Herbal Drug Incorporated Antibacterial Nanofibrous Mat Fabricated by Electrospinning: An Excellent Matrix For Wound Dressings

S. Suganya,[1,2] T. Senthil Ram,[1] B. S. Lakshmi,[2] V. R. Giridev[1]

[1]*Department of Textile Technology, A.C.Tech, Anna University Chennai, Chennai 25*
[2]*Centre for Biotechnology, A.C.Tech, Anna University Chennai, Chennai 25*

Received 11 August 2010; accepted 2 December 2010
DOI 10.1002/app.33915
Published online 29 March 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Nano-components and nano-systems for health care and medical applications are the focus of many research projects worldwide. Nanofibrous membranes are highly soft materials with high surface-to-volume ratios, and therefore can serve as excellent carriers for therapeutic agents that are antibacterial or accelerate wound healing. PCL/PVP Nanofiber mat containing chloroform: methanol (4:1) crude bark extract of *Tecomella undulata*, a medicinal plant widely known for its traditional medical applications including its wound healing ability, were prepared and evaluated for their antibacterial properties. With good drug stability and high drug-loading efficacy, the incorporation of herbal extract in the polymer media did not appear to influence the morphology of the resulting fibers, as both the drug-free and the drug-loaded nanofibers remained unaltered, microscopically. Activity was tested against standard strains of Pseudomonas aeruginosa MTCC 2297, *Staphylococcus aureus* ATCC 933, *Escherichia coli* (IP-406006). Extract loaded PCL/PVP nanofiber mat were able to inhibit the growth of the bacterial strains 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. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 2893–2899, 2011

Key words: electrospinning; nanofiber; PCL; PVP; herbal drug; antibacterial activity; wound dressing

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 the wound from infection and dehydration. Therefore, based on these requirements, a candidate for wound dressing materials must be acting as good barrier and should have good oxygen permeability.[1]

Electrospinning has been used since the 1930s in the textile industry for the manufacturing of nonwoven fiber.[2] 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.[3] 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.[4] 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.[5]

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.[6] 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.[7] In another work bioabsorbable nanofiber membranes of poylactic acid targeted for the prevention of surgery-induced adhesions, were also used for loading an antibiotic drug Mefoxin.[8] Currently, many studies have been focused on incorporating drugs with blended polymer-based electrospun nanofibers. By adjusting the blending ratio of polymers, the property of the fibrous material and drug release behavior could be monitored.[9]

The production and designing of effective antimicrobial materials has become a highly desired objective in maintaining primary health care. Medicinal plants represent a rich source of antimicrobial

Correspondence to: S. Suganya (suganyashamage@gmail.com).

Contract grant sponsor: DST-PURSE, Department of Science and Technology, India; contract grant number: 110001/PD2/2008.

agents. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Plants those were found to 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.[10]

Recently studies have been focused on incorporating natural extracts with polymer-based electrospun nanofibers for various biomedical applications. For example the potential of electrospun gelatin fiber mats as carriers for topical delivery of a methanolic crude extract of *Centella asiatica* a medicinal plant that heals ulcerous abnormalities of the skin have been investigated.[11] Similarly in another work the feasibility and potential of PCL nanofibres as a delivery vehicle loaded with curcumin for skin related disease was investigated.[12]

In the current study *Tecomella undulate (T.undulate*) was chosen, which is locally known as Rohida, found in Thar Desert regions of northwest and western India and it possess excellent antibacterial properties. The bark obtained from the stem contains tecomin which is used as a remedy for wound healing. It is also used in curing urinary disorders, enlargement of syphilis, gonorrhoea, leucoderma, and liver diseases. Seeds are used against abscess.[13]

Polycaprolactone (PCL) and polyvinyl pyrrolidone (PVP) are the most popular polymers and are known for their biodegradable nature, hydrophilicity, chemical, and thermal stability. Both PCL and PVP have been widely used as a controlled drug releasing carrier, because of its good tissue compatibility, solute permeability and excellent electrospinnability[14,15]. PCL offers strength to the matrices and PVP works as an excellent drug delivery medium. In the present study PCL/PVP nanofiber mats containing herbal extracts from *T. undulata,* were fabricated and bactericidal property were evaluated against common pathogenic bacteria like *Pseudomonas aeruginosa (P.aeruginosa*), *Staphylococcus aureus (s.aureus*), *Escherichia col (E.coli).* The herbal drug encapsulated nanofibers with potential antibacterial property can be used as effective drug delivery system and in developing wound dressings for infants, elderly and infirm people to protect them against common infections.

