Journal of Academia and Industrial Research (JAIR)
Volume 2, Issue 11 April 2014

ISSN: 2278-5213

RESEARCH ARTICLE

Wound Healing Ability of Herbal Drug Incorporated PCL (Poly(ε-caprolactone)) Wound Dressing

K.P. Chellamani*, R.S. Vignesh Balaji, D. Veerasubramanian and J. Sudharsan
The South Indian Textile Research Association (SITRA), Coimbatore-641014, India
sitraindia@dataone.in, kpc@sitra.org.in*; 91422-4215347

Abstract

PCL nanomembrane containing herbal drugs *Tecomella undulata* (TU), *Glycyrrhiza glabra* (GG), *Asparagus recemosus* (AR) and *Linum usitatissimum* (LU) were prepared and evaluated for their antibacterial properties, moisture vapour transport rate, *in vitro* drug release and wound healing ability. The incorporation of herbal drugs in the PCL polymer media did not appear to influence the morphology of the resulting fibers, as both the drug-free and the drug incorporated nanomembrane remained unaltered microscopically. Antibacterial properties of nanomembrane were evaluated and herbal drug incorporated PCL nanomembrane were able to inhibit the growth of the bacteria which indicate that it could not only act 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. The extent of wound healing provided by the SITRA developed herbal drug incorporated wound dressings (TU, GG, AR and LU wound dressing) is 50% faster as compared to that of commercial wound dressing.

Keywords: PCL nanomembrane, antibacterial properties, wound healing, herbal drug, wound dressing.

Introduction

In recent years, the electrospinning process has attracted a great deal of attention due to its ability to produce ultrafine fibres with diameters in the range of nanometers to sub-micrometres and high surface area to volume or mass ratios. The principle of electrospinning process is to use electrostatic force as the main driving force for fibre formation. The morphology of the as-spun fibres depends on a number of parameters such as solution concentration, solution conductivity, applied electrostatic field strength, collection distance and collection time. Among others, some potential uses of electrospun fibres in medicine are immobilization of enzyme, tissue engineering scaffolds, DNA and drug delivery systems. One of the obvious advantages of the electrospinning process over the conventional film-casting technique is the highly porous structure of electrospun fibre mats which exhibit much greater surface area that assumingly could allow drug molecules to diffuse out from the matrix much more conveniently (Taepaiboon *et al.,* 2007). The efficiency of drug delivery to various parts of the body is directly affected by particle size. Nanostructure-mediated drug delivery, a key technology for the realization of nanomedicine has the potential to enhance drug bioavailability, improve the timed release of drug molecules and enable precision drug targeting. Nanoscale drug delivery systems can be implemented within pulmonary therapies, as gene delivery vectors and in stabilization of drug molecules that would otherwise degrade too rapidly.

Additional benefits of using targeted nanoscale drug carriers are reduced drug toxicity and more efficient drug distribution (Hughes, 2005). The effects of plant extracts on bacteria have been studied by researchers in different parts of the world. Plants those have antibacterial efficacy against the test organism may have some antimicrobial phytochemicals or alkaloids. The presence of flavonoids in *Fagonia cretica*, Tannins in *Aerva persica* and Tecomin in *Tecomella undulata* has been reported. Recent studies have focused on incorporating natural extracts with polymer-based electrospun nanofibers for various biomedical applications (Suganya *et al*., 2011). In the present study, PCL nanomemrane containing herbal extracts from *Tecomella undulata* (TU), *Glycyrrhiza glabra* (GG), *Asparagus recemosus* (AR) and *Linum usitatissimum* (LU) were fabricated and bactericidal property were evaluated against common pathogenic bacteria namely *Staphylococcus aureus* and *Klebsiella pneumoniae*. The herbal drug encapsulated nanofibers with potential antibacterial properties can be used as an effective drug delivery system and in developing wound dressings for infants, elderly and infirm people to protect them against common infections.

