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Fibro-Porous Polycaprolactone Membrane Containing Extracts of *Biophytum sensitivum*: A Prospective Antibacterial Wound Dressing

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ABSTRACT: A fibro-porous wound dressing with antibacterial activity was fabricated from polycaprolactone (PCL) solution containing crude extract of *biophytum sensitivum* (BS) a potential antibacterial herbal drug. Scanning electron microscopy revealed the smooth fibrous morphology of the PCL membrane, whereas the drug-loaded PCL formed fibers with more interconnective junctions with an average fiber diameter between 1 and 3 μm. Physical characterization of the membrane revealed that it has excellent mechanical stability, water vapor transmission rate and that it promotes water uptake. The release characteristics by total immersion method in phosphate buffer and acetate buffer displayed an increase in drug release with time. Finally, the antibacterial activity of the membrane was tested against standard strains of *Staphylococcus aureus* and *Escherichia coli*. PCL membranes loaded with the drug extracts were able to inhibit the growth of bacterial strains which indicated that this fibro-porous membrane could act as a potential wound-dressing material to treat various wounds. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 2280–2286, 2013

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INTRODUCTION

In the treatment of burns, donor site wounds, and surgical wounds immediate coverage of wound surface with medicated dressings is compulsory for rapid and proper healing.[1–3] Many substitutes such as autografts, allografts, and xenografts have been employed for wound healing. However, these approaches have disadvantages, which include high cost, limited availability of skin grafts in severely burned patients, and problems of disease transmission and immune response.[4–6]

An ideal wound-dressing material should have several characteristics like flexibility, gas permeability, durability, moderate water vapor transmission rate, high level of exudates absorption capability, antibacterial efficiency, and non toxicity.[7] Fibro-porous systems have shown tremendous promise as drug delivery system for wound healing.[8] A number of techniques such as phase separation, self-assembly, and electrospinning have been developed to fabricate nano fibrous membranes with unique properties.[9,10] Among these methods, electrospinning has been portraying immense attention due to its effortlessness in producing fibers from materials with diverse origin, including polymers with characteristic diameters ranging from several microns down to tens of nanometers.[11]

Drug delivery systems using electrospun fibro-porous membranes are able to improve therapeutic efficacy due to greater surface area, lower toxicity, and enhance compliance of the patients by delivering drugs over longer period of time to the site of action.[12] The porous nature of the electrospun fiber membranes would allow nutrients to diffuse into and at the same time wastes to diffuse out from the cellular construct. In addition, they allow high oxygen permeation, facilitate profusion of exudates, and provide a good protection of wounds from infection and dehydration, which are some of the important characteristics of functional wound dressings.[13]

Currently there are many studies focusing on the incorporation of various herbal pharmacological agents onto fibrous systems for wound-dressing applications.[14–17] *Biophytum sensitivum (BS)* (L.) is an important medicinal plant used in traditional medicine by many people in Asia, Africa, and Pacific islands.[18] The reported beneficial effects of *BS* includes anti-inflammatory, anti-septic, anti-diabetic, anti-pyretic, and diuretic.[19,20] However, to the best of our knowledge there is no reported study on the wound-dressing application of *BS* incorporated polymeric membranes. In this study, *BS* containing polycaprolactone (PCL) solution was electrospun at optimum conditions. Various properties such as surface morphology, mechanical strength, water vapor transmission rate, release properties, and *in vitro* cytotoxicity of both the neat and the drug-loaded electrospun PCL fiber were investigated. The antibacterial properties of the membranes were studied against common pathogenic bacteria namely *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Materials and Preparation of Herbal Extract

The PCL (M_w-70,000–90,000) used in this study was supplied by Sigma Aldrich (India). Dichloromethane (DCM specially dried) and N, N, dimethyl formamide (DMF, specially dried) were supplied by Merck, India. They were used directly without any further purification.

BS leaves were first cleaned, dried, and finally grounded into fine powder. A 50 g of the fine powder was then dissolved in ethanol and kept overnight with continuous stirring. The solution was first filtered with crude filter paper and then the solvent was removed by distillation utilizing rotary evaporator. After ridding off ethanol (as much as possible) from the supernatant liquid, a pasty substance (~ 5 g), dark green/brown in color, was obtained.