EXPERIMENTAL

Preparation of herbal extract

Bark pieces of *T.undulata* obtained from local traditional herbal shop was used for the present study. The bark of *T. undulata* was powdered and dried. About 10 g of bark powder was dissolved in chloroform (40 mL) and methanol (10 mL) and was kept overnight in stirrer. Then the solution was filtered with crude filter paper followed by whatmann paper. Fine filtrate was used for electrospinning. The concentration of the antimicrobial substance in the polymeric system was 7.5% on the weight of the polymer.

Nanofiber fabrication

PCL (MW-70,000-90,000) and PVP (MW- 40,000) provided by Sigma-Aldrich were used. Herbal drug-loaded PCL/PVP solutions were obtained by dissolving 0.5 g of PCL and 0.5 g of PVP to 10 mL of herbal extract. Drug-free PCL/PVP solutions were obtained by dissolving 0.5 g of PCL and 0.5 g of PVP to 10 mL of chloroform : methanol (4:1). Prior to electrospinning, the solutions were stirred for 2 h. The drug-free and drug-loaded PCL/PVP solutions were carefully placed into a 5-mL syringe with an internal diameter of 0.5 mm and mounted on pump (KD Scientific). The tip of the needle was connected to high voltage source (Gamma high voltage). Electrospinning was carried out under a constant electric field of 12 kV with tip to collector distance of 10 cm. The solution feeding rate was kept at 0.75 mL/h. Experiments were carried out at room temperature. The fibers were collected on the aluminum plate in the form of nonwoven matrices. Nanofibers were than vacuum dried to remove residual chloroform, methanol and other volatile impurities.

Scanning electron microscope analysis

The morphology of the nanofiber mats was observed using scanning electron microscope (SEM; HITACHI). 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 porometry

The pore size distribution and bubble point measurements of nanofibers used in this study were determined using a capillary flow porometer (Porous Materials Inc, USA) as per the procedure suggested in literature.[16] The relationship between the pore size and the corresponding pressure is given by the Young-Laplace equation:

$$D = \frac{4\gamma \mathrm{Cos}\theta}{\Delta P} \quad (1)$$

Where D is the diameter of the pore, ΔP is the differential gas pressure, γ is the surface tension of the wetting liquid, Galwick (γ 15.9 dynes/cm), and θ is

the wetting angle and the pore size distribution was then calculated from this data.

In vitro drug release assay

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 of 655 nm, 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.

Fourier transform infrared spectroscopy analysis

The Fourier transform infrared spectroscopy (FTIR) was carried out using Bruker optik GMBH FTIR spectrometer in the range of 500 cm^{-1} to 4000 cm^{-1} to confirm the incorporation of herbal drugs in the polymeric nanofibers. KBr pellets mixed with the sample were prepared and used for testing.

Thermo gravimetric analysis

The thermal behavior of the electrospun fiber mat samples with and without drug was examined by SDT Q 600 V8.0 Build 95 thermo gravimetric/differential thermal analyser (TG-DTA). Measurements were conducted over a temperature range of 40–800°C, at a heating rate of 20°C/min under nitrogen purge.

Antibacterial activity

The antibacterial activity of the electrospun fiber mats against three pathogenic bacteria commonly found on burn wounds: *P. aeruginosa* MTCC 2297 (Gram-negative) *S.aureus* ATCC 933 (Gram-positive) and *E.coli* (IP-406006) (Gram-negative) were investigated. The assessment was conducted based on the disc agar diffusion method. A 100 μL aliquot of bacteria reconstituted in nutrient broth and previously subcultured was spread onto an agar plate. Both the drug-free and drug-containing PCL/PVP fiber mats were cut into circular discs (15 mm in diameter) and placed on the top of the agar plate. The plates were incubated at 37°C for 24 h. If inhibitory concentrations were reached, there would be no growth of the microbes, which could be seen as a clear zone around the disc specimens. The zone was then recorded as an indication of inhibition against the microbial species.

