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Electrospun nanofibrous polymeric scaffold with targeted drug release profiles for potential application as wound dressing

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ABSTRACT

We have successfully fabricated a dual drug release electrospun scaffold containing an anesthetic, lidocaine, and an antibiotic, mupirocin. Two drugs with different lipophilicities were electrospun from a poly-L-lactic acid (PLLA) solution with a dual spinneret electrospinning apparatus into a single scaffold. The release of the drugs from the scaffold showed different profiles for the two drugs. Lidocaine hydrochloride exhibited an initial burst release (80% release within an hour) followed by a plateau after the first few hours. Mupirocin exhibited only a 5% release in the first hour before experiencing a more sustained release to provide antibacterial action for over 72 h. For comparative purposes, both drugs were spun from a single spinneret and evaluated to determine their release profiles. The scaffold maintained its antibiotic activity throughout the processes of electrospinning and gas sterilization and supported cell viability. It has been reported in the literature that interactions between polymer and drug are known to govern the pattern of drug release from electrospun scaffolds. Here, it was found that the presence of the two drugs in the same polymer matrix altered the release kinetics of at least one drug. Based on the release profiles obtained, the dual spinneret technique was the preferred method of scaffold fabrication over the single spinneret technique to obtain a prototype wound healing device.

1. Introduction

The ideal wound dressing should minimize infection and pain, prevent excessive fluid loss, maintain a moist healing environment, promote epithelial restoration, and be biocompatible. In addition, dressings should have adequate adherence to the wound area and must be easy to apply and remove to ensure patient compliance and comfort. In addition to the application of dressings, wound treatment includes irrigation of the affected area with an anesthetic solution followed by application of prophylactic antibiotics to prevent wound infection. Reapplication of the antibiotic formulation and repeated changing of the dressings by either the healthcare provider or the patient continues until the wound heals. This is often an inconvenient process and needs some basic improvement. While dressings currently available in the market satisfy one or more of these requirements, no single dressing addresses all the above issues. Based on these ideas, the concept of a dual drug scaffold wound dressing was put forward. Such a scaffold would offer a unique combination of the inherent properties of electrospun scaffolds, such as promoting cell proliferation, and simultaneously provide anesthetic and antibiotic activity for pain relief and healing. The challenge in fabricating the above scaffold lay in obtaining two different drug release profiles from a single medical device. The final aim was to have sustained delivery of an antibiotic over at least 3 days for prophylaxis against bacterial infections and to have immediate delivery of the anesthetic for pain alleviation.

In recent years, researchers have utilized electrospinning in an effort to promote faster restoration and increase the biocompatibility of the wound dressing. Electrospun scaffolds from poly(ethylene-co-vinyl alcohol) (Kenawy et al., 2003), collagen (Rho et al., 2006), polyurethane (Khil et al., 2003), and collagen-PEO (Huang et al., 2001) have been investigated for potential application as wound dressings. Such substrates have high void volumes and thus accommodate a significant amount of exudates while increasing the breathability of the applied dressing. Previous studies aimed at creating wound-healing scaffolds through electrospinning have relied on such inherent properties of the electrospun material rather than the addition of drugs to provide the required protection and pain management.

There also has been increasing interest in the incorporation of drugs in electrospun fibers in areas other than wound healing. For example, studies have been performed in the areas of cancer therapy and heart disorders (Kenawy et al., 2002; Verreck et al., 2003; Zeng et al., 2003, 2005; Brewster et al., 2004; Jiang et al., 2004, 2005;

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Kim et al., 2004; Chew et al., 2005; Xu et al., 2005; Cui et al., 2006; Luong-Van et al., 2006). While investigations have been aimed at achieving desired drug release profiles and sustaining the bioactivity of the incorporated active compound, few have been able to elucidate the mechanisms behind the release characteristics (Zeng et al., 2005).

The drugs of choice for inclusion in our scaffold were lidocaine hydrochloride and mupirocin. Lidocaine is a routinely used anesthetic in wound related pain management. It has a lower incidence of allergic reactions than the ester-type anesthetics such as procaine and tetracaine (Popescu and Salcido, 2004). In addition, lidocaine has some antibacterial action against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Aydin et al., 2001). Mupirocin (Bactroban®) is a commonly used antibiotic in wound care for prophylaxis against cutaneous infection and elimination of carriage of *Stapylococcus aureus* (a pseudomonic acid—a fermentation product of *Pseudomonas fluorescens*) inhibits protein synthesis, actively preventing the incorporation of isoleucine into protein by binding to isoleucyl transfer-RNA synthetase. Due to this unique mechanism of action, there is no *in vitro* incidence of cross-reactivity with other antimicrobials. Mupirocin is effective against aerobic gram positive and some gram negative flora. The low incidence of adverse effects and rare cases of resistance add to its attractiveness as an antibiotic of choice. Mupirocin is typically formulated as a 2% cream or ointment that is applied three times daily from 3 to 10 days as required; the wound is covered with gauze after application (Source: Bactroban® monograph).

