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Research paper

Controlled release of metronidazole benzoate from poly ε-caprolactone electrospun nanofibers for periodontal diseases

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ABSTRACT

Poly ε-caprolactone (PCL) nanofibers containing metronidazole benzoate (MET) were successfully electrospun and evaluated for periodontal diseases. Solutions of 10.5% w/v PCL and 5–15% w/w MET in mixtures of dichloromethane (DCM)/*N*,*N*-dimethylformamide (DMF) with ratios of 90:10, 80:20 and 70:30 v/v were prepared, and the nanofibers were produced by electrospinning technique. Scanning electron microscopy (SEM) was used to investigate the morphology and average diameter of the electrospun nanofibers. DSC results indicated a molecular dispersion of MET in the PCL nanofibers and showed a decrease in crystallinity of PCL nanofibers by adding MET. Results showed that an increase in the DCM:DMF ratio led to a decrease in the solution conductivity and an increase in the solution viscosity as well as in the nanofibers diameter. Also increasing metronidazole benzoate concentration caused an increase in the solution conductivity and a decrease in the solution viscosity as well as in the nanofibers diameter. *In vitro* drug release studies in phosphate buffer solution (pH 7.4) showed that the drug release rate was affected by the solvents ratio and the drug concentration. Moreover, the burst release was low, and sustained drug release was prolonged to at least 19 days.

1. Introduction

Periodontal disease is a general term for a number of pathological conditions characterized by inflammation and degeneration of the gums (gingival), supporting bone (alveolar bone), periodontal ligament and cementum [1]. The extension of inflammation from marginal gingival into the supporting periodontal tissues marks the transition from gingivitis to periodontitis [2]. One of the most important clinical features of periodontitis is periodontal pocket [3]. The epithelium of the gingiva migrates along the tooth surface forming 'periodontal pocket' that provides an ideal environment for the growth and proliferation of microorganisms [1]. The aim of current periodontal therapy is to remove the bacterial deposits from the tooth surface and to shift the pathogenic bacteria to one compatible with periodontal health by mechanical cleaning and systemic or local application of antimicrobial agents [4]. Although systemic administration of antibiotics is useful, high oral doses are necessary to achieve effective concentrations in the gingival fluid. However, long-term use may lead to the development of resistant bacterial strains. These drawbacks have led researchers worldwide to focus on localized delivery of antibiotics directly at the diseased site [5]. The effectiveness of local delivery but not controlled release is that it reaches the base of periodontal pocket and is maintained for an adequate length of time for the antibiotic effect to occur. But there is not any mechanism to retain therapeutic levels for a prolonged period of time. Controlled delivery systems are designed to release drug slowly for more prolonged drug availability and sustained drug action [3]. These systems can be divided into two main categories: nondegradable devices and degradable devices. The latter offer the advantage of not requiring the patient to revisit for the removal of the device. Therefore, high patient compliance will be afforded. It persuaded researchers to focus on developing different types of degradable delivery systems such as films, fibers, microcapsules.

The first literature about using monolithic fibers as a drug delivery system for periodontal diseases was by Goodson et al. who studied on ethylene vinyl acetate fibers incorporated tetracycline hydrochloride (TCL), which exhibited *in vitro* drug release up to 9 days [6]. Afterwards, other researchers attempted to develop controlled release devices using various polymers and antibiotics and evaluated *in vitro* or *in vivo* for the treatment of periodontal diseases. Among antibiotics, MET is a front line chemotherapeutic agent for treating infections by anaerobic bacteria associated with periodontal diseases due to the low minimum inhibitory concentration (MIC) it requires [4]. The chemical structure of MET is shown in Fig. 1. Poly

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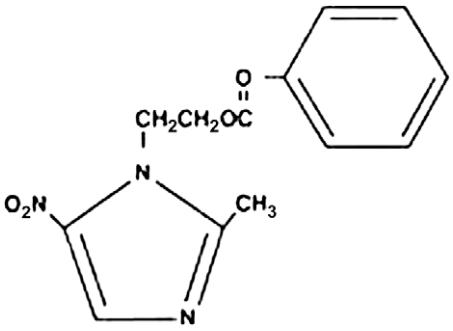

Fig. 1. Chemical structure of metronidazole benzoate.

