Encapsulation of metronidazole benzoate in poly ε−caprolactone nanofibers

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Introduction

Periodontal diseases are localized infections and inflammatory conditions that are associated with anaerobic Gram-negative bacteria and affect teeth-supporting structures. The aim of current periodontal therapy is to remove the bacterial deposits from the tooth surface and to shift the pathogenic micro-organisms to one compatible with periodontal health (Kamel 2007). Local controlled delivery of antimicrobial agents is used for periodontal therapy. These systems are designed to release drug slowly for more prolonged drug availability and sustained action. The degradable controlled release devices have the advantage of requiring the patient to pay only a single visit to the therapist for the insertion of the device (Divya 2006). Metronidazole is a front-line chemotherapeutic agent for treating infections by anaerobic bacteria such as *Porphyromonas gingivalis* because of the low minimum inhibitory concentration (MIC) required (Kamel 2007).

Recently, the electro-spinning method has attracted a great deal of attention to produce polymeric fibers with diameters in the range of nano-to a few microns using electrically driven jet of polymer solution or melt (Kenawy 2007). Medicated ultra fine fibers can be fabricated by electro-spinning from a mixture of solutions of a drug and a polymer to encapsulate the drug and production of a controlled drug delivery system (Xu 2005). The aim of this study was production of nanofibers of poly ε-caprolactone which is a biodegradable polymer, by electro-spinning method to encapsulate metronidazole benzoate for controlled delivery of this drug in periodontal diseases. Furthermore, the release properties of the drug from the non-woven web were assessed and compared with the nanofibers.

Materials and Methods

Matherials: poly ε-caprolactone (PCL) (Aldrich, Germany), dichloromethane (DCM) and N,N-dimethylformamide (DMF) (Merck Chemical Company, Germany) and metronidazole benzoate was supplied from Amin Pharmaceutical Company, Iran.

Methods: nanofibers were electro-spun from solutions of 10.5% w/v PCL and 5-15% w/w metronidazole benzoate in a mixture of DCM and DMF with the ratios of 90:10, 80:20 and 70:30. For electro-spinning, the solution was transferred to a 10 ml syringe attached to a flat-end metal needle. A steady flow of the solution from 1.82 to 2.14 ml/hr was carried out using a syringe pump. A high voltage power supply was used to create an electric field between the needle and a metal collector plate. Drug release studies were carried out in phosphate buffer medium (pH 7.4). The amount of drug dissolved in the medium was determined spectrophotometrically. Scanning electron microscopy (SEM) was used to investigate the effect of solvents ratios and drug concentration on the morphology and average diameter of the electro-spun nanofibers. DSC analyses were also applied for assessing the physical structure and probable chemical interaction of the polymer and drug.

Results and discussion

Morphology of electro-spun nanofibers

To determine the morphology of the electro-spun nanofibers, an analysis of SEM images was performed. Fig. 1 shows the effect of solvents ratios on morphology of the electro-spun nanofibers. As illustrated in this figure, electro-spinning of the solution with DCM:DMF ratio of 70:30, produced beaded nanofibers. The fiber morphology was dramatically enhanced by increasing the DCM:DMF ratio to 80:20. But at DCM:DMF ratio of 90:10, bimodal diameter distribution was observed. Average diameter of the resultant nanofibers by electro-spinning from solutions with DCM:DMF ratios of 70:30, 80:20 and 90:10 were 360, 363 and 999 nm, respectively. Measurements showed that increasing DCM:DMF ratio led to a decrease in the solution conductivity and an increase in the solution viscosity. Zhang et al. (2007) also showed that there are many parameters such as viscosity and conductivity that will influence the morphology of the electro-spun nanofibers.

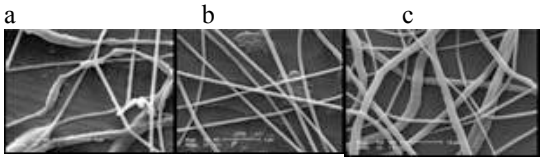

Figure 1 : SEM photographs of electro-spun PCL nanofibers containing 5% metronidazole benzoate with different DCM:DMF ratio: a) 70:30 b) 80:20 c) 90:10

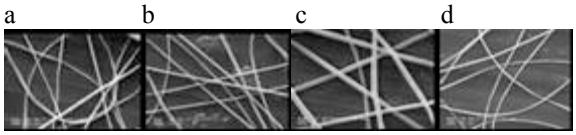

Figure 2: SEM photographs of electro-spun PCL nanofibers with DCM:DMF 80:20 and different drug concentrations: a) 0% b) 5% c) 10% d) 15%

The formation of beaded fibers at solvent ratio of 70:30 was due to low viscosity of this solution. Also, it has been suggested that bimodal distributions are developed by repeated splaying of the fiber jets in solutions with high viscosity (Hsu et al., 2004), as the solution with solvent ratio of 90:10. Resistance of the solution to be stretched by the charges on the electro-spinning jet is increased when the solution viscosity is increased (Zhang et al., 2007). Also, by reducing the solution conductivity, the decreased charges carried by the solution will decrease the stretching of the electro-spinning jet, hence, the fiber diameter increases (Zhang et al., 2007).

Fig. 2 shows the effect of drug concentration on the electro-spun nanofibers. As illustrated in this figure, the surface of formed fibers at 0%, 5%, 10% and 15% w/w drug concentration was smooth without any defect. Also, by increasing drug concentration from 0% to 5%, 10% and 15%, the average fiber diameters decreased from 399 to 363, 331 and 313 nm, respectively. Measurements showed that increasing the metronidazole benzoate concentration led to an enhancement of the solution conductivity and a reduction in the solution viscosity. As mentioned above, these changes in viscosity and conductivity decreased the fiber diameter. Furthermore, the SEM images of all electro-spun nanofibers showed no drug crystals on the fiber surface and this observation demonstrated the solubility and compatibility of the drug in the polymer-drug-solvent system and distribution of drug in polymeric matrix.

