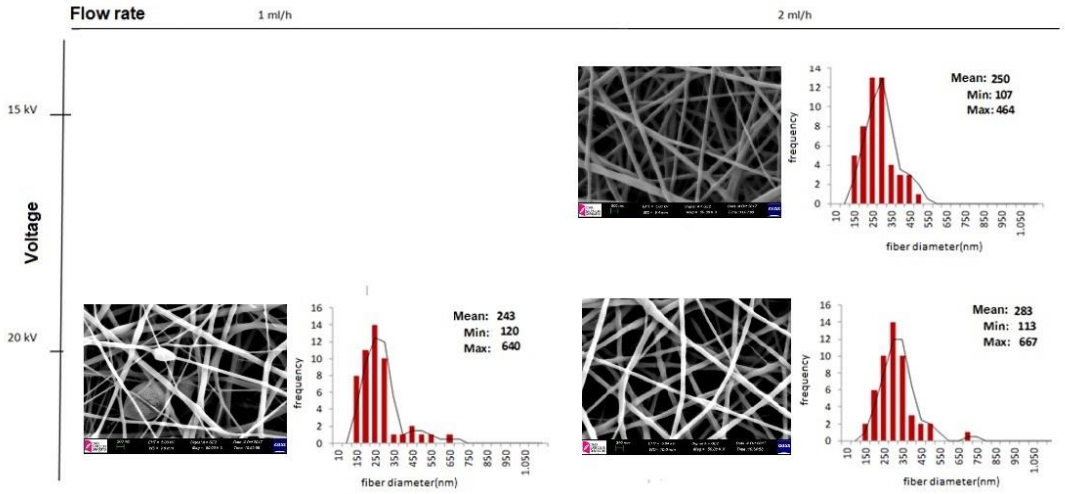

Fig. 2. Morphology and average fiber diameter of PCL/SF nanofibers produced at voltages of 15 and 20 kVs and flow rates of 1 and 2 ml/h (50000 X).

Fig. 3 shows the morphology of curcumin loaded (3% w/w) PCL/SF nanofibers. Curcumin loaded nanofibers showed bead free morphology, but the loading of curcumin significantly increased the diameter of fibers. While the mean diameter of PCL/SF nanofibers was approximately 250 nm upon the incorporation of curcumin the mean diameter of PCL/SF nanofibers increased to approximately 1000 nm. Increasing flow rate and voltage increased the mean fiber diameter slightly.

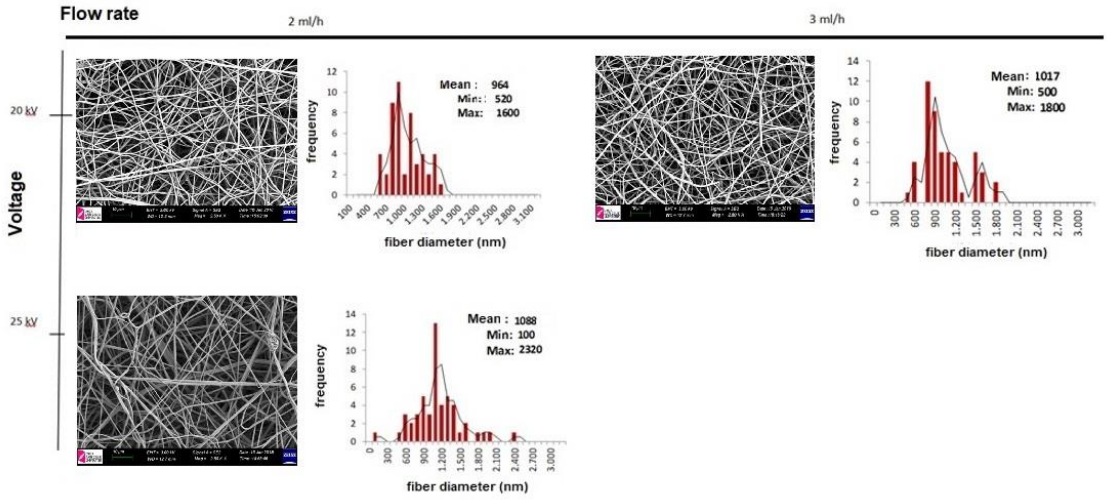

Fig. 3. Morphology and average fiber diameter of curcumin loaded PCL/SF nanofibers produced at voltages of 20 and 25 kVs and flow rates of 2 and 3 ml/h (50000 X).