ε-caprolactone is a semi-crystalline biodegradable aliphatic polyester which is well known for its slow biodegradability, high biocompatibility and good drug permeability [7]. Unlike commonly used biodegradable polymers such as poly (D,L-lactic-co-glycolic acid), PCL does not produce a local acidic environment as it degrades. This, along with its comparatively low cost, renders PCL an attractive biomedical polymer [8]. However, PCL, in the form of homopolymer, has not been used for periodontal diseases successfully yet. It has been tested *in vitro* as a matrix in the form of fiber for TCL delivery and as a film for minocycline delivery in periodontal therapy. Clinically, the fibers released their TCL content very rapidly with a half-life of 11 h [1], and the films had a high burst release within the first 2–3 h and a steady state release for 7 days [9].

In the present study, MET-eluting electrospun PCL nanofibers are prepared for the treatment of periodontal diseases. In the recent decade, electrospun fibers, the fibers fabricated by electrospinning, have been used in several biomedical applications such as controlled drug delivery systems [10–17]. Electrospinning is an inexpensive method that creates polymeric fibers with diameters in the range of nano to a few microns through electrically charged jet of polymer solution or polymer melt. When charges within a polymer droplet at the tip of a needle reach a critical amount, a fluid jet will erupt from the droplet. The electrospinning jet will travel towards a grounded collector. As the solvent evaporates, the jet solidifies and the polymeric fibers collect on the grounded target [18]. Drugs can be capsulated directly into electrospun fibers by electrospinning of a mixture solution of a drug and polymer. As demonstrated in Refs. [11,12], solubility and compatibility of drugs in the drug–polymer–solvent system are the effective factors on drug release behavior although both hydrophobic and hydrophilic drugs can be incorporated in electrospun fibers. Electrospun nanofibers offer advantages such as higher drug loading efficiency in comparison with some other methods like encapsulation. Furthermore, the drug release profile can be tailored by a modulation on the morphology, porosity and composition of nanofibers [15]. Very small diameter of nanofibers can provide a short diffusion passage length [19], and their high surface area is helpful to mass transfer and efficient drug release [13].

In this study, the nanofibers were electrospun from PCL solutions in different mixtures of DCM:DMF containing various amounts of MET. MET is practically water insoluble and freely soluble in DCM [20], which is a good solvent for PCL. Therefore, it was supposed that MET would be successfully incorporated in PCL electrospun nanofibers.

2. Materials and methods

2.1. Materials

PCL ($\overline{M_n} = 80,000$ g/mol) was purchased from Sigma–Aldrich (US). Dichloromethane (DCM), dimethylformamide (DMF), sodium hydroxide (NaOH) and potassium phosphate monobasic (KH_2PO_4, Cryst.extra pure) were purchased from Merck Chemical Co. (Germany). Metronidazole benzoate was kindly provided by Amin Pharmaceutical Co. (Iran).

2.2. Methods

2.2.1. Electrospinning

Electrospinning was carried out using 10.5% w/v solution of PCL in DCM:DMF mixtures with ratios of 90:10, 80:20 and 70:30 v/v. Then, MET, which is freely soluble in DCM, was added to the polymer solution. The drug concentration was in the range of 5–15% w/w with respect to the polymer used. The resulted clear solution was transferred to a 10-ml syringe pump with a right angle-shaped needle of 0.6 mm in inner diameter attached to it. The flow rate of the polymer solution was 1.82–2.14 ml/h, and the applied positive voltage was in the range of 14–17 kV. The resulting fibers were collected on a grounded aluminum plate. The distance between the needle tip and the grounded target was 23 cm. The thickness of all nanofibers webs ranged from 300 to 340 μm.

2.2.2. In vitro drug release studies

The medicated electrospun nanofibers webs were cut into about 2.5×2.5 cm^2 pieces. The samples were accurately weighed, and then both sides of the webs were rinsed with 200 ml of distilled water to wash out the superficial drug. Then, the samples were placed in 10 ml of phosphate buffer solution (pH 7.4) at 37 °C. At predetermined time intervals, the nanofibers sample was taken out from the incubation buffer and put in another fresh buffer solution. The amount of released drug was determined spectrophotometrically using a Shimadzu UVmini 1240 spectrophotometer (Japan). The UV absorbance of MET in buffer solution was determined at λ_{max} = 231 nm and converted to the MET concentration according to the calibration curve of MET in the same buffer. The results were reported as an average of three determinations.

