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RESEARCH ARTICLE

Antibacterial Properties of Allopathic Drug Loaded Polycaprolactone Nanomembrane

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Abstract

Biodegradable polymer nanomembrane have high porosity with interconnected pores and high surface area-to-volume ratio that makes them ideal for wound dressing and drug delivery applications. In this study, biodegradable Polycaprolactone (PCL) nanomembrane and PCL Nanomembrane containing allopathic drug (Tetracycline hydrochloride) were successfully prepared by the electrospinning technique and nanomembranes were characterized using Scanning Electron Microscope (SEM), Capillary flow porometer and Fourier Transform Infrared Spectroscopy (FTIR). The antibacterial properties of nanomembrane were evaluated by AATCC 100-2004. The incorporation of allopathic drug in PCL nanomembrane did not influenced the morphology of the resulting fibers, as both the drug-free and the drug loaded nanomembrane remained unaltered, microscopically. Drug loaded PCL nanomembrane was able to inhibit the growth of the bacteria which indicate that it could act not only as a drug delivery system but also in the treatment of wound healing or dermal bacterial infections thereby proving a potential application for use as a wound dressing.

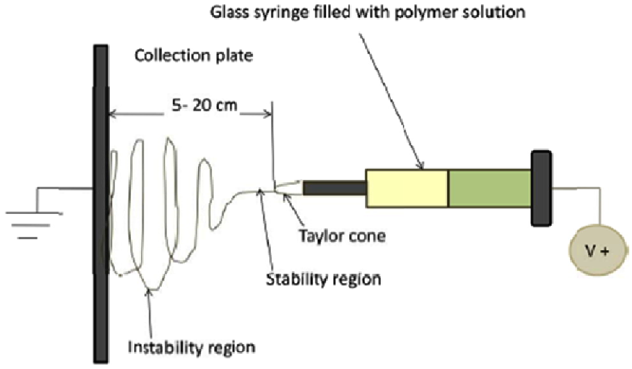
Keywords: Nanomembrane, polycaprolactone, electrospinning technique, antibacterial properties.

Introduction

An ideal wound dressing material should have abilities for removal of exudate and protection of the wound apart from having high porosity for gas permeation and being a good barrier for protection of wound from infection and dehydration. Therefore based on these requirements, a candidate for wound dressing materials must be acting as a good barrier and should have good oxygen permeability. Electrospinning has been used since the 1930s in the textile industry for the manufacturing of non-woven fiber (Suganya *et al*., 2011). Electrospinning process has attracted a great deal of attention due to its ability to produce ultrafine fibers from polymer solutions with diameters in the range of nanometer to submicrometers that exhibit high surface area-to-volume using electrostatic forces. Electrospun fibers has been the focus of research for applications such as filtration of subatomic particles, composite reinforcement, multifunctional membranes, tissue engineering scaffolds, wound dressings, drug delivery, artificial organs and vascular grafts (Luu *et al.,* 2003; Yoshimoto *et al.,* 2003; Bolgen *et al.,* 2007).

Mechanism behind the electrospinning technique is utilization of high electrostatic potential to produce nanofibers. It consists of four parts: syringe pump to control flow rate, syringe with needle which act as one of the electrode to charge the polymer solution, power supply to generate electric field and collector which act as other grounded electrode to collect fibers. Under the influence of electric field, charge is developed in the polymer solution. At low electric field strength, a pendant drop emerged out from the tip of needle which is balanced by the surface tension of solution. As the voltage increased, charges on solution repel each other which results in elongation of drop into conical shape known as taylor cone due to electrostatic forces in opposite direction to surface tension. As the voltage reached to its critical value, all equilibrium forces on drop get distorted and the electrostatic forces overcomes the surface tension due to which, a jet is emerged out of the cone and get deposited on grounded electrode (Monika Rajput, 2012) (Fig. 1). Drug delivery systems using nanofibers are able to improve therapeutic efficacy, reduce toxicity and enhance compliance of the patients by delivering drugs at a controlled rate over a period of time to the site of action.

Fig. 1. Electrospinning setup (Monika Rajput, 2012).