Sample Preparation

PCL pellets were dissolved in DCM : DMF (80 : 20) mixed solvent at ambient conditions with a fixed concentration of 10% w/v. The crude ethanolic extract of *BS* (L.) (10% of the polymer weight) was then added to the PCL solution under constant stirring for 15 min. The neat and drug-loaded PCL solutions were placed in a 20 mL syringe capped with a 21 gauge needle. The positive potential of the power supply (Gamma High voltage, USA) was connected to the syringe using an alligator clip. The opposite potential was connected to a mandrel rotating at 500 rpm. At the onset of electrospinning, the syringe pump was set to deliver the source solution at a rate of 1 mL/h and simultaneously voltage was applied between the syringe and the grounded mandrel (8–10 KV).

Scanning Electron Microscope Analysis

Morphology of the neat and drug-loaded membranes was analyzed using scanning electron microscopy (SEM) (Hitachi-model-S-2400). Thin sections of the materials were observed in SEM after gold palladium sputtering to understand the fiber morphology. The average fiber diameter was assessed using Image J software utilizing the SEM image.

Mechanical Properties

Mechanical properties in terms of stress at maximum load, tensile strength, and elongation at break of the neat PCL and *BS* loaded PCL membranes were assessed using a uniaxial load test machine (Model 3345, Instron Corporation, and Issaquah, WA) at 25 ± 2°C equipped with a load cell of 100*N*. The deformation rate was 100 mm min^{-1} with the sample thickness varying from 0.1 to 0.4 mm (sample size $n = 5$).

Water Vapor Transmission Rate (WVTR)

The WVTR across the membranes was measured by utilizing a boiling tube which was filled with distilled water and the sample membranes were fixed onto its opening. The mouth defined the area of the test membrane exposed to the environment. Then the assembly was kept up-side-down in an isothermal bath at 37 ± 0.5°C. After 24 h, the evaporation of water through the membrane was monitored by the weight change of the boiling tube and the WVTR was calculated by the following equation.

$$\text{WVTR} = (A1 - A2)/S$$

where ($A1$ – $A2$) denotes the weight change of the boiling tube with the membrane (g) and S stands for the area of the cup mouth (m^2).[21] The reported data were mean ± standard deviation of five parallel runs.

Phosphate Buffer Saline and Water Adsorption

The phosphate buffered saline (PBS) and water absorption content of the neat and *BS* loaded PCL membranes were determined by putting the samples in pH = 7.4 of PBS and deionized water at 37 ± 0.5°C. The membranes were first weighed and placed in the PBS and water. After 24 h, the membranes were weighed immediately after being blotted with filter paper to remove excessive surface solution. The percentage water absorption of the membranes in the PBS media and deionized water was calculated according to the following equation.

$$\text{Water absorption percentage} = (W_s - W_o)/W_o \times 100$$

where W_s denotes the weight of swollen sample and W_o stands for the initial sample weight.[21] The reported data were mean ± standard deviation of five parallel runs.

Fourier Transform Infrared Spectroscopy Analysis

The Fourier transform infrared spectroscopy (FTIR) was carried out using Jasco FTIR-6300 spectrometer in the range of 400–4000 cm^{-1} to confirm the incorporation of herbal drugs in the polymeric membranes.

Porosity Measurements

The porosity (ε) of the membranes was measured at room temperature by using the liquid intrusion method.[22] Briefly, the electrospun membranes were cut into 8-mm discs, then weighed and subsequently immersed in ethanol overnight on a mechanical shaker to allow the liquid to penetrate into the scaffold voids. The density of ethanol (δ_{ETH}) is 0.780 g mL^{-1} while the density of PCL for the neat and drug-loaded membranes (δ_{PCL}) was taken as 1.45 g cm^3. The surface of the membrane was blot dried and weighed once again to determine the weight of the ethanol present within the scaffold. The porosity was calculated using the following equation

$$\varepsilon = V_{ETH}/(V_{ETH} + V_{PCL})$$

V_{ETH} is the volume of intruded ethanol and was calculated as the ratio between the observed mass change after intrusion and δ_{ETH}. V_{PCL} is the volume of the PCL fibers and was calculated as the ratio between the dry membranes mass before intrusion and δ_{PCL}.