2.2.3. Entrapment efficiency

Entrapment efficiency was determined by dissolving a known mass of rinsed sample in DCM:DMF with ratio of 80:20 v/v and monitoring the absorbance at λ_{max} = 318 nm. The amount of MET was obtained from the calibration curve of MET in the same solution. The entrapment efficiency was calculated as:

$$\text{Entrapment efficiency}\,\% = \frac{\text{weight of drug in the web}}{\text{theoretical weight of drug loading in the web}} \times 100 \quad (1)$$

2.2.4. Viscosity and conductivity of solutions

Solution viscosities were determined by BROOKFIELD DV-II+-PRO Rheometer (USA) at 25 °C and 5 rpm. Conductivity measurements were taken using a JENWAY 3540 (Germany) electric conductivity meter. All results were reported as an average of three determinations.

2.2.5. Scanning electron microscopic (SEM) studies

The morphology of electrospun nanofibers was studied by a Philips XL30 (Netherlands) scanning electron microscope.

2.2.6. Differential scanning calorimetric (DSC) studies

The nature of the drug in the nanofibers was assessed by performing DSC on pure MET, pure PCL nanofibers, 5% w/w MET-loaded nanofibers and 15% w/w MET-loaded nanofibers. DSC measurements were taken using a METTLER DSC30 (Germany) instrument. About 5 mg of the samples were sealed in an aluminum pan and were heated from 15 °C to 180 °C at a rate of 5 °C/min.

2.2.7. Statistical analysis

Statistical analysis was carried out using the SPSS 13 statistical package. Analysis of variance was followed by the least significant difference (LSD). Post hoc test was used for comparison of different data. $P < 0.05$ was considered as the significant level.

The drug release mechanism was investigated using Peppas equation:

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

where M_t/M_∞ is the drug fraction released at time t, K is a constant depending on the structural and geometric characteristic of the system, n is the diffusional coefficient related to the release mechanism [21].

3. Results and discussion

DCM is a good solvent for both PCL and MET. But it is not a suitable solvent for electrospinning of PCL because of its medium dielectric constant (9.1 at 25 °C) and poor electric conductivity [22]. While DMF is a nonsolvent for PCL, but it has a high dielectric constant (36.7 at 25 °C) [22,23]. DMF was used to enhance electrospinning process in some other investigations [22,23]. Therefore, a mixture of DCM:DMF was used as a solvent in this study.

3.1. Physical characteristics of the electrospun nanofibers

Electrospinning of PCL was carried out from DCM:DMF mixtures with ratios of 90:10, 80:20 and 70:30 v/v to investigate the effect of the solvents ratio. The amount of DMF added to the solution had a significant effect on the electrospun nanofibers morphology as well as on the nanofibers diameter, as shown in Fig. 2. By increasing the amount of DMF in the mixture of solvents, the fibers diameter became lower due to enhanced conductivity and reduced viscosity of the solution as shown in Fig. 3. These effects were previously demonstrated by other workers [22].

Generally, when conductivity of a solution increases, more electric charges are carried by the electrospinning jet. Thus, higher elongation forces are imposed to the jet under the electrical field [18]. On the other hand, by increasing the solution conductivity, bending instability can be increased during the electrospinning. So, the jet path becomes longer and more stretching of the solution is induced [18]. Both higher elongation forces and greater bending instability resulted in fibers with lower diameter. Moreover, by decreasing the viscosity of the electrospinning solution, the fluid jet is stretched and elongated more easily. As a result, the fibers diameter is decreased.