To achieve the proposed dual release, a novel electrospinning technique with simultaneous electrospinning from dual spinnerets has been investigated in this study. This would yield a single scaffold with two polymer fibers containing two different drugs. The drug release profiles, scaffold morphology, drug content, thermal properties, and antibiotic activity of the scaffold are presented. This work enables us to understand polymer–drug interactions and provide insight into correlations between drug release and electrospinning conditions.

2. Materials and methods

2.1. Chemicals

Lidocaine hydrochloride (LH), mupirocin, and hexafluoroisopropanol (HFIP) were purchased from Sigma–Aldrich (St. Louis, MO). Poly-L-lactic acid (PLLA) Resomer L 206 was purchased from Boehringer Ingelheim Chemicals (Petersburg, VA). Human dermal fibroblasts (HDF) and CellTiter96™ AQueous assay (MTS) were purchased from Cascade Biologics (Portland, OR) and Promega Corp (Madison, WI), respectively. Dulbecco's Phosphate Buffered Saline, Trypsin EDTA, and Gibco™ Newborn Calf Serum were purchased from Invitrogen (Carlsbad, CA). *Staphylococcus aureus* ATCC® 25923 was purchased from American Type Culture Collection (Manassas, VA). Tryptic soy broth and agar were purchased from BD Diagnostic Systems (Sparks, MD). Phosphate buffered saline (PBS) tablets were purchased from MP Biomedicals, Irvine, CA. All the other chemicals and solvents were of analytical grade.

2.2. Polymer solutions and electrospinning parameters

PLLA was dissolved in HFIP (Badami et al., 2006) and gently shaken with a vortex mixer for 3 h until the polymer was completely dissolved. A solution of LH or mupirocin in HFIP was slowly added without any visible precipitation; the resulting mixture was shaken for 30 min. The homogeneous drug/polymer solution was then electrospun with the parameters listed in Table 1.

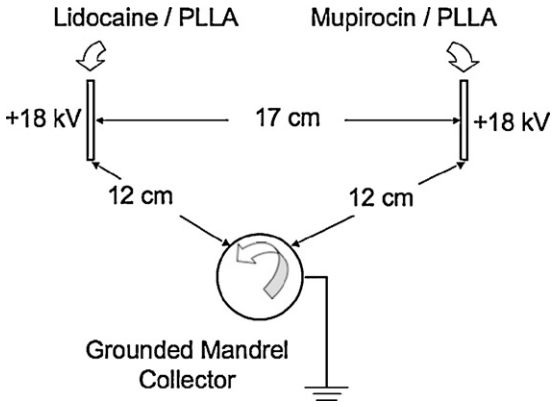

Fig. 1. Schematic of the dual spinneret electrospinning apparatus.

For characterization of fibers, solutions A, B, and C were electrospun separately. Solutions A and B were electrospun with the dual spinneret (DS) system (Fig. 1) into a single scaffold to study release properties. Solution C was electrospun with a single spinneret (SS) apparatus for the purpose of comparison of release profiles with the DS scaffold. The final scaffolds were sterilized for 14 h with Anprolene AN74i ethylene oxide sterilizer (Anderson Products Inc., NC) and purged for an additional 4 h before drying under vacuum for 36 h.

2.3. Electrospinning procedures

The dual spinneret electrospinning apparatus (Fig. 1) assembly is described as follows: Polymer solutions were loaded into two syringe pumps, each connected to a 19-gauge needle. The syringe pump controlled the flow rate of the polymer–drug solution to the respective needles. The tip-to-collector distance was 12 cm, and the distance between the two needles was 17 cm. A high voltage power supply (Gamma High Voltage Research Inc., Omaha Beach, FL) was used to charge the metal needle. Fibers spun from both spinnerets were simultaneously collected on a 5-cm diameter, grounded, aluminum mandrel, which was rotated at approximately 120 rpm.