In Vitro Drug Release Profile

Circular discs ($n = 5$) were placed in a boiling tube filled with 5 mL of the release medium (phosphate buffer and acetate buffer). Drug release was carried out at 37°C. The phosphate buffer releasing medium has a pH of 7.4 and the acetate buffer has a pH of 5.5.[7] At appropriate time intervals, 1.5 mL of the medium was taken and mixed with 1.5 mL DMF. The calibration curve was made from DMF : PBS (50 : 50) and DMF : acetate buffer (50 : 50) at a wavelength of 233 nm and a linear equation was derived by a curve-fitting method. In the assessment of drug release behavior, the cumulated amount of the drug released was calculated and plotted against time.

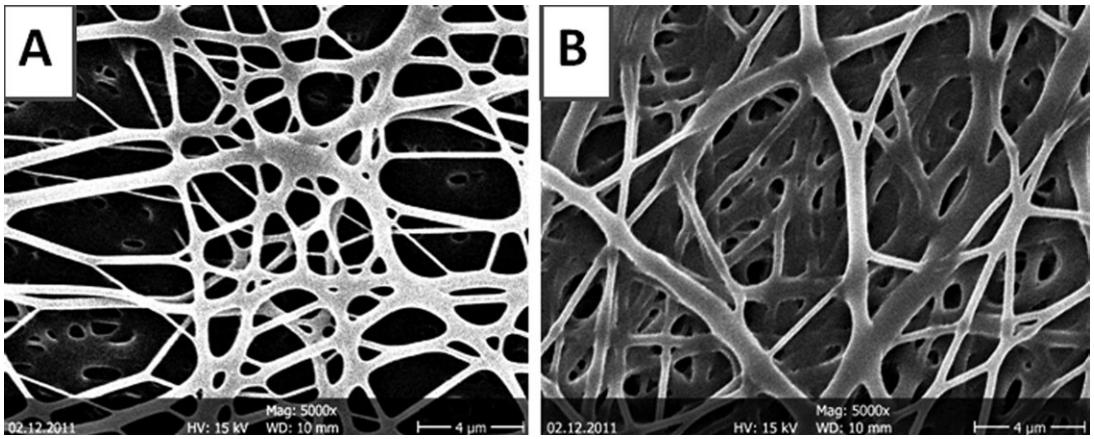

Figure 1. Scanning electron micrographs of the electrospun fibers (A): Neat PCL; (B): *Biophytum sensitivum* incorporated PCL fibers.

In Vitro Cell Viability via MTT Assay

In vitro cytotoxicity experiment of both the neat PCL and the drug-loaded electrospun PCL was conducted in adaptation with the ISO 10993-5 standard test method in a 96-well tissue culture polystyrene plate using L929 fibroblast cells (American type culture collection). After incubation overnight (37 ± 1°C, 5% CO_2), the medium in each well was aspirated off and loaded with 100 μL of fresh medium containing extraction medium with different *BS* concentration. After 24 h, the medium containing biophytum extract was aspirated off and replaced with fresh medium containing 10 μL of MTT reagent. The cells were incubated for 3 h at 37°C. The solution was then aspirated and 200 μL/well of DMSO was added to dissolve the formazan crystals. Finally, after 20 min of rotary agitation, the absorbance at a wavelength of 570 nm was measured.

In Vitro Antibacterial Study

The antibacterial efficacy of the electrospun membranes were investigated by disc diffusion method against pathogenic bacteria such as *S. aureus* ATCC 933 (Gram-positive) and *E. coli* NCTC 9002 (Gram-negative). PCL was used as the control material. BS containing PCL membranes were cut into circular discs of 8-mm diameter and placed on top of the bacterial culture. The plate was incubated for 24 h at 37°C. 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

Scanning electron micrographs of the neat PCL and *BS* loaded fibro-porous PCL membranes are presented in Figure 1. The fiber diameter and the morphology of the fibers were strongly influenced by spinning solution composition. No drug crystals were detected on the polymer fiber surface. This suggested that the drug was dispersed homogeneously in the electrospun PCL fibers. Furthermore, it was noticed that with the incorporation of the drug in the PCL solution, the resultant fiber morphology drastically changed. The fiber diameter increased with the formation of larger interconnective junctions. This increase in fiber diameter and junctions may be due to the incorporation of *BS* which reduced solvent evaporation rate. When the solvent evaporation rate reduces, excess solvent may cause the fibers to merge where they contact to form junctions resulting in inter and intra layer bonding[23] as shown in Figure 1. This interconnected fibrous mesh may provide additional strength to the resultant membrane. The fiber diameter was measured using Image J software from the SEM images and the dimensions of the fibers were in the range 1 μm for the drug free membrane and the fiber diameter shifted to a higher scale on incorporation of the drug.