3.3. Chemical/structural properties

The FTIR spectra of electrospun PCL, curcumin loaded PCL, PCL/SF and curcumin loaded PCL/SF nanofibers are shown in Fig. 3. Some characteristics absorption bands of PCL can be seen in the spectra. These are Carbonyl (C=O) peak at 1724 cm-1, CH2 stretching at 2867 cm-1, and C-O-C stretching at 1169 cm-1 .In the case of the PCL/SF nanofiber, C = O stretching of pure PCL was observed at 1729 cm-1, C-O-C stretching at 1173 cm-1'de and CH2 stretching at 2879 cm-1. Likewise C = O stretching (amide I, random coil) of pure silk fibroin was appeared at 1653 cm-1 and N-H bending (amide II) at 1560 cm-1. With the incorporation of curcumin the intensity of the peak (C = O stretching) at 1725 cm-1 increased and a broad absorption band at 3317 cm−1 was observed due to the phenolic O–H stretching.

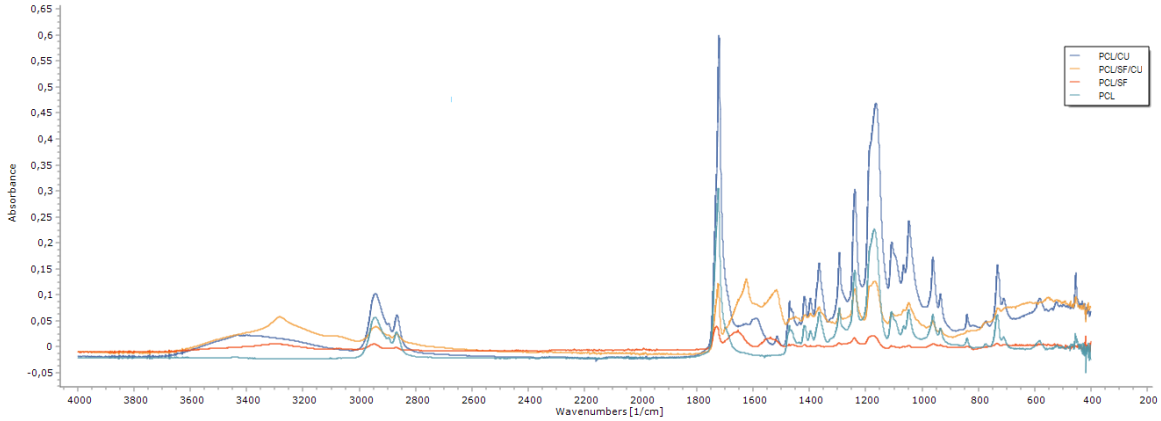

Fig. 4. FTIR spectra of PCL, PCL/SF, curcumin loaded PCL and curcumin loaded PCL/SF nanofibers.

3.4. Mechanical properties

Table 2 shows the stress–strain values of the electrospun PCL, PCL/SF and curcumin loaded PCL/SF nanofibers. Pure PCL nanofiber had a tensile strength of 3.5 MPa. With the addition of SF tensile strength of nanofibers decreased almost 50%. This was expected since during the regeneration process the crystal structure of silk fibers is disrupted and fibroin molecule is degraded resulting poor mechanical properties. The incorporation of curcumin into structure further decreased the tensile strength. Both pure PCL and PCL/SF nanofibers had similar elongation at break values, but like in tensile strength curcumin reduce the elongation.

Table 2. Mechanical properties of PCL, PCL/SF and curcumin loaded PCL/SF nanofibers.