Materials and methods

Materials: Poly (ε-caprolactone) was purchased from Sigma-Aldrich with a molecular weight Mn=80,000 in pellet form. Herbal drugs like *Tecomella undulata, Glycyrrhiza glabra, Asparagus recemosus* and *Linum usitatissimum* were used for this study.

Preparation of nanomembrane

PCL solution preparation: A chloroform: methanol solution in the ratio of 3:1 is prepared and in this solution, PCL pellet is added to get a 15% PCL concentration solution (To get clear solution, continuous stirring for 1 h is essential).

Herbal drug incorporated solution: Herbal drugs like *Tecomella undulata, Glycyrrhiza glabra, Asparagus recemosus* and *Linum usitatissimum* are dissolved in chloroform and methanol and kept overnight in stirrer. Then, the solution was filtered with Whatmann paper. Fine filtrate is used for electrospinning. Drug-loaded PCL solutions are obtained by dissolving PCL to herbal extract. Prior to electrospinning, the solutions are stirred for 2 h. Herbal drug incorporated nanomembranes were prepared at 3 different combinations (Table 1).

Table 1. Combination of herbal drug with PCL.

Name of Herbal drug	Combination of herbal drug with PCL
Tecomella undulata (TU)	2%* TU/15% PCL, 2.5% TU/15% PCL and 3% TU/15% PCL
Glycyrrhiza glabra (GG)	2% GG/15% PCL, 2.5 GG/15% PCL and 3% GG/15% PCL
Asparagus recemosus (AR)	2% AR/15% PCL, 3% AR/15% PCL and 5% AR/15% PCL
Linum usitatissimum (LU)	2% LU/15% PCL, 2.5 LU/15% PCL and 3% LU/15% PCL

*The concentration of the herbal drug in the PCL polymeric system was 2% on the weight of the PCL polymer.

Process optimization for electrospinning: In order to control the bead formation, one can change either the applied voltage (10-22 Kv) or the capillary tip-collector distance (8-15 cm) or flow rate of solution (1.0-5 mL/h). The optimum process parameters to be maintained during electrospinning for drug free and herbal drug loaded PCL nanomembrane were finalized and they are as follows:

1. Applied voltage: 20 Kv
2. Capillary tip to collector distance: 10 cm
3. Flow rate: 3 mL/h

Scanning electron microscope analysis: The morphology of the nanofiber mats was observed using scanning electron microscope (SEM). 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.

Determination of antibacterial property of test specimen (AATCC test method 100-2004): Swatches of test and control specimens are inoculated with *Staphylococcus aureus* (1.5×10^8 Cfu/mL) and *Klebsiella pneumoniae* (1.5×10^8 Cfu/mL). After inoculation, the specimens are incubated for 18 h.

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.

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

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.

Moisture vapour transmission rate (MVTR): The MVTR is an important criterion for a wound dressing material. The liquid formed inside the wound layer first changes to vapour state and then transported to atmosphere. This moisture vapour transmission helps to heal the wound. MVTR was determined according to BS EN 13726-2:2002. A test sample of 40 mm diameter is taken and fixed over a container of 35.7 mm inner diameter, containing 20 mL of distilled water. The test sample container is weighed (W_1) before the start of the test. Then the container is kept inside in an incubator for 24 h (Temperature 37±1ºC and RH 20%). After 24 h, the container is taken out and again weighed (W_2). MVTR is calculated based on the formula:

$$X = (W_1 – W_2) \times 1000 \times 24/T$$

Where, X is MVTR (g/m^2/24 h), W_1 is the mass of the container, sample and liquid in grams, W_2 is the mass of the container, sample and liquid in grams after the test duration and T is the test period in hours.