Various biodegradable and biocompatible polymeric materials have been electrospun into nanoscale fibers and have been demonstrated for their potential as effective carriers for drug delivery. Controlled drug delivery of tetracycline hydrochloride based on the fibrous delivery matrices of poly ethylene-co-vinyl acetate, polylactic acid and their blend have been developed (Kenawy, 2002; Kanani and Bahrami, 2010). Against these backdrops, biodegradable PCL nanomembrane and PCL nanomembrane containing allopathic drug (Tetracycline hydrochloride) were successfully prepared by the electrospinning technique and nanomembranes were characterized using SEM, Capillary flow porometer and FTIR.

Materials and methods

Materials: Poly (ε-caprolactone) was purchased from Sigma-Aldrich with a molecular weight of Mn=80,000 in pellet form. Allopathic drug Tetracycline hydrochloride antibiotic was used for this study.

Preparation of nanomembrane

PCL solution preparation: Chloroform: Methanol solution in the ratio of 3: 1 is prepared and in this solution PCL pellet is added to get a 10% PCL concentration solution (To get clear solution, continuous stirring for 1 h is essential).

Allopathic drug loaded solution preparation: Allopathic drug viz. Tetracycline hydrochloride was dissolved in methanol. With this, chloroform was added to get a Tetracycline hydrochloride drug solution. With the Tetracycline hydrochloride drug solution, PCL was added and the solution was stirred continuously for 1 h. Using Tetracycline hydrochloride drug incorporated PCL solution, nanomembranes were prepared at 4 different combinations and they are:

1. 0.5%* Tetracycline hydrochloride/ 10% PCL
2. 1 % Tetracycline hydrochloride/10% PCL
3. 1.5% Tetracycline hydrochloride/10% PCL and
4. 2% Tetracycline hydrochloride/10% PCL

*The concentration of the tetracycline hydrochloride in the PCL polymeric system was 0.5% on the weight of the PCL polymer.

Process optimization for electrospinning: In order to control the bead formation, changed either the applied voltage (10-15 Kv), the capillary tip-collector distance (8-15 cm) and Flow rate of solution (0.3-1.0 mL/h, the optimum process parameters to be maintained during electrospinning for allopathic and herbal drug were finalized and they are given in Table 1. (i) The drug-free and (ii) drug-loaded (allopathic drug and herbal drug) PCL solutions were placed into a 5 mL syringe with an internal diameter of 0.5 mm and mounted on a pump. The tip of the needle was connected to a high voltage source. Electrospinning was carried out under a electric field with tip collector distance of 10 cm.

Table 1. Optimum process variables in electrospinning to spin drug free and allopathic drug incorporated PCL nanomembrane.

Drug concentration	Applied voltage (kV)	Flow rate (mL/h)	Tip to collector distance (cm)
PCL 10%	13	0.3	10
0.5% TH/10% PCL	13	0.3	10
1% TH/10% PCL	13	0.3	10
1.5 % TH/10% PCL	13	0.3	10
2 % TH/10% PCL	15	0.3	10

The solution feeding rate was kept at 0.3 mL/h. The experiments were carried out at room temperature. The fibers were collected on an aluminium plate in the form of non-woven matrices.

Scanning electron microscopy (SEM): The morphology of the nanofiber mats was observed using scanning electron microscopy. The electrospun fibers were sputtered with thin layer of gold prior to SEM observation. In the basis of SEM images, the average diameter of the electrospun fibers could be measured.

Capillary flow porometer studies: Electrospinning produces membranes with randomly laid fibers with varying pore size and distribution. The electrospun membranes were subjected to capillary porometer.

Fourier transform infrared spectroscopy (FTIR): Fourier transform infrared spectroscopy is an analytical tool to identify the nature of chemicals that are coated on the fabric specimen. It also helps to know to what extent the molecules of the finishing chemicals are attached with fiber molecules of the specimen. FTIR uses infrared radiation to determine the chemical functionalities present in a sample. When an infrared beam hits a sample, chemical bonds stretch, contract and bend, causing it to absorb IR radiation in a defined wave number. The FTIR spectra were recorded over the 4000-400 cm^{-1} range to confirm the incorporation of allopathic drugs in the polymeric nanofibers.