	Stress MPa (N/mm2)	Strain %
PCL	3.5	63.3
PCL/SF	1.82	64.53
Curcumin loaded PCL/SF	0.65	42.46

3.5. Water contact angle test

The hydrophilicity of the materials is one of the desirable property for wound dressings. It is usually characterized by water contact angle testing. The contact angle values of PCL, PCL/SF and curcumin loaded PCL/SF nanofibers and the representative water drop images in contact are displayed in Figure 5. The presence of silk fibroin improved the hydrophilicity of the pure PCL

nanofibers. The water contact angles decreased around 20–25 degree. Incorperation of curcumin into the structure had much bigger effect on the water contact angle. The contact angle sharply decreased to 17 degree. In general, curcumin has poor water solubility, but the oxygen in the chemical structure of curcumin is a proton donor and can easily bond with water[9]. This explains the sharp decreased observed in the contact angles when curcumin was added to structure.

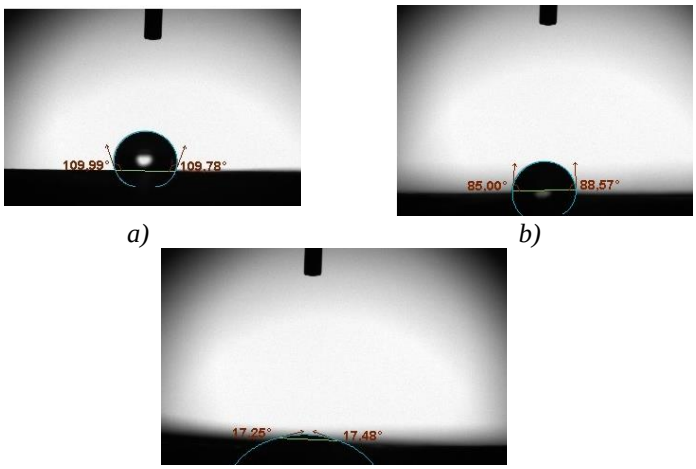

Fig. 5. Water contact angle a) PCL, b) PCL/ SF c) Curcumin loaded PCL/SF.

3.6. Water uptake ratio

During wound healing controlling the moisture balance between wound and wound dressing is critical. Low humidity might result in dry wound and excess humidity might cause infection [25]. Table 3 shows the water uptake values of nanofibers. Since PCL is a hydrophobic polymer PCL nanofibers had the lowest water up take rate. The addition of SF into structure immediately improved the water uptake values. Curcumin loaded fibers had the highest water absorption rate. As explained previously the oxygen in the chemical structure of curcumin tends to bonds with water molecules. In addition, it is likely that the increased fiber diameter resulted in bigger pores and increased porosity in the structure which might have improved the water absorption.

Table 3. Water Uptake values of PCL, PCL/SF and curcumin loaded PCL/SF nanofibers.

	Wd (mg)	Ww (mg)	Water Uptake (%)
PCL	31.6	31.8	0.6
PCL/SF	33.7	34.5	2.4
Curcumin loaded PCL/SF	38.2	41	7.3

3.7. Release study

The release profile of curcumin loaded PCL/SF nanofibers was shown in Fig. 6. The release percentage of curcumin from the samples was calculated based on the calibration curve and absorbance of testing samples. Approximately 87 % of the loaded amount of curcumin was released to the release medium during the first 3 hours. This burst release could be attributed to the

presence of curcumin on or near the surface of the nanofibers. Then, curcumin release slowed down and it was completed in 240 h. Generally, a burst release is not desirable for drug release applications, but a burst release of therapeutic agent at wound site might be advantages in case of severe infections or right after surgery.

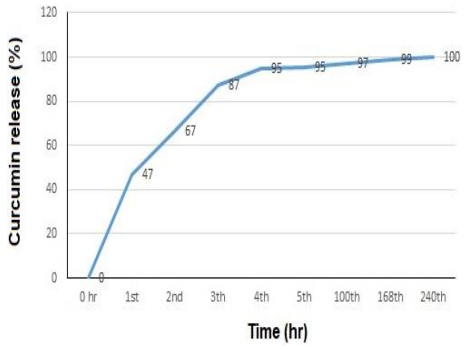

Fig. 6. Release profile of curcumin loaded PCL/SF nanofibers.

4. Conclusions

The purpose of this study was to develop an active wound dressing material from polycaprolactone (PCL), silk fibroin(SF) and curcumin (CU) by electrospinning technique. PCL is a widely used polymer for biomedical application, but its high hydrophobicity limits its use in wound dressings. In order to overcome this limitation PCL was blended with SF. Curcumin was incorporated into the blend as therapeutic agent. Blending of PCL with SF improved hydrophilicity and water take up capacity, but also decreased the strength.