Interestingly, at DCM:DMF ratio of 90:10 v/v, electrospinning of the solution resulted in fibers with a broad bimodal diameter caused by multiple splaying of the jet in highly viscoelastic solutions [23]. This event was observed during the electrospinning of this solution due to its high viscosity as shown in Fig. 3. DCM:DMF ratio of 80:20 v/v dramatically reduced diameter and produced uniform nanofibers. Also, no beads were formed in the electrospun nanofibers. By increasing DMF content to 30% v/v, a number of beaded nanofibers were observed because the solution viscosity dramatically decreased (Fig. 3). Also, the rate of conductivity increase slows down when the DMF content increases from 20% to 30% v/v. Thus, the decrease in fibers diameter was not as noticeable as that induced by altering the DMF content from 10% to 20% v/v. According to statistical analysis (LSD results), the difference between average diameters at 20% and 30% v/v DMF content was not significant. The average fibers diameter of electrospun nanofibers obtained from solutions containing 10%, 20% and 30% v/v DMF content was 999, 363 and 360 nm, respectively. Therefore, the

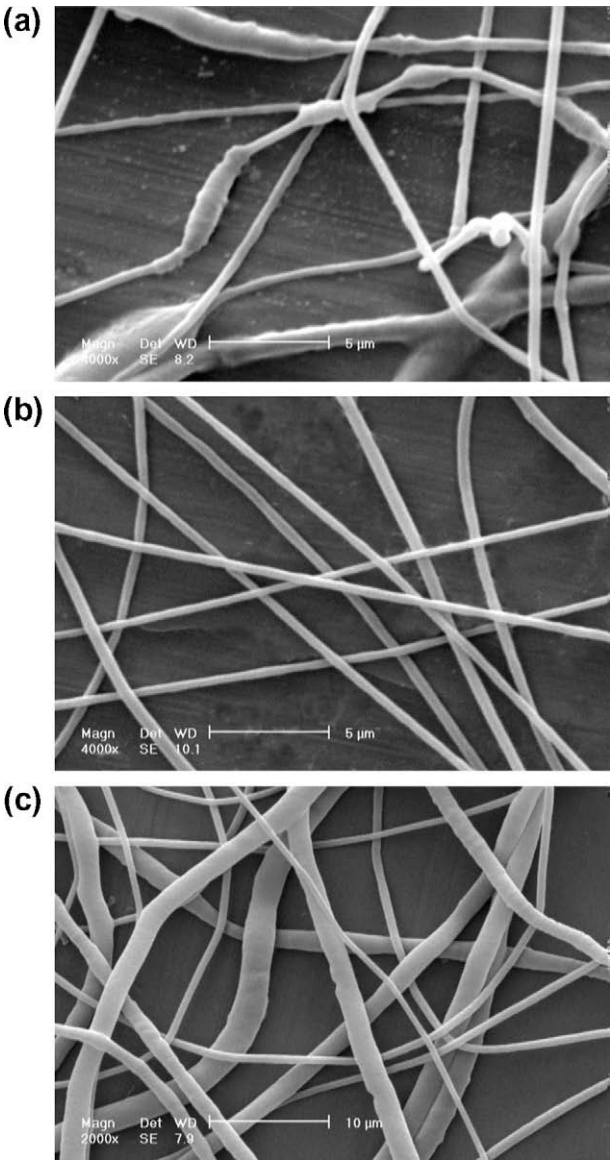

Fig. 2. SEM photographs of electrospun PCL nanofibers containing 5% w/w MET as a function of DCM:DMF ratio (v/v): (a) 70:30, (b) 80:20, and (c) 90:10.

DCM:DMF ratio of 80:20 v/v was selected as optimum ratio for further experiments.

Moreover, electrospinning of PCL solutions containing various amounts of MET were carried out from DCM:DMF ratio of 80:20 v/v. The results of measuring the electric conductivity and viscosity of solutions are shown in Fig. 4. By increasing the amount of MET, the viscosity of PCL solution decreased while its conductivity increased. Although MET molecules do not ionize in the solution, addition of MET significantly increased the conductivity similar to DMF effect due to the enhanced polarity of the solution. The possible reason for the reduction in the viscosity caused by addition of MET is that the presence of MET molecules could act as a plasticizer for PCL chains and consequently lowers the viscosity.