Antibacterial property of test specimen (AATCC test method 100-2004): Swatches of test and control specimens are inoculated with *Staphylococcus aureus* and *Klebsiella* pneumoniae (1.5 x 10^8 Cfu/mL). After inoculation, the specimens are incubated for 18 h and after incubation; the bacteria are eluted from the specimen swatches by shaking in known amounts of neutralizing solution. The number of bacteria present in this liquid is determined and the percentage reduction by the specimens is calculated by the below formula:

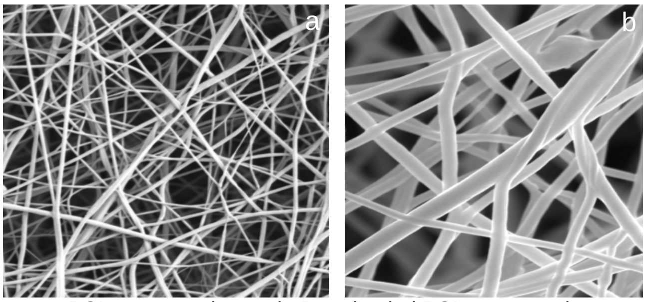
$$R = (100\,(C - A))/C$$

Where, A is the number of bacteria recovered from the inoculated test specimen swatches incubated over the desired contact period and C is the number of bacteria recovered from the inoculated control specimen swatches immediately after inoculation.

Results and discussion

Morphology of drug-free and drug-loaded PCL nanomembrane: SEM morphologies of electrospun fibrous mats are presented in Fig. 2a and b. The fibers possess the common features of being round-shaped with smooth surface and the drug-free and the drug-loaded PCL nanofiber appeared smooth and no drug crystals were detected on the polymer surface. This suggested that drug was dispersed homogeneously in the electrospun fibers. Furthermore, it was noticed that incorporation of the drug in the PCL solutions did not affect the morphology of the resulting fibers. The dimensions of the fiber were in the range of 80-100 nm for drug free fibers and the diameters of the fiber shifted to the higher side (100-150 nm) on incorporation of drugs.

Fig. 2. SEM morphologies of electrospun fibrous mats.



a. PCL nanomembrane, b. Drug loaded PCL nanomembrane.

Fig. 3a. Pore size distribution of drug free PCL nanomembrane.

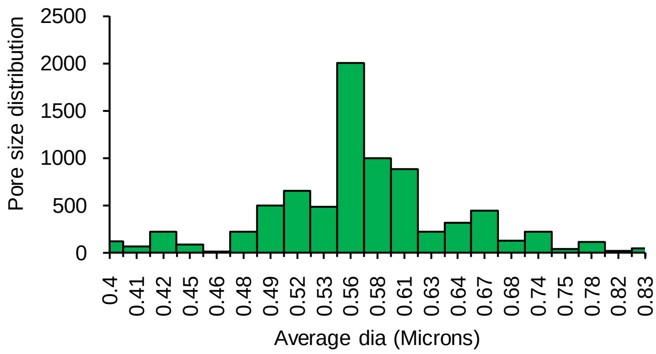


Fig. 3b. Pore size distribution of drug loaded PCL nanomembrane.

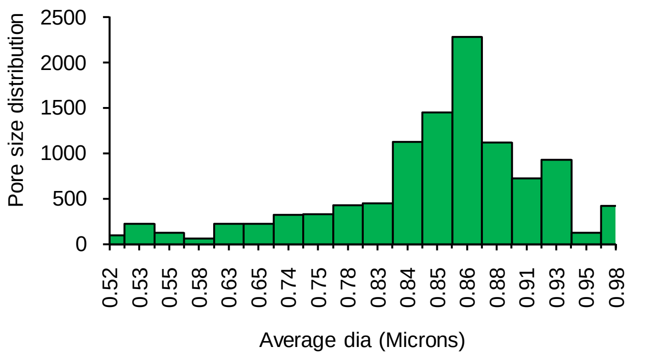
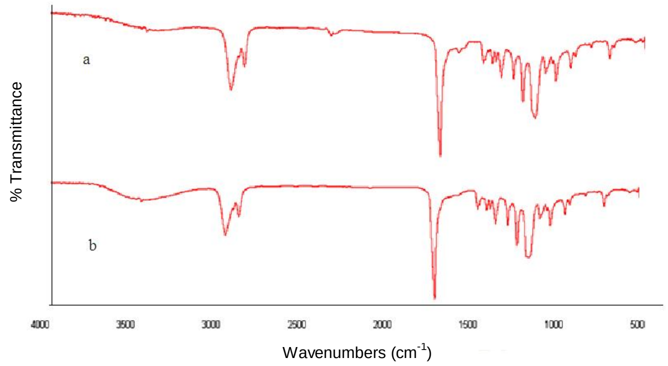


