A novel nanofiber Cur-loaded polylactic acid constructed by electrospinning

Thi Thu Trang Mai¹, Thi Thu Thuy Nguyen², Quang Duong Le¹,³, Thi Ngoan Nguyen¹, Thi Cham Ba³, Hai Binh Nguyen¹, Thi Bich Hoa Phan¹, Dai Lam Tran¹, Xuan Phuc Nguyen¹ and Jun Seo Park²

¹ Institute of Materials Science, Vietnam Academy of Science and Technology, Hanoi, Vietnam
² Division of Chemical Engineering, Hankyong National University, Ansung, Korea
³ University of Technology and Management, Hanoi, Vietnam

E-mail: trangmt23@gmail.com and trandailam@gmail.com

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Abstract
Curcumin (Cur), extracted from the Curcuma longa L. plant, is well known for its anti-tumor, anti-oxidant, anti-inflammatory and anti-bacterial properties. Nanofiber mats of polylactic acid (PLA) loading Cur (5 wt%) were fabricated by electrospinning (e-spinning). Morphology and structure of the fibers were characterized by field emission scanning electron microscopy (FE-SEM) and Fourier transform infrared (FTIR) spectroscopy, respectively. The diameters of the obtained fibers varied from 200 to 300 nm. The release capacity of curcumin from curcumin-loaded PLA fibers was investigated in phosphate buffer saline (PBS) containing ethanol. After 24 h, 50% of the curcumin was released from curcumin-loaded PLA fibers. These results of electrospun (e-spun) fibers exhibit the potential for biomedical application.

Keywords: nanofibers, electrospinning, curcumin, polylactic acid

Classification numbers: 5.08, 5.10, 5.16

1. Introduction
Electrospinning (e-spinning), a simple but advanced technique, has attracted widespread attention for fabrication of nanofibers and application in tissue engineering and drug delivery systems in recent years [1–8]. E-spinning possesses great advantages including simplicity of use, potential scale up and versatility in spinning a wide variety of polymeric fibers. This method involves the introduction of a high voltage source to obtain fibers whose diameters range from several nanometers to microns. These advanced fibers have a high ratio of length and diameter, superior mechanical property and tunable surface morphologies, and could therefore enhance cell attachment and be applicable for supplementing the physical structure of the extracellular matrix of biological tissues [9]. Additionally, these electrospin (e-spin) fibers, which are highly porous, exhibit high functional surface areas that could allow drug molecules to diffuse out from the matrix, thus enhancing mass transfer capacity of drugs and opening up a novel class of drug carriers [10, 11].

Polylactic acid (PLA), polyglycolic acid (PGA) and polylactic-co-glycolic acid (PLGA) with their biodegradability and biocompatibility are typical polymers utilized in tissue engineering scaffolds and biomedical applications [11,12]. Recently, much attention has been paid to the e-spinning of these polymers for their application in drug delivery, surgical implantation and tissue engineering [4, 13–16]. Cur (chemical structure shown in figure 1), a natural compound in the Curcuma longa L. plant, is well known for its anti-tumor, anti-oxidant, anti-microbial, anti-bacterial and anti-inflammatory properties [17–20]. Thanks to these remarkable properties, Cur was widely used for biomedical applications including wound healing, anti-bacterial, anti-coagulation, anti-oxidation and anti-proliferation [21–23].

Many publications on Cur or e-spun nanofibers of PLA (and several other polymers) have separately been reported in the literature. However, there are only very few reports about combination of Cur and PLA. The purpose of this study is to fabricate by e-spinning Cur-loaded PLA nanofibers having...
decreasing nanosized diameters and high loading/releasing capacity of Cur to overcome the low solubility at neutral environment of Cur, therefore being applicable to biomedical treatments. Morphology and structure of the obtained fibers were investigated to ascertain the formation. The nanosized Cur-loaded PLA fibers were also tested in vitro to evaluate their Cur-release capacity for biomedical applications. Further study on Cur- and modified-Cur-based nanofibers and their specific biomedical activities are expected to be conducted in the near future.

2. Materials and methods

2.1. Materials

PLA ($M_w = 88,000$) was purchased from Acros Organics Co. Curcumin was supplied by Institute of Chemistry, Vietnam. Acetone and dimethylacetamide (DMAc) were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade and used without further purifications.

2.2. Fabrication of Cur-loaded PLA nanofibers

Nanosized Cur-loaded PLA fibers were manufactured by the e-spinning method. A schematic of an e-spinning system is shown in figure 2 [24]. A weight amount of PLA was dissolved in acetone/DMAc (2:1 v/v) to prepare a PLA solution at a concentration of 8% w/v. Cur was loaded in PLA solution by dissolving its powder at the amount of 5 wt% compared to PLA. The mixture was stirred overnight at room temperature to achieve homogenous solution. The polymer solution was poured into a standard 5 ml syringe that was attached to a steel needle. E-spinning was carried out by introducing a high voltage to the needle as a positive electrode and an opposite rotating sheet of aluminum as negative electrode. The distance between needle tip and collector was set at 12 cm. Once positive electrode reached a critical voltage of 10 kV, spinning head extruded polymer solution and the e-spun fibers were collected on the aluminum sheet. The emitting rate of the polymer solution was controlled at 0.5 ml/h by means of a syringe pump.

2.3. Characterization

2.3.1. Morphological characterization. Morphological structure of Cur-loaded PLA nanofibers was observed by field emission scanning electron microscope (FE-SEM Hitachi S4800). The structure of the nanosized fibers was examined using Fourier transform infrared (FTIR) spectrometer (FTIR–Nicolet 6700) in the region of 400–4000 cm$^{-1}$ with the resolution of 4 cm$^{-1}$.