The mechanical properties of *BS* loaded electrospun PCL membranes and the neat PCL membranes were investigated and the results are summarized in Table I. The neat PCL membrane and the drug-loaded PCL membranes are elastic in nature, with a tensile strength ranging from 4.7 ± 0.8 MPa to 17.1 ± 0.7 MPa and elongation at break from 177% to 74%. The stress vs. strain curves of both BS loaded PCL and neat PCL membranes are shown in Figure 2. The modulus at 10% strain increased from 0.8 MPa to 4.4 MPa after the incorporation of the drug. The addition of *BS* increased the tensile strength and reduced the elongation at break of the electrospun PCL membrane. This increase in mechanical strength may be due to the better membrane integrity of drug-loaded membrane due to reduced solvent evaporation. The tensile strength of the drug-loaded membrane was comparable to that of two commercial tissue regenerative membranes (Resoluts LT, Biofixs) and a wound-dressing material (Beschitins W; Unitica).[24,25] This indicates that the *BS* loaded PCL membranes provided a similar mechanical stability and could be applied in the wound healing applications.

Table I. Mechanical Integrity of PCL (neat) and *Biophytum sensitivum* Loaded PCL Fibrous Membranes ($n = 5$)

Material	Tensile strength (MPa)	Elongation at break (%)	Modulus at 10% strain (MPa)
PCL (neat)	4.7 ± 0.8	177 ± 19	0.8 ± 0.2
BS Loaded PCL	17.1 ± 0.7	74 ± 8	4.4 ± 0.6

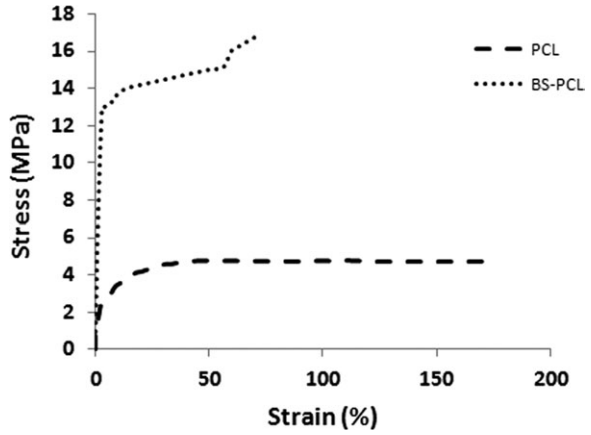

Figure 2. Stress–strain curve of the membranes.

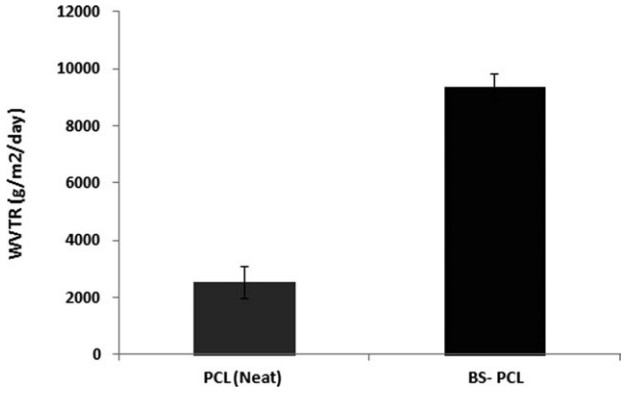

Figure 4. Water vapor transmission rate (WVTR) of the membranes ($n = 8$).

To be used as a high quality wound dressing, the dressing material should have the ability to absorb excessive extrudate from the wound site and keep a suitable moist environment for the regeneration of the skin. Figure 3 shows the fluid absorbing capabilities of the membranes. PBS absorption for BS loaded membrane was 241% and for deionised water it was 223%, respectively. Zahedi et al. reported that typical film dressings only show water absorption of 2.3%.[26] Yu et al. reported that for polyurethane thick membranes the water absorption were only 21–26%.[27] Obviously *BS* loaded membrane performed better on absorption of deionised water than the value reported for the films indicating the better extrudate absorption capacity of the drug-loaded membrane. These high values were enough to prevent wound beds from exudates accumulation according to previous research.[27]

Another important property of an ideal wound dressing would be to control the rate of water evaporation in a controlled manner. The rate of water evaporation for normal skin is 204 g/m^2 per day, while that for injured skin it can range from 279 g/m^2 per day for a first-degree burn to 5138 g/m^2 per day for a granulating wound. Figure 4 shows the WVTR of the membranes. With the incorporation of *BS* the WVTR increased drastically. This may be due to the incorporation of hydrophilic drug into the membrane. In terms of Queens recommendations,[28] PCL membrane with 10 % *BS* was quite suitable as wound-dressing membranes without risking exudates accumulation or total dehydration of the wound surfaces.