Both the increase in conductivity and the decrease in viscosity of the solution caused by addition of MET resulted in a decrease in nanofibers diameter as shown in Fig. 5. By increasing the drug percentage from 0% to 15% w/w, average diameter of medicated electrospun nanofibers decreased from 399 to 313 nm. The SEM photographs demonstrated that no beaded fibers were obtained

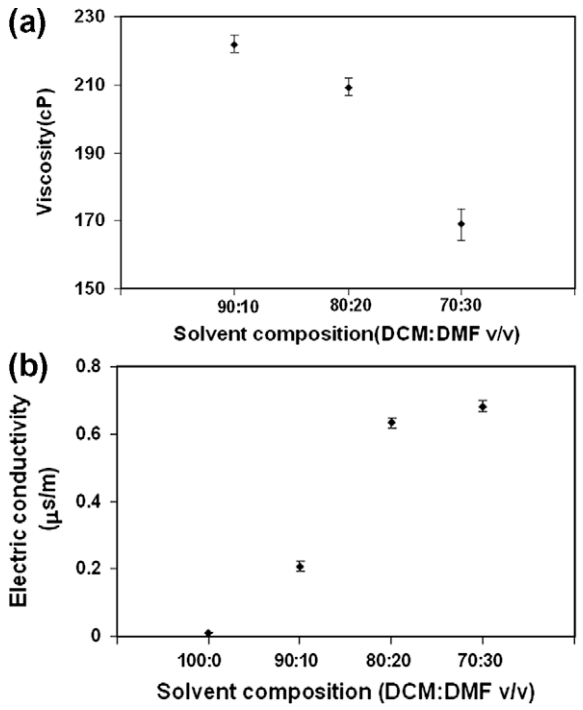

Fig. 3. Effects of DCM:DMF ratio on the solution properties: (a) solution viscosity and (b) solution conductivity.

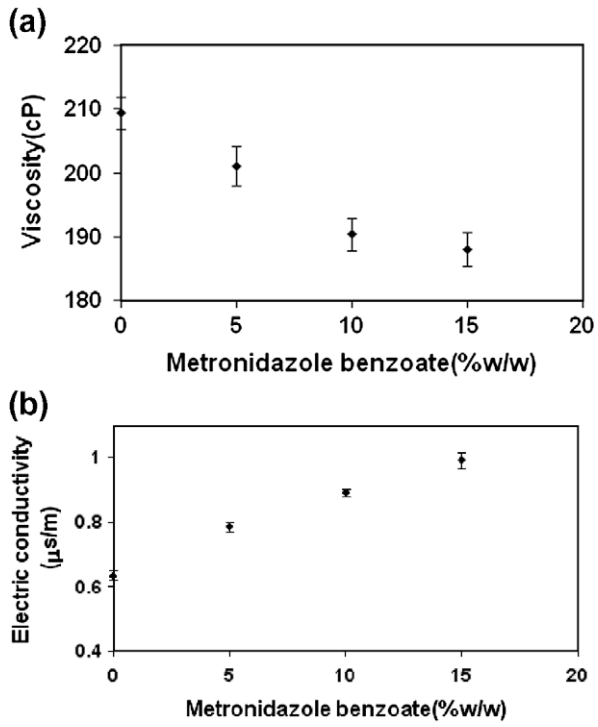

Fig. 4. Effects of MET concentration on the solution properties: (a) solution viscosity and (b) solution conductivity.

by electrospinning of these solutions containing various amounts of MET.

All electrospun nanofiber mats produced in this study were desirably smooth and flexible and yet retained the general mechanical characteristics of PCL such as elasticity as was studied physically by touch. This flexibility, in addition to hydrophobicity, provides easy handling ability during implantation and a comfortable texture for use.

3.2. Differential scanning calorimetric study

DSC studies were performed to understand the physical state of the drug in the electrospun nanofibers. The thermogram of crystalline MET showed an endothermic sharp peak at 103 °C and an enthalpy (ΔH) of 790 J/g due to melting temperature of MET (Fig. 6a). Also, an endothermic melting peak at 57 °C was observed for pure PCL nanofibers (ΔH = 1979.2 J/g), (Fig. 6b). The melting endotherm peak of MET was detected neither in the 5% w/w MET-loaded (Fig. 6c) nor in 15% w/w MET-loaded (Fig. 6d) nanofibers. The absence of detectable crystalline domain, even at high concentration of MET, indicates that drug was molecularly dispersed in amorphous state in polymeric matrix. On the other hand, by adding MET to the solutions, the melting enthalpy of PCL nanofibers became lower and appeared at almost the same temperature of 57.1 °C. By increasing the MET content of nanofibers, the melting enthalpy of PCL nanofibers decreased from ΔH of 1979.2 J/g to ΔH of 1222 J/g and ΔH of 888.3 J/g for 5% and 15% w/w MET content, respectively. These results show that increasing the amount of MET in the semi-crystalline PCL nanofibers caused a reduction in the crystallinity.