RESULTS AND DISCUSSION

Morphology of drug-free and herbal drug-loaded PCL/PVP fiber mats

SEM morphologies of electrospun fibrous mats are presented in Figure 1. The fibers possess the common features of being round-shaped with smooth surface and the drug-free and the drug-loaded PCL/PVP fibers 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/PVP solutions did not affect the morphology of the resulting fibers. The diameter range of fibers was measured using Image J software and the dimensions of the fiber were in the range of 100 to 300 nm for drug-free fibers and the diameters of the fiber shifted to the higher side (200-450 nm) on incorporation of drugs.

Capillary flow porometer studies

Electrospinning produces membranes with randomly laid fibers with varying pore size and pore size distribution. The 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 0.35 microns to 0.65 microns with addition of the herbal drug and the pore size distribution shifted to the higher side [Fig. 2(a,b)]. This may due to the coarseness of the fibers making the membrane.

In vitro drug release study

A cumulative percentage of herbal drug released from the drug-loaded nanofibers was *in vitro* examined for a period of 24 h, and a relationship between the cumulative percentage and releasing time was plotted in Figure 3. 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 because the drug came

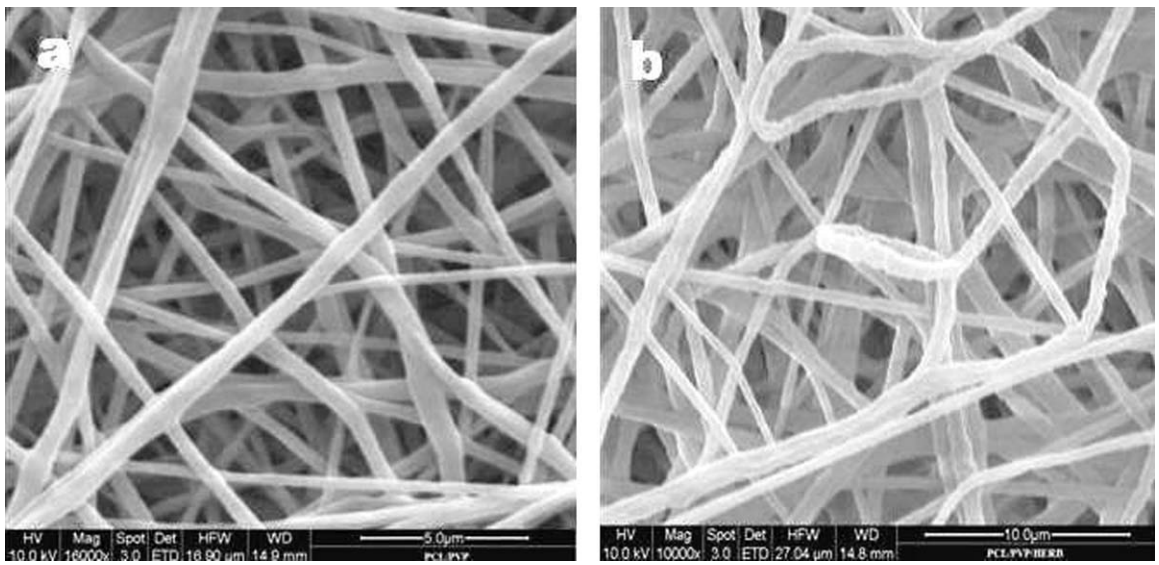

Figure 1 SEM morphologies of (a) Drug-free and (b) Drug-loaded electrospun PCL/PVP nanofiber.