2.3.2. In vitro curcumin release studies. The release profile of Cur from the Cur-loaded PLA nanofibers was investigated in PBS at pH 7.4 containing 30% v/v ethanol. A certain amount of the sample (5 mg) was immersed into 10 ml of release medium and kept at 37°C over specific time intervals. The amount of Cur released at various times, up to 24 h, was determined using an ultraviolet-visible (UV–Vis) spectrometer at excitation of 431 nm. With the aid of the calibration curve of Cur measured in the same condition, the percentage of Cur release was calculated and plotted versus time according to the equation

$$\text{Release} \ (% \%) = \frac{\text{Release curcumin}}{\text{Total loaded curcumin}} \times 100\%.$$

3. Results and discussion

3.1. Morphological characterization of e-spun fibers

Morphology of these e-spun fibers is characterized and shown in figure 3. It can be observed that the fibers are commonly round-shaped, randomly arrayed and highly porous. The surfaces of fibers are homogeneous, having no defects or spots of Cur. The diameter of the e-spun Cur/PLA fibers varies in the range of 200–500 nm. To the best of our knowledge so far, this diameter is comparable to other e-spun fibers reported recently in reference [9] (PLA sizing from 315 to 670 nm and cellulose acetate (CA) sizing from 500 to 595 nm) and [25] (PLA sizing at approximately 700 nm). Further investigation of the effect of blend ratio on the size and therefore the drug loading/releasing capacity of the blends will be performed.

The FTIR spectroscopy was performed to elucidate the combination of Cur and PLA in the nanofibers, shown in figure 4. In figure 4, shifts of typical peaks of Cur and PLA are clearly depicted. As for Cur, a broad absorption band at 3446 cm$^{-1}$ is due to the phenolic O–H stretching vibration [26], and this band was observed at 3450 cm$^{-1}$ in Cur-loaded PLA. Additionally, sharp peaks at 1514 and 1430 cm$^{-1}$ are respectively typical for stretching vibration of C=C of benzene ring and olefinic bending vibration of C–H bound to the benzene ring of Cur [26, 27]. The shifts of these peaks were observed at 1594 and 1504 cm$^{-1}$, respectively, confirming that Cur and PLA were bound together. Moreover, a peak at 861 cm$^{-1}$, assigned for vibration...
of C–O in –C–OCH₃ of phenyl ring [26], was also observed at 860 cm⁻¹ in Cur-loaded PLA. It is reasonable that a peak at 1290 cm⁻¹, also assigned as vibration of C–OCH₃ [26], was not observed in Cur-loaded PLA because of the eclipse by strong peaks of PLA at that wavenumber range due to a high proportion of PLA in the fibers. On the other hand, due to high ratio PLA/Cur, typical peaks of PLA were observed clearly and strongly in both neat PLA and Cur-loaded PLA. Three characteristic regions of PLA and their shifts were identified based on analysis in the literature [28]. A needle-like peak at 1737 cm⁻¹ is assigned as carbonyl stretching C=O in the –CO–O– group of PLA and was observed to shift to 1758 cm⁻¹. Another needle-like peak at 1165 cm⁻¹, assigned as stretching vibration of –C–O– in –CH–O– in PLA polymer chains, shifted to 1185 cm⁻¹. A mountainous triplet peak at 1110, 1072 and 1025 cm⁻¹, corresponding to C–O stretching vibration in –CO–O– group in polymer chains, shifted accordingly to 1126, 1094 and 1031 cm⁻¹, respectively. Briefly, all typical peaks of both Cur and PLA have blue shifted in Cur-loaded PLA, ascertaining that Cur and PLA have bound together to form a more stable structure.

3.2. Curcumin release studies

To investigate the capacity of Cur release from the Cur-loaded PLA fibers, release experiments were carried out using a PBS solution with 30% ethanol at pH 7.4 and controlled temperature of 37°C. The calibration curve of Cur is shown in figure 5 and the following equation

\[
\text{Concentration (mg ml}^{-1}\text{)} = 0.0003 + 0.0228 \times \text{Absorbance.}
\]

Based on the calibration curve of Cur and absorbance of testing samples, released Cur concentration was calculated, and the release profile is shown in figure 6. Cur showed a burst release from the e-spun nanofibers during the first 2h, releasing nearly 50% of the loaded amount of Cur. This burst release could be attributed to the presence of Cur on or near the surface of the fibers. Then, Cur released slowly to reach a peak of 55% after 8h of release in the medium. From that point, the amount of Cur release began to reduce gradually to around 50% after 24h of immersion in release buffer. This percentage of Cur release was similar to the Cur release from cellulose acetate fiber mats reported by Suwantong et al [29].
4. Conclusion

Cur-loaded PLA nanofibers were e-spun and had comparatively small diameter in comparison to other drug loaded e-spin fibers. FTIR spectra elucidated the fabrication of the nanofibers. FE-SEM images also implied homogeneously fine but porous surface. The saturated releasing capacity of Cur was only 50% of the loaded Cur. However, this value was still interesting thanks to the fact that (i) the loading capacity of Cur onto PLA is very high (data not shown), and (ii) Cur was added at a low concentration of 5 wt% of PLA, resulting in decreased release kinetics of Cur. Higher concentration of loading Cur and in vitro biological assay are expected to be fulfilled in the near future.

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