2.4. Uniformity of distribution of fibers

To confirm uniform spraying and mixing of the fibers in the matrix with the DS technique, Texas Red was used to stain one of the fibers. Briefly, 1% w/v of Texas Red in ethanol was suspended in a 17-wt % PLLA solution in HFIP and loaded into one syringe pump. The other syringe pump contained a non-fluorescent solution of 17 wt% PLLA in HFIP. Electrospinning conditions were similar to those for solution A. After drying, the fibers were viewed under a fluorescence microscope (Zeiss Axiovert 200, Thornwood, NY).

2.5. Characterization of fibers

Surface morphology of the electrospun scaffolds before and after drug release was observed on an AMRAY 1830 I scanning electron microscope (SEM). Samples for SEM were dried under vacuum, mounted on aluminum stubs, and sputter-coated with gold–palladium. Histograms of fiber diameter were generated by the measurement of approximately 160 individual fibers in 3000X SEM images using NIH-ImageJ software (http://rsb.info.nih.gov/ij/). Incorporation of drugs and polymer–drug interactions were studied by differential scanning calorimetry (DSC). The fibers were

Table 1
Parameters for the electrospinning of drug–polymer solutions

Apparatus	Solution	Polymer (% w/v)	Drug concentration as % w/w of polymer	Voltage (kV)	Distance (cm)	Flow rate (mL/h)	Needle gauge
Dual Spinneret	A	PLLA (17%)	Mupirocin 7.5%	18	12	0.50	19
	B	PLLA (17%)	LH 80%	18	12	0.43	19
Single Spinneret	C	PLLA (17%)	Mupirocin 3.75% LH 40%	18	12	0.10	19

heated in DSC 2920 (TA instruments) with a heating rate of 10 °C/min from −10 to 200 °C. The compositions of electrospun scaffolds were quantified by Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR). Briefly, 3% w/v solutions of the DS and SS electrospun scaffolds were prepared in deuterated chloroform. Spectra were obtained with a 300-MHz Varian Mercury spectrometer (Palo Alto, CA). Spectrum acquisition and integration were repeated five times to assess the precision of the technique.

2.6. Drug release from the scaffolds

The electrospun scaffolds were placed in 5 ml of pH 7.4 phosphate buffered saline (PBS) in vertical Franz diffusion cells (Permegear Inc., Bethlehem, PA) with five replicates for each scaffold. The scaffold was immersed completely in the receptor compartment of the diffusion cell below the sampling port. The scaffold exhibited good wettability and remained completely immersed without additional support throughout the experiment. The outer jacket of the Franz cells was maintained at 37 °C and stirred at 600 rpm, and the inner compartments were covered with Parafilm®. At appropriate intervals from 1 to 72 h, 200 μl samples were withdrawn from the sampling port and replenished with an identical volume of fresh buffer. The drug concentrations were determined by high performance liquid chromatography (HPLC) with a Hewlett Packard 1100 system (Agilent Technologies) equipped with degasser (G1379A), autosampler (G1313A), quaternary pump (G1311A), and a UV–visible diode array (G1315A). Previously established HPLC methods were used for detection of both LH (Kang et al., 1999) and mupirocin (Echevarria et al., 2003). In all cases, drug concentration values were corrected for the progressive dilution occurring because of the sampling pattern (Meidan et al., 2003). Statistical analysis involved application of a two-tailed, unequal variance Student's *t*-test.

2.7. Antibiotic activity of mupirocin in the scaffold

Bacterial viability tests were conducted using the rapid, modified Kirby–Bauer Disc method (Boyle et al., 1973). A 100-μl aliquot of *Staphylococcus aureus* reconstituted in nutrient broth and previously subcultured was spread onto an agar plate. Sections (0.5 cm diameter) of DS and SS fiber scaffolds were placed on agar plates, allowing sufficient time for the drug to diffuse into the surroundings. The plate was incubated for 6 h at 37 °C, sprayed with 0.025% MTS reagent, and visualized after 10–15 min. The zones were then measured and compared against previously established interpretative criteria (Finlay et al., 1997). Controls with no mupirocin loading were maintained separately using the same procedure.

2.8. Cell proliferation and morphology

Human dermal fibroblasts (500 cells/μl) were used to study cell viability on the scaffolds. Electrospun fiber scaffolds were punched (0.6 cm in diameter) and placed in sterile 96-well tissue-culture Costar® plates (Corning Incorporated, NY); 10 μl of cell suspension and 90 μl of Dulbecco's Modified Eagle Medium (DMEM) were added to each plate; the plates were then incubated for 3, 4, and 6 days at 37 °C. The controls contained either fibroblasts in media without a scaffold or an electrospun scaffold with media but no fibroblasts. MTS assays were performed on 3, 4, and 6 days postseeding. Briefly, fresh media was added to each scaffold after aspiration of the old media, and 20 μl of MTS solution was added per well. After 3 h, the supernatant was analyzed colorimetrically using a multiwell plate reader (Powerwave, Bio-Tek Instruments) at 490 nm.