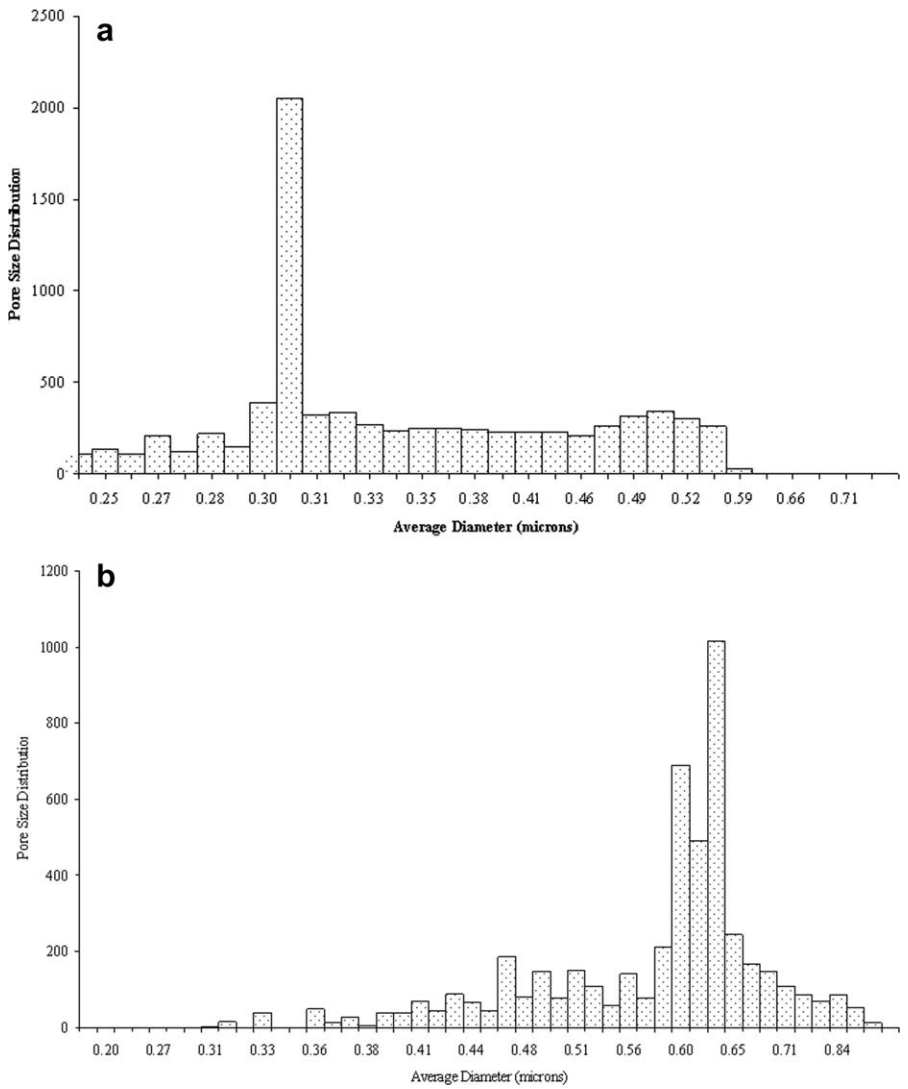

Figure 2 Pore size histogram of (a) Drug-free and (b) Drug-loaded electrospun PCL/PVP nanofiber.

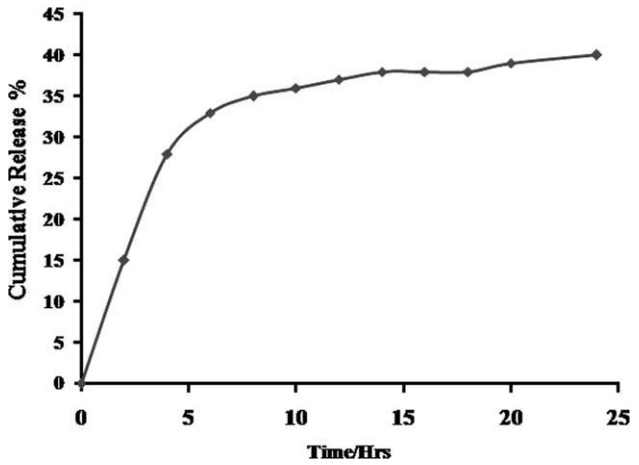

Figure 3 The release pattern of the herbal drug from nanofiber mat.