3.3. In vitro drug release

Calculated values of entrapment efficiency of different formulations after rinsing are presented in Table 1.

The results display a systematic dependence of the entrapment efficiency to the amount of drug incorporated, while the solvents composition did not have a significant effect on it. It is believed that reduced entrapment efficiency observed by increasing the amount of drug was mainly due to further exposure of the drug to the water. It means that at higher concentration of MET, a more portion of MET was located on the web surface. Therefore, it can dissolve in the rinsing water and is washed out easily.

Figs. 7 and 8 show the release profiles of MET from various electrospun PCL nanofibers. As illustrated in these figures, drug release continued for a period of at least 19 days. Indeed, the drug release from PCL nanofibers is ideally prolonged in comparison with various controlled drug release systems that have previously been studied for periodontal diseases [1,2,4,9,24–27]. Furthermore, none of the electrospun mats showed high burst release implying the perfect inclusion of the drug inside the fibers.

Since MET is highly soluble in DCM which is the main portion of solvents mixture, the solution remains homogenous during the electrospinning process without separation of drug crystals. On the other hand, both MET and PCL have hydrophobic properties. Thus, the affinity and compatibility between the drug and polymer is vital for perfect encapsulation of the drug inside the electrospun nanofibers during the rapid stretching and quick solvent evaporation of electrospinning process [11]. In this research, successful encapsulation of drug inside the nanofibers was the main cause of ideally prolonged drug release as well as low burst release. Short period of drug release and initial burst caused by incompatibility of drug–polymer–solvent system were reported in some investigations [10–13,15,16].

Fig. 7 shows the effect of solvents ratio on the release profiles of MET from electrospun PCL nanofibers. It can be seen that drug release profiles were not significantly affected by decreasing DMF content from 30% to 20% v/v. It means that morphological changes of nanofibers caused by altering DCM:DMF ratio from 70:30 to 80:20 v/v did not have a significant effect on release profiles and these formulations released MET at approximately the same rates. However, for 10% v/v DMF content, nanofibers showed a different