SEM was used to examine the morphological characteristics of cells cultured onto the nanofibrous structure. Electrospun scaffolds in culture plates seeded with HDF were cultured for 3, 4, or 6 days. Loosely adherent or unbound cells were removed from the experimental wells by aspiration, and the bound cells were fixed in 4% formaldehyde in a buffer (pH 7.4) for 20 min. After aspiration of the fixative and repeated washings with buffer and water, electrospun nanofibers were dehydrated in gradient ethanol solutions (50%, 70%, 85%, 95%, and 100%) for 15 min each. After critical point drying, samples were sputtered with gold–palladium and examined by SEM.

3. Results and discussion

3.1. Characterization of fiber scaffolds

Fiber scaffolds containing fibers of two unique compositions were obtained using the DS electrospinning apparatus. Macroscopically the scaffold was a conformable and resilient structure with the appearance of an ultrafine cloth. Fluorescence microscopy of the scaffold, which contained one fiber doped with Texas Red and another fiber without Texas Red, showed homogenous distribution of the two fibers (Fig. 2). One can observe two larger diameter fibers, one of which is clearly fluorescent, fiber 1, and another which is not fluorescent, (fiber 2). The DS electrospinning apparatus could be used to electrospin a hybrid mesh of materials of varying degradation rates, mechanical properties, or chemical functionality. Here, the technique was used to create a mesh where one fiber was loaded with an antibiotic and a second fiber was loaded with an anesthetic.

Simultaneous electrospinning of solutions A and B from the DS apparatus produced the scaffold pictured in Fig. 3A. Fig. 3B depicts the scaffold resulting from electrospinning solution C from the SS apparatus. Though all solutions contained the same concentration of PLLA, the DS technique produced a scaffold with two different fiber diameter populations, while SS produced a single population of fibers with an intermediate fiber diameter (Fig. 4). This result is not surprising since solution B had a much higher ionic strength than solution A due to the higher concentration (80 wt %) of LH, a salt. Solution C, which contained 40 wt % LH, had a fiber diameter between that observed from the electrospinning of solutions A and B. Fong, Chun, and Reneker (Fong et al., 1999) have previously demonstrated that increasing ionic strength induces more charge into the polymer solution and reduces the diameter of electrospun fibers.

The drug-loading of the DS and SS electrospun fiber scaffolds was confirmed via Proton NMR, after observation of dripping of the LH solution, solution B, from the needle during electrospinning. As expected, the LH content of the DS scaffold was lower than the

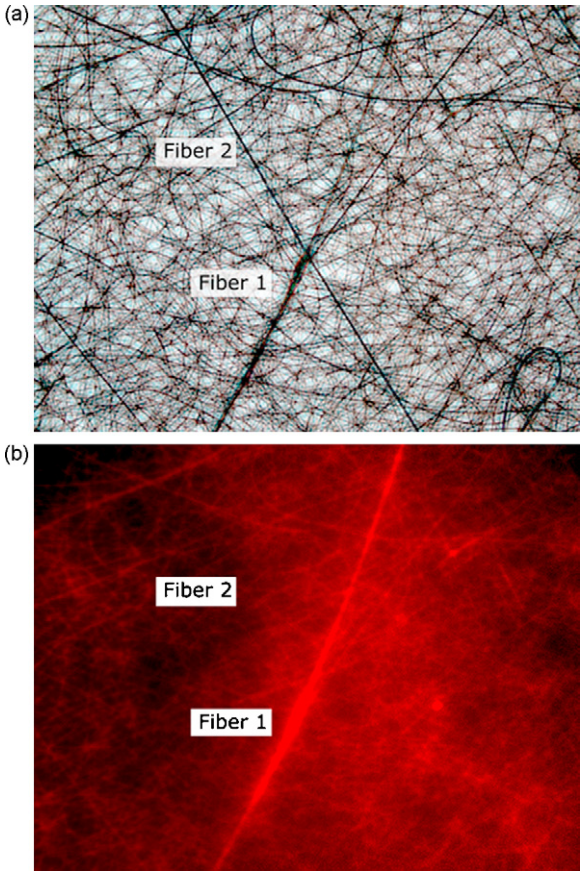

Fig. 2. Light microscopy (a) and fluorescence (b) images of electrospun fiber scaffolds fabricated by DS technique. Fiber 1 is clearly visible by both imaging techniques, while fiber 2 is not visible by fluorescence microscopy.

amount of LH dispensed from the spinneret (Table 2). These fibers consequently had an elevated PLLA and mupirocin content. The SS scaffold, which was electrospun at 0.1 ml/h, contained the amount of drug originally added, as there was no loss due to dripping.