The ideal wound dressing should have a high porosity for vapor permeation and should act as a good barrier for protection of the wound from infection and dehydration.[29] Electrospinning produces non woven fibrous membranes with varying percentage porosity. The average estimates of porosity for both the membranes were determined by liquid intrusion analysis. The percentage porosity reduced from 84% to 72%. This slight reduction in percentage porosity may be due to reduced solvent evaporation after the incorporation of *BS* (Figure 5). This can also be confirmed by SEM image analysis. The reduced solvent evaporation results in higher packing density and thus reducing porosity. Even though there was a reduction in percentage porosity the drug-loaded membranes had very good extrudate absorption capability and water vapor transmission rate when compared to neat PCL.

Incorporation of drug into the matrix is confirmed by FTIR spectroscopy. FTIR spectra for PCL, herbal drug, and drug-loaded PCL electrospun membranes are shown in Figure 6. FTIR spectrum of PCL membrane is shown in Figure 6(b). The peak at 2948 and 2985 cm^{-1} corresponds to asymmetric and symmetric vibrations of CH_2 group and C=O vibration of the ester group of the polymer occurs at 1726 cm^{-1}. The peak at 1101, 1065, and 960 cm^{-1} are due to O—C vibrations with CH_2

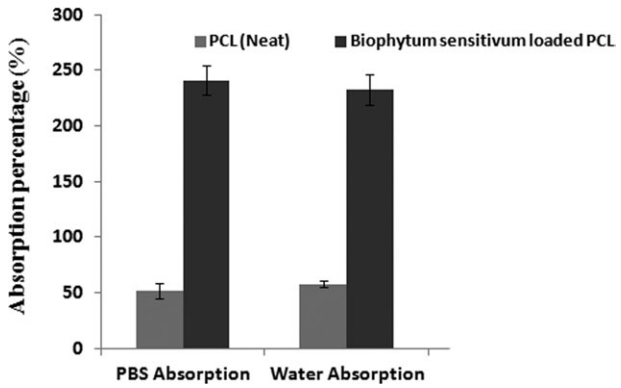

Figure 3. Phosphate buffer and water absorption of the membranes.

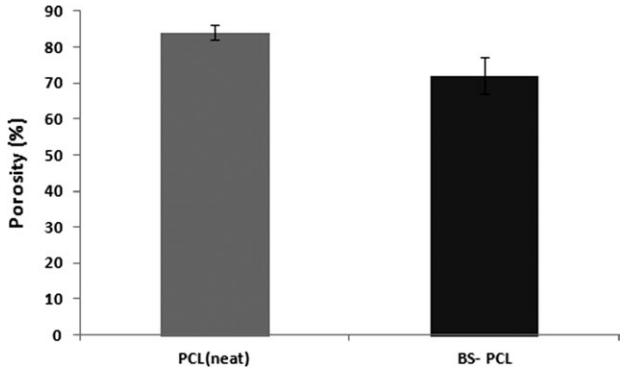

Figure 5. Percentage porosity of the membranes by liquid intrusion technique ($n = 5$).