out only when the polymer started to degrade or after water penetrated sufficiently into the nanofibers. Prior to investigating the release characteristics of herbal drug from the drug-loaded nanofibers, the total concentration of herbal extract in the blend nanofiber was determined at a peak absorption wavelength of 655 nm. The concentration of herbal drug in the polymeric system was 7.5% on the weight of the polymer. The release profile from drug loaded nanofiber exhibited a drug release of about 24% in the first 4 h and around 40.9% of the total drug in the later 24 h. Correspondingly, a cumulative release of about 3.5 ± 0.4% of the drug from the blend nanofibers was obtained. Hence nanofibers from blend electrospinning are suitable for a release model to kill bacteria in a short period of time that requires a large amount of drug.

FTIR spectra

FTIR spectra for PCL, PVP, PCL/PVP blend, herbal drug, and drug loaded PCL/PVP were shown in Figure 4. FTIR for PCL is shown in spectrum (a).The peaks at 2945 and 2980 cm^{-1} corresponds to asymmetric and symmetric vibrations of CH_2 group and the C=O vibration of ester occurs at 1728 cm^{-1}. The CH_2 band vibrations of the polymer are present at 1362, 1407, and 1465 cm^{-1} and the ester COO vibrations occur at 1181 and 1238 cm^{-1}.The peak at 1099, 1047, and 961 cm^{-1} are due to O-C vibrations with the CH_2 rocking vibration occurring at 729 cm^{-1}. The FTIR of PVP is shown in spectrum (b) and the peak at 3509 cm^{-1} is due to OH stretching vibrations of water. The CH_2 vibrations occur just below 3000 cm^{-1} and the C=O stretch occur at 1262 cm^{-1}.The CH_2 bending give peak at 1431 cm^{-1}.The C—N vibration occurs at 1385 cm^{-1}.The FTIR of PCL/PVP blend is shown in spectrum (c). It carries features of spectrum (a) and (b).The peak corresponding to C=O is broadened. The FTIR spectrum of *T.undulata* is shown in spectrum (d). It carries CH_2 vibrations at 2925 and 2856 cm^{-1}.The C=O vibration occurs at 1713 cm^{-1}.There is a broad envelope between 1650 and 1000 cm^{-1} it is due to overlapping of peaks due to CH_2 bends COO and O=C vibrations. This tentatively assigned to ester groupings. The FTIR spectrum of Herbal drug loaded PCL/PVP is shown in spectrum (e). The spectrum displays all features of (a) and (b), but the resolution observed for C=O stretching vibration in spectrum (c) is nearly absent in this spectrum. It is ascribed to overlapping of C=O vibrations of polymers blend and herb. It appears to be the main evidence for the presence of herb in the polymer blend. In addition CH_2 bend modes and COO vibrations modes are well resolved in this spectrum compared with spectrum (c).This resolution is again taken as evidence for the presence of herb in the polymer blend.Herb stays in between the polymer this results in resolution of peaks due to COO and CH_2 bending modes. As the herb has CH_2 groups similar to PCL and PVP, and COO groups similar to PCL It can be said that herb is very well associated with polymers.

Thermo gravimetric analysis (TGA)

The thermal degradation of fibers obtained from PCL/PVP blend and herbal drug loaded PCL/PVP were analyzed by thermo gravimetric analyzer (TGA) and the TGA curves are presented in Figure 5. Totally, 1.5 mg of PCL/PVP blend and herbal drug loaded PCL/PVP mat was taken during heating from 40 to 800°C at a rate of 20°C per min under nitrogen purge 200 mL per min. The herbal drug loaded PCL/PVP degradation gives a first weight