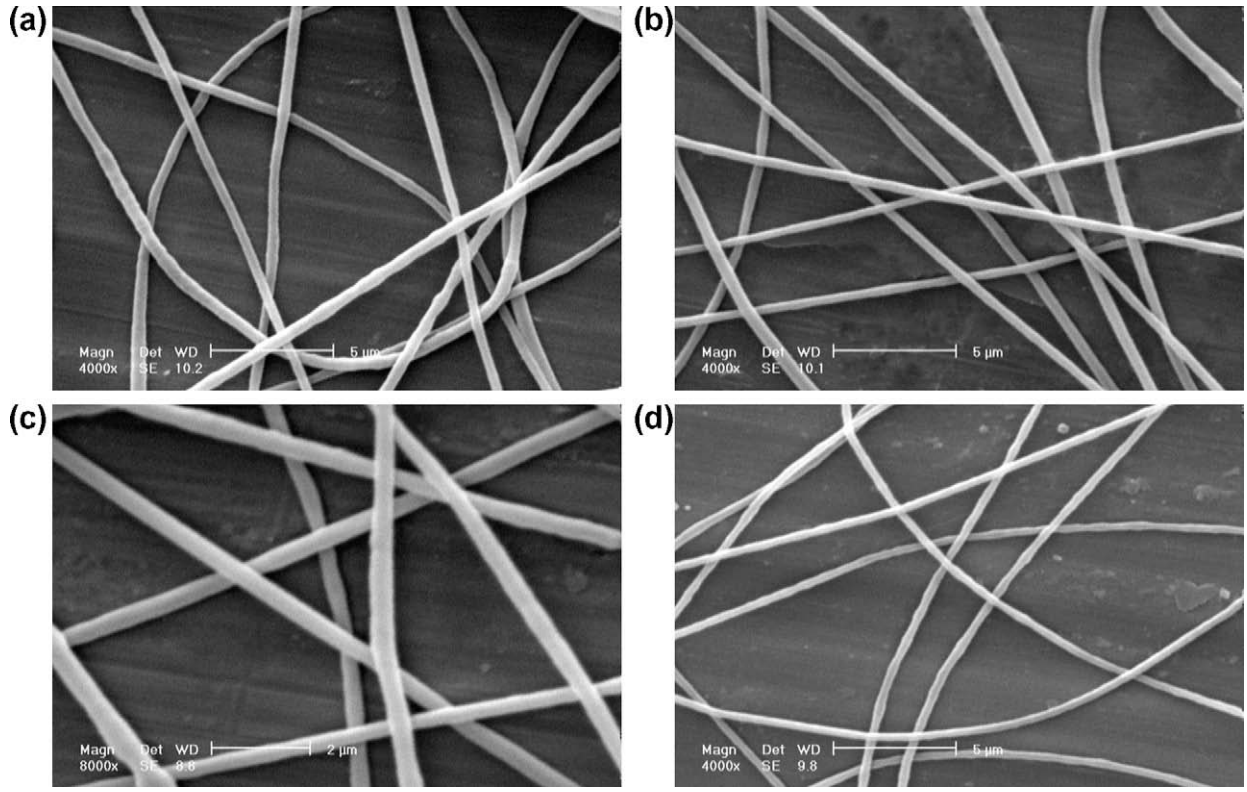

Fig. 5. SEM photographs of electrospun PCL nanofibers (DCM:DMF 80:20 v/v) as a function of drug concentration (% w/w): (a) 0%, (b) 5%, (c) 10%, and (d) 15%.

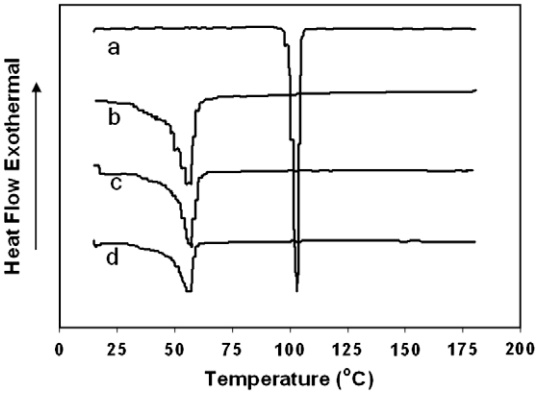

Fig. 6. DSC thermograms of (a) pure metronidazole benzoate, (b) pure PCL nanofibers, (c) 5% w/w drug-loaded nanofibers, and (d) 15% w/w drug-loaded nanofibers.

Table 1
Entrapment efficiency of different formulations after rinsing ($n = 3$).

DCM:DMF (v/v)	Theoretical drug loading (% w/w)	Entrapment efficiency (%)
70:30	5	76.4 ± 2.5
90:10	5	74.3 ± 3.2
80:20	5	78.2 ± 2.5
80:20	10	67.8 ± 2.4
80:20	15	62.4 ± 1.8

behavior. In fact, after a period of 13 days, in the case of 30% and 20% v/v DMF contents, the drug release rates slowed down until accumulated drug release became almost steady. But the electrospun nanofibers at solvents ratio of 90:10 v/v released the drug much more rapidly than the other samples after this period of time.

Fig. 8 gives the release profiles of nanofibers containing various amounts of MET which were electrospun from DCM:DMF ratio of 80:20 v/v. It shows that increasing the amount of MET from 5% to 10% w/w significantly affected the drug release rate. The greater the drug content, the faster the drug release rate. But the drug release rate did not significantly increase by addition of more MET up to 15% w/w. Furthermore, within the first 4 h, about 11.5 ± 0.65%, 13.7 ± 1.9% and 15.4 ± 1.5% of MET was released from 5%, 10% and 15% w/w samples, respectively.

It is believed that at higher concentrations of MET, the solved drug in the polymer solution had more tendency to migrate to the surface or near the surface of nanofibers during the electrospinning process. Therefore, the exposure and diffusion of MET to the buffer solution became higher leading to a faster drug release rate. The other reason may be related to physical and structural properties of PCL nanofibers. As mentioned in DSC studies (Fig. 6), increasing amount of MET in PCL nanofibers caused a reduction in the crystallinity of the polymer carrier. Drugs may be incorporated into the amorphous regions of semi-crystalline polymers such as PCL, and therefore, drug release occurs firstly from these regions [8]. Thus, the lower crystallinity of PCL nanofibers containing a higher amount of MET supports the faster release rates from these formulations. These results are in agreement with lower entrapment efficiency at higher drug content (Table 1).