Fig. 4. FTIR spectra of (a) PCL, (b) Allopathic drug loaded PCL.



Capillary flow porometer: Electrospun membranes were subjected to capillary porometer studies and the mean flow pore diameter (the mean flow pore diameter is such that 50% of flow is through pores larger than the mean flow pore diameter) and the pore size distribution was measured. The mean flow pore diameter increased from 0.56 microns to 0.86 microns with addition of the Tetracycline hydrochloride drug and the pore size distribution shifted to the higher side (Fig. 3a and b). This may due to the coarseness of the fibers making the membrane.

FTIR evaluation: FTIR spectra for PCL and drug loaded PCL were shown in Fig. 4. FTIR for PCL is shown in spectrum a. The peak at 2865 and 2943 cm^{-1} corresponds to asymmetric and symmetric vibrations of CH_2 group and the C=O vibration of ester occurs at 1721 cm^{-1}. The CH_2 band vibrations of the polymer are present at 1365, 1418 and 1470 cm^{-1} and the ester COO vibrations occur at 1170 and 1240 cm^{-1}. The peak at 1107, 1045 and 960 cm^{-1} are due to O-C vibrations with the CH_2 rocking vibration occurring at 731 cm^{-1} (Suganya *et al.*, 2011). The FTIR spectra for Tetracycline hydrochloride is shown in Fig. 4b. The most important vibrations modes are (Ogunniran *et al.*, 2008): C-N at 2944 cm^{-1}, C=O and C=C of aromatic ring between 1722.00 to 1470 cm^{-1} at 1418-1365 cm^{-1} related to OH, C-C and C-C and finally the bands at 1293-1239 cm^{-1} corresponding to N-H and C-N. These spectra confirm the Tetracycline hydrochloride presence on the developed substrate.

Antibacterial activity: The percentage reduction in the number of bacteria present in the SITRA's developed wound dressings against gram positive and gram negative organisms are given in Table 2. The allopathic drug loaded PCL nanomembrane to be an efficient wound dressing should be able to inhibit the growth of the bacteria responsible for severe wound infection thereby aiding wound healing. Drug free nanofiber did not show any bacteria reduction whereas drug loaded specimen gave a 100% (2% TH/10% PCL) bacterial reduction.

Table 2. Antimicrobial activity of allopathic drug incorporated PCL nanomembrane.

Drug concentration	Bacterial reduction (%)	
	Staphylococcus aureus	*Klebsiella pneumoniae*
1% TH/10% PCL	74	78
1.5 % TH/10% PCL	93	91
2 % TH/10% PCL	100	100

Findings indicate that allopathic drug loaded PCL nanomemrane possess efficient antibacterial property and can be used in the treatment of wound healing or dermal bacterial infections thereby proving a potential application for use as a drug delivery and as a wound dressing agent.

Conclusion

SITRA has developed allopathic drug incorporated PCL nanomembrane for wound care applications. SITRA's nanomembranes have strong antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The produced electrospun allopathic drug incorporated PCL nanomembrane has great potential in biomedical applications. The drug loaded PCL nanomembrane retained its biological functionality even after it had been subjected to a high electrical voltage, indicating that the developed electrospun mat have the great potential in drug delivery, wound healing as well as promising materials for treating surfaces that contain pathogenic microorganisms especially in hospital environment.

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