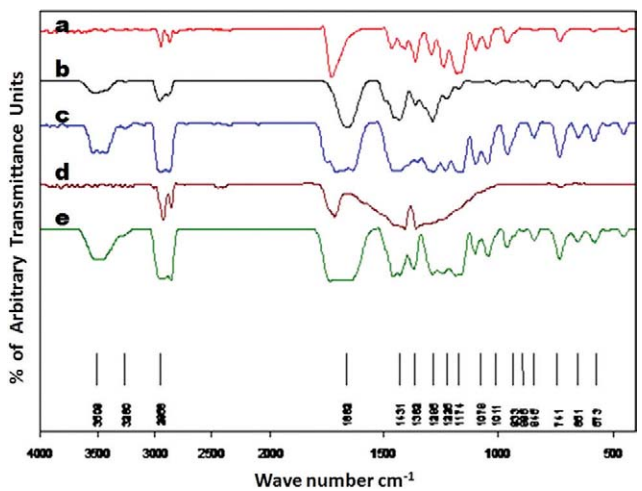

Figure 4 The FTIR spectra of (a) PCL, (b) PVP, (c) PCL/PVP blend, (d) Herbal drug, (e) Herbal drug loaded PCL/PVP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

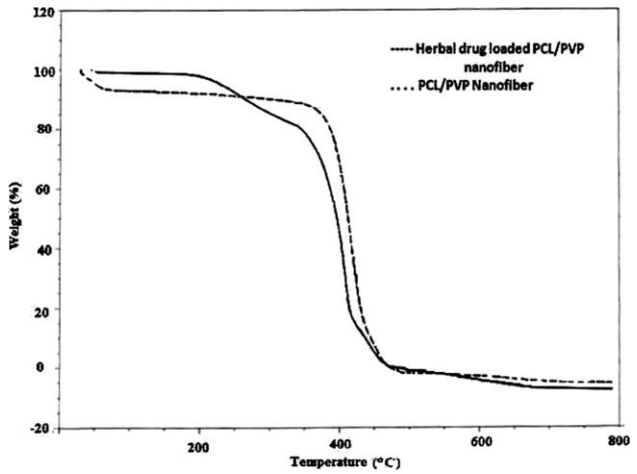

Figure 5 TGA curves of PCL/PVP blend and herbal drug loaded PCL/PVP.

loss, starting from 212 to 281°C and second weight loss from 385 to 413°C whereas the PCL/PVP blend without herbal drug gives first weight loss only from 391 to 431°C.It shows that the interaction between PCL/PVP blend was interfered by the herbal drug components which results in the change of its thermal stability. Moreover drug-loaded nanofibrous mat is stable enough to withstand the heat applied during decontamination processes that are common in medical applications.

Antibacterial activity

The herbal drug loaded PCL/PVP nanofiber mat to be an efficient wound dressings should be able to inhibit the growth of the bacterial strains responsible for severe wound infection thereby aiding wound healing. The predominant pathogenic bacteria responsible for severe burn wound infections are *P.aeruginosa*, *S. aureus*, and *E. coli*. Hence antibacterial activity of herbal drug-loaded PCL/PVP fiber mats were assessed against these typical pathogenic bacteria respectively, *P.aeruginosa* MTCC 2297, *S. aureus* ATCC 933 and *E. coli* (IP-406006). The activity of drug-free PCL/PVP fiber mats synthesized using chloroform: methanol (4:1) against these bacteria was used as control. As reported in Figure 6 after 24 h of contact intervals, drug free nanofiber did not show any zone of inhibition whereas drug loaded specimen gave a 30 mm diameter zone for *P.aeruginosa*, a 24 mm diameter zone for *S. aureus* and a 28 mm diameter zone for *E.coli* (the diameter of specimen is 15 mm), respectively. Hence the nanofibers were bactericidal to the testing microorganisms due to the strong antibacterial ability of bark extract from *T.undulata*. The results indicate that herbal drug loaded PCL/PVP nanofiber mat 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.

CONCLUSIONS

Herbal drug incorporated PCL/PVP nanofibers were prepared by electrospinning technique. The nanofibers were bactericidal to the testing microorganisms due to the strong antibacterial ability of bark extract from *T.undulata*. The produced electrospun nanofiber has great potential in biomedical applications. The loaded-herbal drug retained its biological functionality even after it had been subjected to a high electrical voltage, indicating that the medicated fibers developed by our system 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.