Drug release study

The release profiles of metronidazole benzoate from various electro-spun samples are shown in figures 3 and 4. None of the electro-spun samples showed a quick burst of drug release and the drug release was prolonged to 19-23 days for different formulations.

Fig. 3 shows the effect of solvents ratios on release profiles of metronidazole benzoate. Results show that morphological changes caused by altering DCM:DMF ratio from 70:30 to 80:20 did not have a significant effect on release profiles. But in comparison to two other samples, although the

electro-spun sample at solvent ratio of 90:10 released fewer drug at initial times, but more amounts of drug were released at longer times.

Fig. 4 shows the effect of drug concentration on release profiles of metronidazole benzoate. Results show that increasing drug concentration led to a higher release rate. When the drug concentration is increased, the drug molecules may aggregate more near the fiber surface, which would lead to an even larger initial burst of drug and higher release rate (Kim et al., 2004) as seen in the PCL nanofibers with 10% and 15% drug.

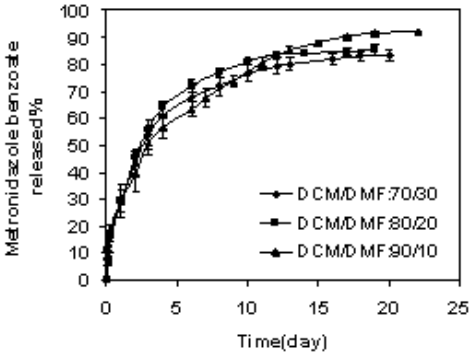

Figure 3: Effect of solvents ratios on release profiles from electro-spun nanofibers containing 5% metronidazole benzoate

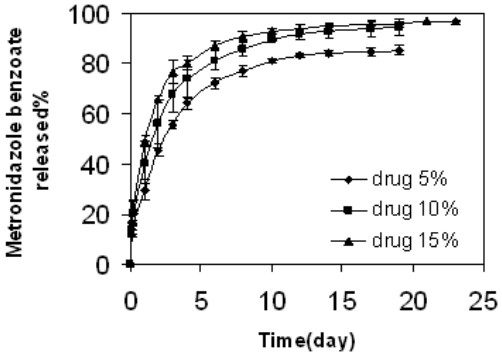

Figure 4: Effect of metronidazole benzoate concentration on release profiles from electro-spun nanofibers with DCM:DMF 80:20

Fig. 5 shows the considerable effect of web thickness on release profiles. For the web with thickness of 0.14 mm, 82% of drug-loaded has been released in 4 hr and drug release continued for 4 days. This can be compared to the web with 0.3 mm thickness, which has released only 11% of drug-loaded in 4 hr and drug release has been prolonged to 19 days.

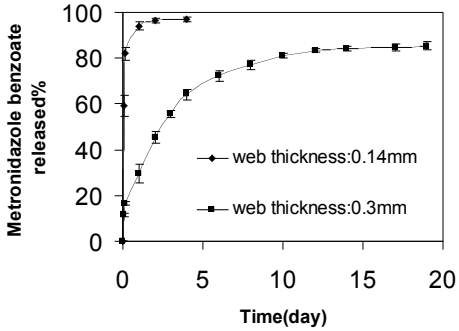

Figure 5: Effect of web thickness on the metronidazole benzoate profiles from electro-spun nanofibers containing 5% drug

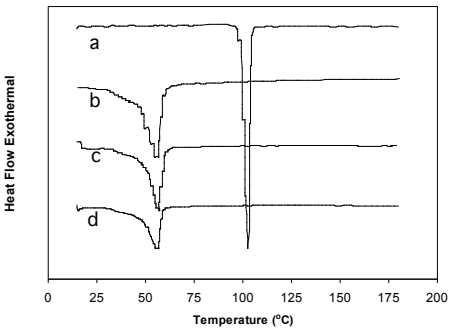

Figure 6: DSC curves of a) pure metronidazole benzoate b) pure PCL c) 5% drug-loaded nanofibers d) 15% drug-loaded nanofibers

DSC analysis

DSC analysis (Fig. 6) was applied for assessing the physical structure and probable chemical interaction of the polymer and drug. As this figure indicates the pure metronidazole benzoate and PCL show melting points at 103ºC and 57ºC, respectively. But the melting endotherm of metronidazole benzoate was not detected in drug-loaded nanofibers. Based upon this data, it is believed that the drug in the incorporated fiber was in the amorphous state. Also the melting endotherm of PCL in drug-loaded fiber was unchanged, indicating no chemical interaction between polymer and drug has occurred (Mundargi et al., 2007).

Conclusions

Biodegradable PCL nanofibers were fabricated by electro-spinning method for controlled release of the encapsulated metronidazole benzoate. Results showed that increasing DCM:DMF ratio will lead to a decrease in the solution conductivity and an increase in the solution viscosity as well as the nanofiber diameter. Also increasing the metronidazole benzoate concentration will lead to an increase in the solution conductivity and a decrease in the solution viscosity as well as the nanofiber diameter. The results showed that the drug release rate was affected by the solvents ratio as well as the drug concentrations. In webs with appropriate thickness, the burst release was low and drug release was prolonged to 19-23 days. Metronidazole benzoate was released by a Fickian diffusion mechanism. Conclusively, poly ε-caprolactone electro-spun nanofiber webs can release encapsulated metronidazole benzoate in a controlled manner and can be used as a locally controlled delivery system for in periodontal diseases.

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