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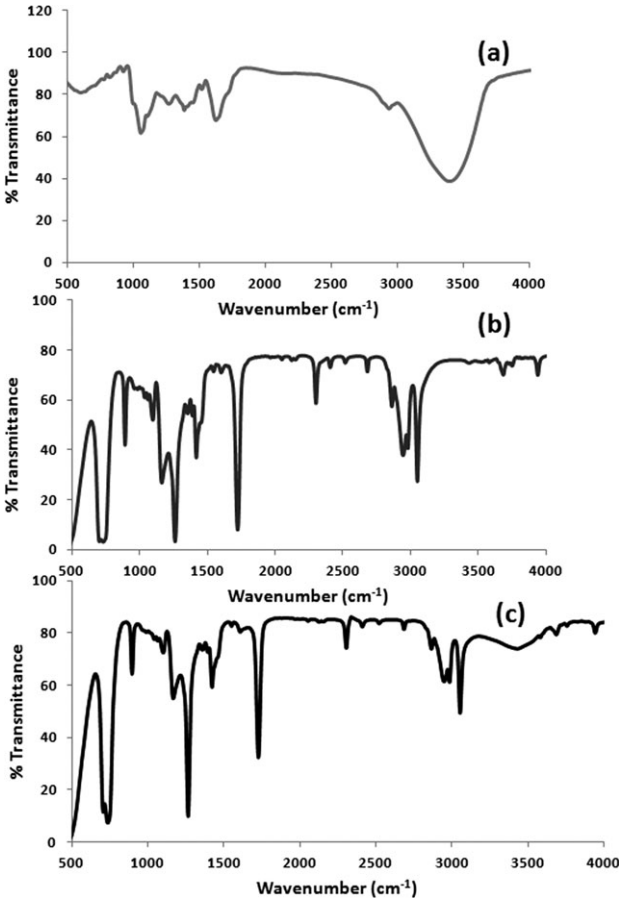

Figure 6. FTIR spectra of (a) *Biophytum sensitivum*, (b) PCL, (c) *Biophytum sensitivum* incorporated PCL membrane.

rocking vibrations occurring at 735 cm^{-1}. Figure 6(a) displays the FTIR spectrum of *BS*. The peak at 1624 cm^{-1} shows the aromatic ring C=C stretching of the phenolic groups in the crude extract. The broad peak between 3000 cm^{-1} and 3400 cm^{-1} maybe due to the presence of N—H groups and also due to the presence of hydroxyl groups present in the drug. The FTIR spectrum of the drug-loaded PCL membrane is shown in Figure 6(c). The spectrum contains all the features of (a) and (b) and the peak at 3432 cm^{-1} appears to be the main evidence for the presence of *BS* in the matrix. All the other peaks of the drugs were masked by the vibrations and stretching of functional groups in PCL. This provides us an indication that the drug was present and well associated with the PCL membrane.

Figure 7 shows the cumulative release characteristics of *BS* from the electrospun PCL membrane by total immersion technique. The experiment was carried out in phosphate buffer and acetate buffer at 37°C. In phosphate buffer, the percentage of drug released from the fibrous membrane rapidly increased during the first 15 h and increased rather slightly afterwards (i.e., to reach the total amount of sensitivum release). Initial rapid release may be because of the absorption of sufficient phosphate buffer solution into the fibrous membranes resulting in the release of the drug. The concentration of *BS* in the polymeric membrane was 10% on the weight of the polymer. The release

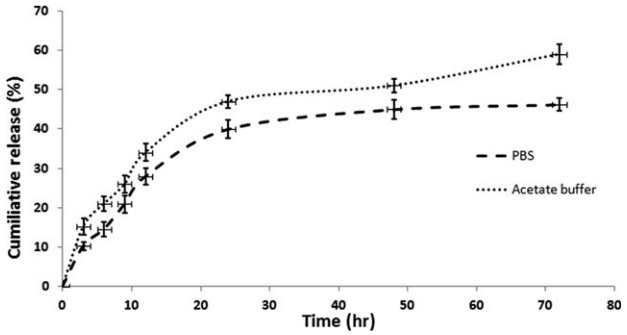

Figure 7. The release profile of *BS* from the membranes.

profile from the drug-loaded membranes exhibited a drug release of about 28% in the first 12 h and around 40% of the total drug in the next 24 h. Later on the release varied very slightly from 40% to 42% after 72 h. In case of acetate buffer, the release of *BS* was slight more than phosphate buffer solution. In the first 12 h, there was around 34% release and after 72 h the release increased to around 59% of the total drug. Hence *BS* loaded PCL membranes could be used as a suitable wound dressing to kill bacteria's for a specific time period.

The cytotoxicity of the *BS* loaded PCL membrane was assessed by MTT assay. Although it's a known fact that most of the herb-loaded materials released no substances in the levels that were harmful to the cells, assessment with regard to cytocompatibility is a prerequisite since toxic solvents are used for the fabrication of the membrane. Mouse fibroblast cells (L929) were used for the assessment. Figure 8 shows the MTT absorbance in cell viability experiments. After 24 h of exposure of the extract to the cells, the cells displayed very good viability. Percentage viability was >70% for the drug-loaded PCL membrane. This confirms that membrane was cytocompatible.