In vitro drug release study: A piece of drug-containing fiber mat (0.1 g) was first placed in a vial filled with 10 mL of release medium acetate buffer. Drug release studies were carried out at 37°C and 100 rotation/min (rpm) in a thermostatical shaking incubator. The releasing medium acetate buffer with pH 5.5 was prepared by dissolving 1.5 g of sodium acetate in 1.5 mL of glacial acetic acid and then the final solution was made up to 100 mL by adding distilled water. In this case, 1.5 mL of sample was taken from the medium after appropriate intervals for about 24 h and then the same volume of fresh release medium was added as replacement. A calibration curve was obtained for the herbal drug concentration at a peak absorption wavelength and a linear equation was derived by a curve-fitting method. In the assessment of drug release behavior, a cumulated amount of the released drug was calculated. The percentages of drug released from the nanofibers were plotted against time.

Skin irritation: The herbal drug incorporated PCL nanomembrane specimens were evaluated for potential skin irritation when they are used for covering the wound. The evaluation was as per ASTM F 719-81 standard.

Table 2. Scoring criteria for test reactions.

Reaction	Description	Score
Erythema (ER)	Erythema and Eschar	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well-defined erythema (pale red in colour)	2
	Moderate to severe erythema (red and area well defined)	3
	Severe erythema (beet redness to slight eschar formation)	4
Edema (ED)	Edema formation	
	No edema	0
	Very slight (barely perceptible)	1
	Slight edema (edges of area well defined by definite raising)	2
	Moderate edema (edges raised approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Exposure of skin to the test material is accomplished by means of a patch test technique employing two intact sites on the back of each of six albino rabbits. Ethical committee approval was obtained for the testing material (KMCRET/MOTRS/01/2013-2014). The skin is clipped free of hair one day prior to testing. The test substance is applied using 0.5 mL for liquids, 0.5 g for solids or semisolids and a 2.5 by 2.5 cm square patch for films. After application, each test site is covered with 2.5 x 2.5 cm gauze flat and the entire trunk is occluded with a polyethylene sleeve. After 24 h, the sleeve, flat and test material are removed and test sites are evaluated for erythema and edema.

Scoring method: Using the criteria given in Table 2, the test sites are scored for Erythema (ER) and Edema (ED) Test sites can also be scored for erythema and edema at 48 h as well as 72 h after removal (as per the usage requirement) using the criteria given in Table 2.

Wound healing rate: The extent of wound healing provided by a given wound dressing was evaluated using the method proposed by Morton and Malone (1972). As per this method, thirty healthy rats were employed for the experimentation and they were separated into 5 groups each with 6 rats. Ethical committee approval was obtained for this study (KMCRET/MOTRS/01/2013-2014). Excision of wounds was made on the rate as per the method suggested by Morton and Malone (1972). The rats were anaesthetized with anaesthetic ether and placed in operation table in their natural position. A square wound of about 1.5 cm (width) x 0.2 cm (depth) was made on depilated ethanol-sterilized dorsal thoracic region of rats. Infection was made on wounds by *Staphylococcus aureus*.

1. Group I rats were treated with commercial wound dressing (CWD).
2. Group II rats were treated with *Tecomella undulata* herbal drug incorporated PCL nanomembranes (TUPN).
3. Group III rats were treated with *Glycyrrhiza glabra* herbal drug incorporated PCL nanomembranes (GGPN).
4. Group IV rats were treated with *Asparagus recemosus* herbal drug incorporated PCL nanomembranes (ARPN).
5. Group V rats were treated with *Linum usitatissimum* drug incorporated PCL nano membranes (LUPN).

Table 3. Average fibre diameter of drug free and herbal drug incorporated PCL nanomembranes.

Type of drug	Drug concentration	Fibre dia (nm)
PCL (drug free)	15% PCL	220
Herbal drug (TU)	2% TU/15% PCL	256
	2.5% TU/15% PCL	274
	3% TU/15% PCL	287
Herbal drug (GG)	2% GG/15% PCL	252
	2.5% GG/15% PCL	278
	3% GG/15% PCL	291
Herbal drug (AR)	2% AR/15% PCL	260
	3% AR/15% PCL	290
	5% AR/15% PCL	315
Herbal drug (LU)	2% LU/15% PCL	259
	2.5%LU/15% PCL	278
	3% LU/15% PCL	292

The dressings were applied on the wounds of the rats every day till the epithelialization was complete. The extent of wound contraction was studied by tracing the raw wound area in a tracing paper on 6^{th} d, 12^{th} d, 18^{th} d and 24^{th} d.