Peppas equation (Eq. (2)) was used to investigate the mechanism of drug release from various formulations. The calculated kinetic release parameters are summarized in Table 2. As indicated by values of n, which varied from 0.31 to 0.44, MET release from all formulations followed Fickian diffusion mechanism. It means that MET release was attributed mainly to the diffusion or permeation of the drug through PCL matrix. The periods of our release experiments were too short to expect significant release of MET

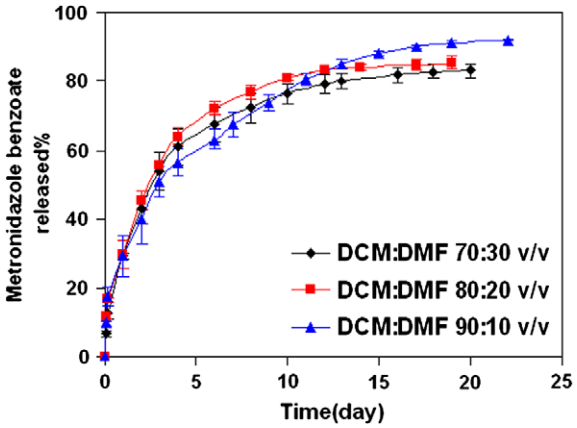

Fig. 7. Effect of solvents ratio on the drug release profiles from medicated electrospun nanofibers containing 5% w/w drug vs. time ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

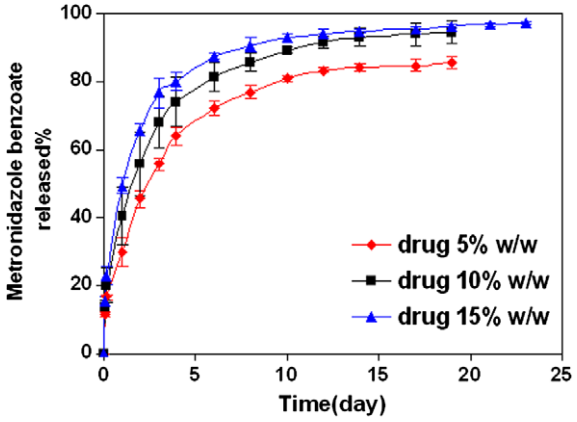

Fig. 8. Effect of drug concentrations on the drug release profiles from medicated electrospun nanofibers at DCM:DMF ratio of 80:20 (v/v) vs. time ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Kinetic parameters of MET release from different electrospun nanofibers using Peppas equation.

Formulations		Peppas parameters		Mechanism of release
DCM:DMF (v/v)	MET (% w/w)	R^2	n	
70:30	5	0.9604	0.44	Fickian diffusion
90:10	5	0.9823	0.39	Fickian diffusion
80:20	5	0.9748	0.38	Fickian diffusion
80:20	10	0.9609	0.36	Fickian diffusion
80:20	15	0.989	0.31	Fickian diffusion

by PCL degradation, as degradation of PCL is slow in aqueous medium due to its semi-crystalline and hydrophobic nature. Thus, drug release from PCL electrospun nanofibers over these periods of time was controlled by diffusion mechanism.

4. Conclusion

MET-loaded nanofibers were successfully fabricated by electrospinning. Results showed that decreasing DMF content in the solvents mixture led to a decrease in the solution conductivity and an increase in the solution viscosity as well as in the nanofibers diameter. Also increasing MET concentration in the electrospinning solution caused reverse effects on the viscosity, conductivity and consequently on the diameter of nanofibers. It was demonstrated that the drug release rate was affected by both the solvents ratio and the drug concentration and a sustained release of MET was achieved from the nanofibers for at least 19 days with low burst release. This could be an ideal treatment period for periodontal diseases. All electrospun nanofibers remained smooth and quite flexible, without shrinkage during the period of our treatments, which may offer a desirable texture to be used comfortably. Moreover, the drug release obeyed the Fickian diffusion mechanism. Conclusively, such PCL electrospun nanofibers can be used as a locally controlled delivery system for MET in periodontal diseases.

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