The potential for use of *BS* loaded electrospun PCL membrane as a functional wound-dressing material was assessed by observing the antibacterial activity against some common bacteria found on burn wounds. The activity of PCL alone was used as the control for antibacterial assessment. As reported in Figure 9, after 24 h of contact interval, PCL membrane showed no zone

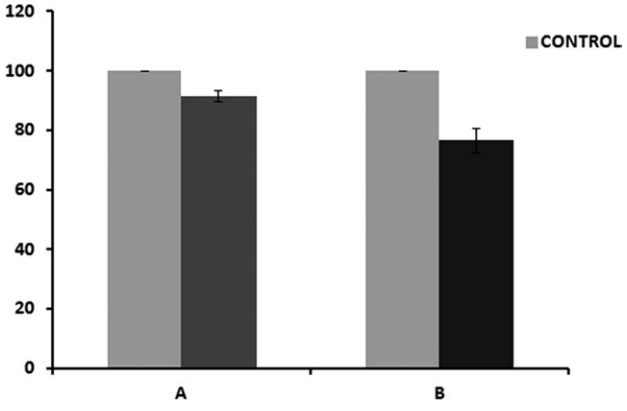

Figure 8. Viability against L929 cell line (A) PCL (B) *BS* incorporated PCL.

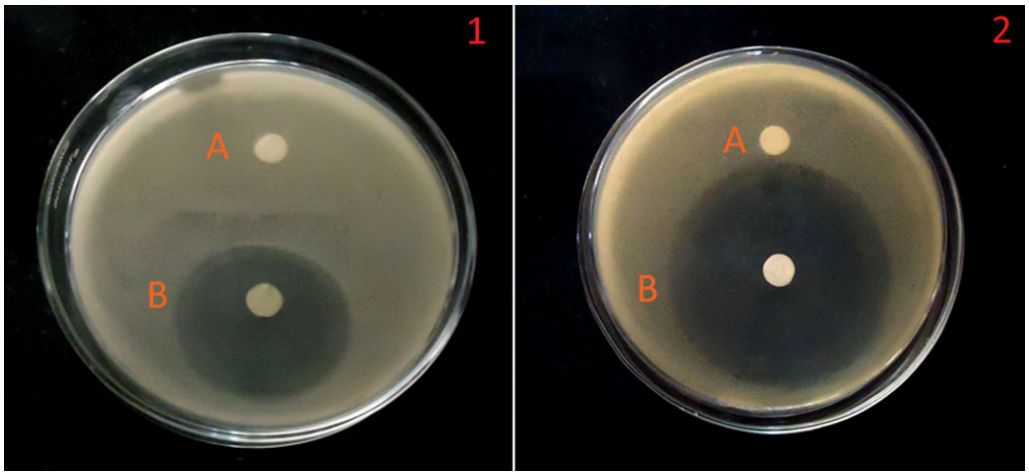

Figure 9. *In vitro* testing for antibacterial activity (A) PCL, (B) *BS* loaded PCL membrane (1) Against *S. aureus.* (2) Against *E. coli.* [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of inhibition against *S. aureus.* and *E. coli*, whereas the *BS* loaded specimen gave a 27 mm inhibition against *S. aureus* and 47 mm inhibition zone against *E. coli.* Hence the *BS* loaded drug membranes were bactericidal to the testing microorganisms. There was a drastic increase in antibacterial activity after the incorporation of the herbal drug this might be due to the strong antibacterial activity of *BS* extracts. The results indicated that the herbal drug-loaded PCL membranes possessed sufficient antibacterial property to be used as a potential wound-dressing material.

CONCLUSIONS

In the present contribution *BS* (L), widely known for its anti-tumor, anti-inflammatory, and anti-diabetic properties, was added to the neat PCL solution. The neat and *BS* loaded PCL solutions were electrospun into fine fibrous membranes under similar conditions. The fibers were smooth and no biophytum crystals were observed on the surface of the fibers, a result indicative of complete incorporation of the drug within the fibers. The membranes displayed good tensile strength, water uptake capability and water vapor transmission rate. In the total immersion method >40% of the drug was released from the *BS* loaded fibrous membranes into the mediums. After the incorporation of the drug, this PCL membrane became antibacterial against *S. aureus* and *E. coli. BS* incorporated PCL membranes showed no toxic effects to L929 cells. The membranes developed in the present study have great potential in drug delivery and wound healing. They are also promising materials for treating surfaces exposed to pathogenic microorganisms.

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