Results and discussion

Morphology of drug-free and drug-loaded PCL nanomembranes: Average fibre diameter of drug free and herbal drug incorporated PCL nanomembranes are presented in Table 3. The fibers possess the common features of being round-shaped with smooth surface. The drug-free and the drug-loaded PCL nanofibres appeared smooth. No drug crystals were detected on the polymer surface of the drug loaded nanomembranes. 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 fibers were in the range of 200-220 nm for drug free fibers and the diameters of the fiber shifted to the higher side (250-315 nm) on incorporation of the drugs.

Antibacterial activity: The percentage reduction in the number of bacteria present in the SITRA developed wound dressings against gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumaoniae*) organisms are given in Table 4.

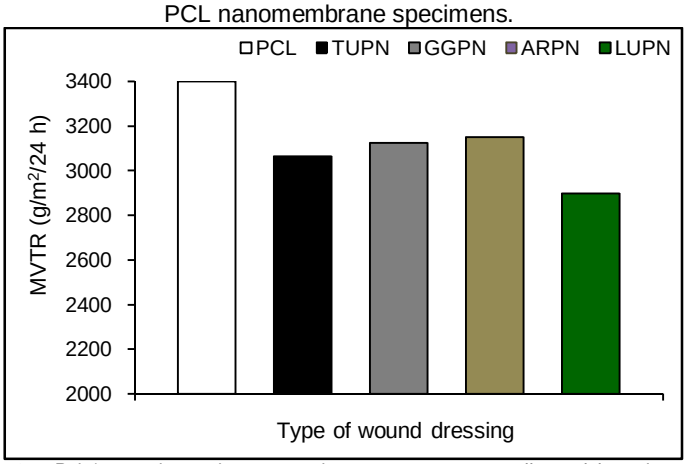
Table 4. Antimicrobial activity of Allopathic and herbal drug incorporated PCL nanomembrane.

Drug concentration	Bacterial reduction (%)	
	Staphylococcus aureus	*Klebsiella pneumoniae*
1% TH/15% PCL	74	78
1.5% TH/15% PCL	93	91
2% TH/15% PCL	100	100
2% TU/15% PCL	55	0
2.5% TU/15% PCL	100	80
3% TU/15% PCL	100	100
2% GG/15% PCL	53	57
2.5% GG/15% PCL	78	85
3% GG/15% PCL	100	100
2% AR/15% PCL	0	55
3% AR/15% PCL	88	100
5% AR/15% PCL	100	100
2% LU/15% PCL	0	60.2
2.5% LU/15% PCL	76	96
3% LU/15% PCL	100	100

The findings indicate that herbal drug loaded PCL nanomemrane possess efficient antibacterial property and can be used in the treatment of wound healing or dermal bacterial infections.

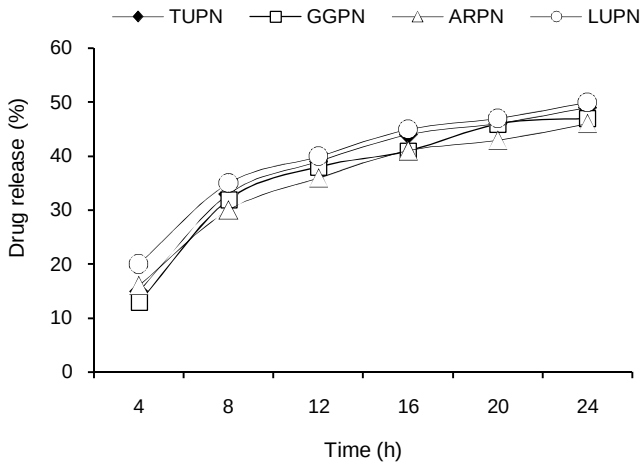
Moisture vapour transmission rate (MVTR): Herbal drug loaded PCL nanomembrane specimen exhibit good MVTR properties in the range of 2900-3100 g/m^2 per day (Fig. 1). For an infected skin, MVTR value of 2000-3000 is good. Hence, all the nanomembrane substrates made in this study can be considered to be suitable for infected skins. The rate of water vapour transmission for normal skin is 700-1200 g/m^2 per day, while for the injured skin it can range from 800-1300 g/m^2 per day and for a third-degree burn, it can go up to 10000 g/m^2 per day. An ideal dressing is expected to control the evaporative water loss from a wound at an optimal rate. The water vapour permeability of a wound dressing should prevent both excessive dehydration and build-up of exudates (Lou *et al.,* 2008).