With the incorporation of curcumin mean fiber diameter increased significantly. Nanofibers became more hydrophilic, but also weaker. In vitro release profile of curcumin showed that an initial burst release, which could be favorable in severe topical infections or surgical wounds right after operation, followed by a sustained release.

References

[1] G. Han, R. Ceilley, Advanced Therapeutics **34**(3), 599 (2017).
[2] S. Das, A. B. Baker, Frontiers in Bioengineering and Biotechnology **4**, 82 (2016).
[3] R. Z. Murray, Z. E. West, A. J. Cowin, B. L. Farrugia, **7**, 2 (2019).
[4] P. Zahedi, I. Rezaeian, S. O. Ranaei-Siadat, S. H. Jafari, P. Supaphol, Polymers for Advanced Technologies **21**(2), 77 (2010).
[5] M. F. Dzikri, T. P. Armedya, S. Q. Khairunisa, S. C. W. Sakti, Y. Raharjo, W. Purnamasari, N Nasronudin, M. Z. Fahmi, Digest Journal of Nanomaterials & Biostructures 14(1), 203(2019).
[6] D. Li, Y. Xia, Advanced Materials **16**(14), 1151 (2004).
[7] M. A. Woodruff, D. W. Hutmacher, Progress in Polymer Science **35**, 1217 (2010).
[8] L. Ghasemi-Mobarakeh, M. P. Prabhakaran, M. Morshed, Biomaterials **29**, 4532 (2008).
[9] M. Fallah, S. H. Bahrami, M. Ranjbar-Mohammadi, Journal of Industrial Textiles **46**(2), 562 (2016).
[10] J. S. Lim, C. S. Ki, J. W. Kim et al., Biopolymers **97**(5), 265 (2012).
[11] L. Chen, Y. Zhu, Y. Li, Y. Liu, J. Yu, Sheng Wu, Gong Cheng, Xue Bao, **27**(6), 831 (2011).
[12] H. Cao, X. Chen, L. Huang, Z. Shao, Materials Science and Engineering C **29**, 2270 (2009).
[13] G. Liu, Q. Tang, Y. Yu, J. Li, J. Luo, M. Li, Polym. Adv. Technol. **25**, (2014).
[14] X. Li, Q. Zhang, D. Ye, J. Zhang, Y. Guo, R. You, S. Yan, M. Li, J. Qu, **57**(2), 206 (2017).
[15] S. H. Mun, D. K. Joung, Y. S. Kim, O. H. Kang, S. B. Kim, Y. S. Seo et al., Phytomedicine **20**, 714 (2013).

[16] T. Phan, P. See, S. Lee, S. Chan, The Journal of Trauma: Injury, Infection, and Critical Care **51**(5), 927 (2001).
[17] T. Ak, I. Gulcin, Chemico-Biological Interactions **174**, 27 (2008),
[18] G. Liang, S. Yang, H. Zhou, L. Shao, K. Huang, J. Xiao et al., European Journal of Medicinal Chemistry **44**(2), 915 (2009).
[19] W. Lee, C. Loo, P. Young, D. Traini, R. Mason, R. Rohanizadeh, Expert Opinion on Drug Delivery **11**(8), 1183 (2014).
[20] B. Joe, M. Vijaykumar, B. Lokesh, Critical Reviews in Food Science and Nutrition **44**(2), 97 (2004).
[21] C. Mohanty, M. Das, S. Sahoo, Molecular Pharmaceutics **9**(10), 2801 (2012).
[22] D. Akbik, M. Ghadiri, W. Chrzanowski, R. Rohanizadeh, Life Science **116**(1), 1 (2014).
[23] G. Basal, G. D. Tetik, G. Kurkcu, O. Bayraktar, I. D. Gurhan, A. Atabey, Digest Journal of Nanomaterials & Biostructures **11**(4), 113 (2016).
[24] A. Haider, S. Haider, I.-K. Kang, Arabian Journal of Chemistry **11**(8), 1165 (2018).
[25] R. Xu, H. Xia, W. He et al., Scientific Reports **6**, 24596 (2016).