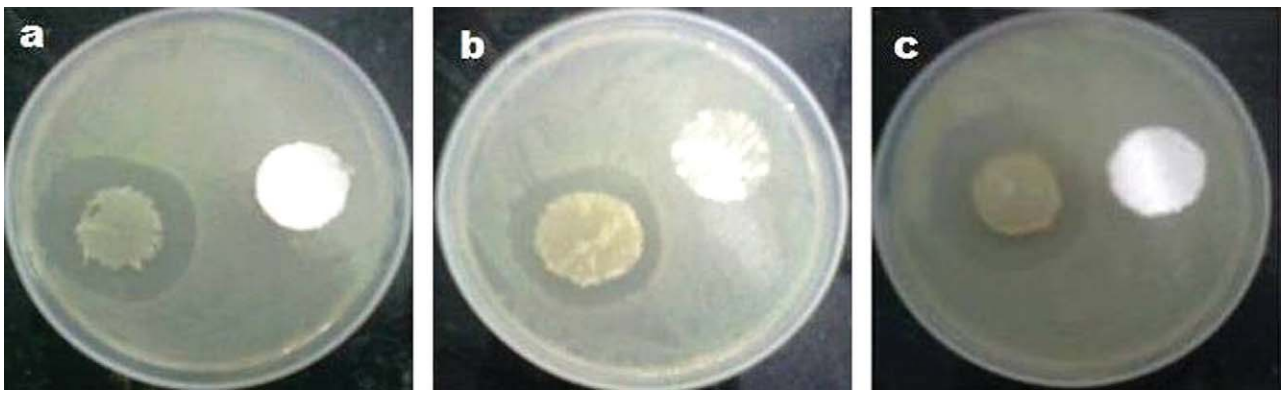

Figure 6 In vitro testing of drug loaded electro spun mats for antimicrobial activity against: (a) Pseudomonas aeruginosa, (b) *Staphylococcus aureus*, and (c) *Escherichia coli*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

References

1. Yoshimoto, H.; Shin, Y. M.; Terai, H.; Vacanti, J. P. Biomaterials 2003, 24, 2077.
2. Luu, Y. K.; Kim, K.; Hsiao, B. S.; Chu, B.; Hadjiargyrou, M. J. Control Release 2003, 89, 341.
3. Frenot, A.; Ioannis, S. C. Colloid Interf Sci 2003, 8, 64.
4. Doshi, J.; Reneker, D. H. J Electrostat 1995, 35, 151.
5. Kenawy, E. R.; Abdel-Hay, F. I.; El-Newehy, M. H.; Wnek, G. E. Mater ChemPhys 2009, 113, 296.
6. Xie, J. W.; Wang, C. H. Pharm Res 2006, 23, 1817.
7. Kenawy, E. R.; Bowlin, G. L.; Mansfield, K.; Layman, J.; Simpson, D. G.; Sanders, E. H.; Wnek, G. E. J Control Release 2002, 81, 57.
8. DeCherney, A. H.; DiZerega, G. S. Surg Clinics North Am 1997, 77, 671.
9. Kim, T. G.; Lee, D. S.; Park, T. G. Int J Pharm 2007, 338, 276.
10. Gehlot, D.; Bohra, A. Indian J Med Sci 2000, 54, 102.
11. Sikareepaisan, P.; Suksamrarn, A.; Supapol, P. Nanotechnology 2008, 19, 015102.
12. Merrell, J. G.; Mclaughlin, S. W.; Tie, L.; Laurencin, C. T.; Chen, A. F.; Nair, L. S. Clin Exp Pharmacol Physiol 2009, 36, 1149.
13. Obayed Ullah, M.; Jashim Uddin, M.; Hamid, K.; Kabir, S.; Azizur Rahman, M.; Choudhuri, M. S. K. J Biol Sci 2008, 11, 2036.
14. Fabre, T.; Schappacher, M.; Bareille, R.; Dupty, B.; Soum, A.; Bertrand-Barat, J.; Baquey, C. Biomaterials 2001, 22, 2951.
15. Jin, W. J.; Lee, H. K.; Jeong, E. H.; Park, W. H.; Youk, J. H. Macromol Rapid Commun 2005, 26, 1903.
16. Gopal, R.; Kaur, S.; Feng, C. Y.; Chan, C.; Ramakrishna, S.; Tabe, S.; Matsuura, T. J Membrane Sci 2007, 289, 210.