Fig. 1. MVTR properties of allopathic and herbal drug loaded PCL nanomembrane specimens.



PCL: Poly(ε-caprolactone) nanomembrane, TUPN: *Tecomella undulata* drug loaded PCL nanomembrane, GGPN: *Glycyrrhiza glabra* drug loaded PCL nanomembrane, ARPN: *Asparagus recemosus* drug loaded PCL nanomembrane, LUPN: *Linum usitatissimum* drug loaded PCL nanomembrane.

Fig. 2. *In vitro* drug release study of drug loaded (allopathic and herbal) PCL nanomembrane specimens.

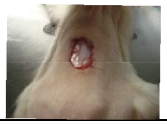
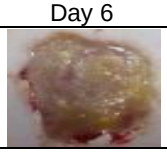
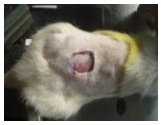
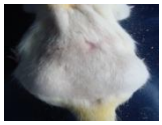
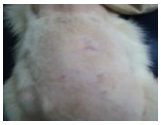
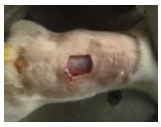
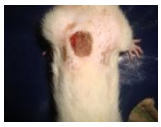
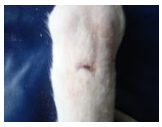
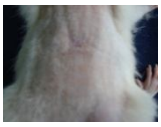
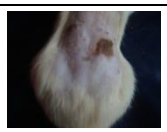
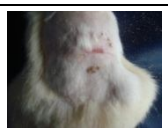
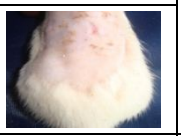
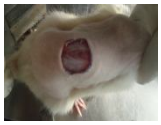
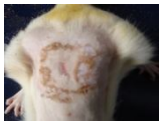
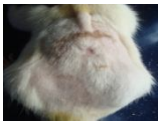
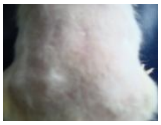


In vitro drug release study: A drug released from the herbal drug-incorporated nanofibers were examined *in vitro* for a period of 24 h and a relationship between the cumulative percentage and releasing time was plotted in Fig. 2. Drug release from the nanofibers showed a low initial rapid release followed by a sustained and slow release over a prolonged period of time. Initial rapid release is as the drug came out only when the polymer started to degrade or after water penetrated sufficiently into the nanofibers. The release profile from drug loaded nanofiber exhibited a drug release of about 12-20% in the first 4 h and around 45-50 of the total drug in the later 24 h. Hence, allopathic and herbal drug loaded are suitable for a release model to kill bacteria in a short period of time that requires a large amount of drug.

Skin irritation: Twenty four healthy rabbits were selected for the study and they were separated into four groups. *Tecomella undulata* herbal drug incorporated PCL nanomembranes (TUPN) was used with group I rabbits, *Glycyrrhiza glabra* herbal drug incorporated PCL nanomembranes (GGPN) was used with group II rabbits, *Asparagus recemosus* herbal drug incorporated PCL nanomembranes (ARPN) was used with group III rabbits and *Linum usitatissimum* drug incorporated PCL nanomembranes (LUPN) was used with group IV rabbits. The study has shown that both allopathic as well as herbal drug incorporated PCL nanomembranes do not cause any skin irritation even after 72 h of contact with the wound.

Wound healing rate: The weight of the traced portions of the wounded area of rats subjected to different treatments (CWD, TUPN,GGPN, ARPN and LUPN treated wound) were measured using electronic balance. Based on the difference in weight, the superiority or otherwise of a particular wound dressing is determined. Figure 3 shows CWD, TUPN, GGPN, ARPN and LUPN treated wound.

Fig. 3. Healing in CWD, TUPN, GGPN, ARPN and LUPN treated wound.

Dressing type	Extent of wound healing						
	Day 0	Day 6	Day 12	Day 18	Day 24	Day 31	Day 35
CWD treated wound							
TUPN treated wound					— Wound healing is 50% faster in the case of ADPN treated, TUPN treated, GGPN treated and ARPN treated wounds than the CWD treated wounds. — Wound healing is 30% faster in the case of LUPN treated wounds than the CWD treated wounds.		
GGPN treated wound							
ARPN treated wound							
LUPN treated wound							

It is clear from Fig. 3, that there is a decrease in wound area with or without the application of the dressings. The reduction in the wound area is faster in the case of wounds dressed using SITRA developed wound dressings. Hence, the extent of wound healing provided by the SITRA developed herbal drug incorporated PCL nanomembranes (TUPN, GGPN, ARPN and LUPN) is better.

Conclusion

SITRA has developed herbal drug incorporated PCL nanomembrane (HDPN) wound dressings and the following are the conclusive points derived from the present study.

- HDPN specimens were developed using 4 types of herbal drugs namely *Tecomella undulata, Glycyrrhiza glabra, Asparagus recemosus* and *Linum usitatissimum.*
- SITRA developed nanomembranes showing strong antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The drug loaded PCL nanomembrane specimens retained their biological functionality even after they were subjected to a high electrical voltage.
- TUPN, GGPN, ARPN and LUPN exhibited good MVTR properties in the range of 2900-3100 g/m^2 per day.
- Wound healing is much faster while using SITRA developed wound dressings (ADPN, TUPN, GGPN, ARPN and LUPN) as compared to commercially available wound dressings, the reduction in time required for wound healing while using SITRA developed wound dressings is as high as 50%.

Acknowledgements

Authors are thankful to Ministry of Textile, Government of India for sponsoring the projects. The authors express their sincere thanks to Dr. Prakash Vasudevan, Director, SITRA for his keen interest in this project.

References

1. BS EN 13726-2. 2002. Test methods for primary wound dressings Moisture vapour transmission rate of permeable film dressings.
2. Hughes, G.A. 2005. Nanostructure-mediated drug delivery. *Nanomed. Nanotechnol. Biol. Med.* 1: 22-30.
3. Lou, C.W., Lin, C.W., Chen, Y.S., Yao, C.H., Lin, Z.S., Chao, C.Y. and Lin, J.H. 2008. Dressing properties evaluation of Tencel/Cotton non-woven fabric coated with chitosan for wound. *Text. Res. J.* 78(3): 248-253.
4. Morton, J.J.P. and Malone, M.H. 1972. Evaluation of vulnerary activity by an open wound procedure in rats. *Arch. Int. Pharm*. 196: 117-126.
5. Standard Test Method ASTM F 719-81. 2012. Standard practices for testing biomaterials in rabbits for primary skin irritation.
6. Suganya, S., Senthil Ram, T., Lakshmi, B.S. and Giridev, V.R. 2011. Herbal drug incorporated antibacterial nanofibrous mat fabricated by electrospinning: An excellent matrix for wound dressings. *J. Appl. Polym. Sci.* 121: 2893-2899.
7. Taepaiboon, P., Rungsardthong, U. and Supaphol, P. 2006. Drug-loaded electrospun mats of poly(vinyl alcohol) fibres and their release characteristics of four model drugs. *Nanotechnol.* 17: 2317-2329.