3.2. Drug release from the scaffolds

The kinetic drug release profiles are shown in Fig. 5. Both the DS and SS electrospun scaffolds eluted LH in a burst-release fashion, with 80% of the LH detected in the first hour. Over the next 71 h, LH diffused out of the polymer matrix, achieving a cumulative release of 90%. No significant difference was found between the percent release from DS or SS fibers at 1 h ($p = 0.90$) and 72 h ($p = 0.63$). While studying the release of tetracycline hydrochloride from different polymers, including PLA, Kenawy et al. (2002) similarly observed a burst release of the drug followed by a plateau.

Though similar LH release was observed in the DS and SS configurations, the SS electrospinning technique caused the undesirable burst release of 28% of the mupirocin at the first hour, while only 5% of the mupirocin diffused from the DS electrospun scaffold ($p < 0.001$). The cumulative release at 72 h was 12% and 36% for the

Table 2
Drug content in mass % ±95% confidence interval by proton-NMR

Component	Dual spinneret	Single spinneret
PLLA	78.7 ± 0.7%	74.6 ± 1.0%
LH	17.9 ± 0.7%	23.3 ± 1.0%
Mupirocin	3.4 ± 0.2%	2.2 ± 0.2%

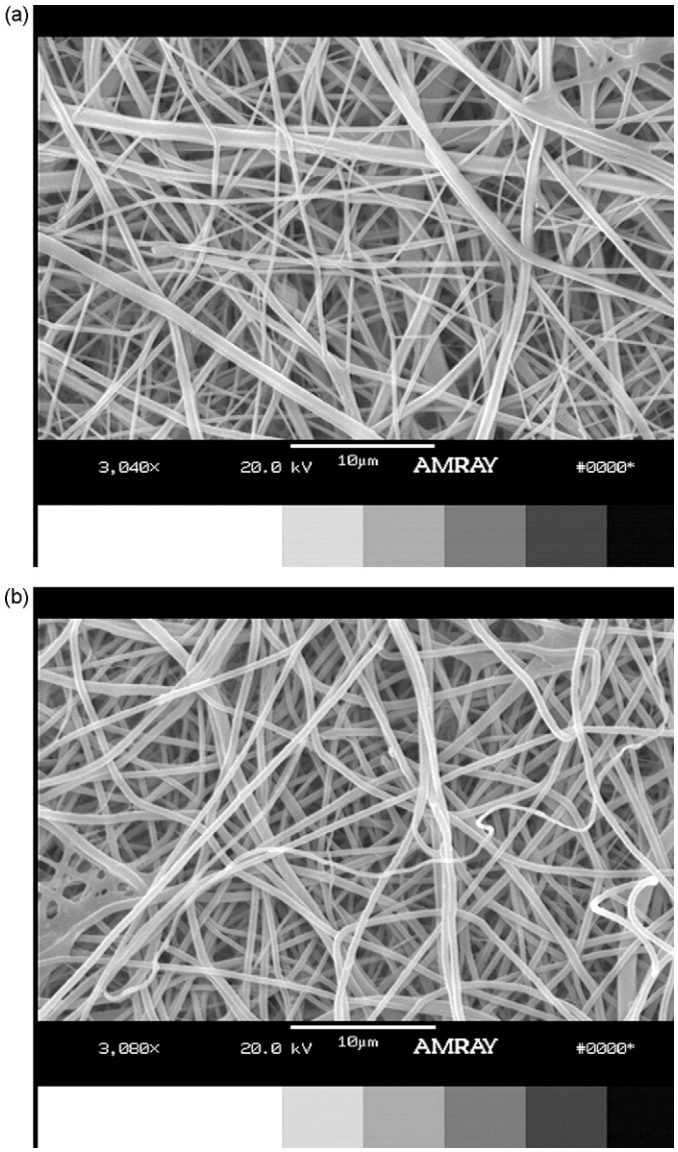

Fig. 3. SEM images of (a) DS electrospun scaffold and (b) SS electrospun scaffold.

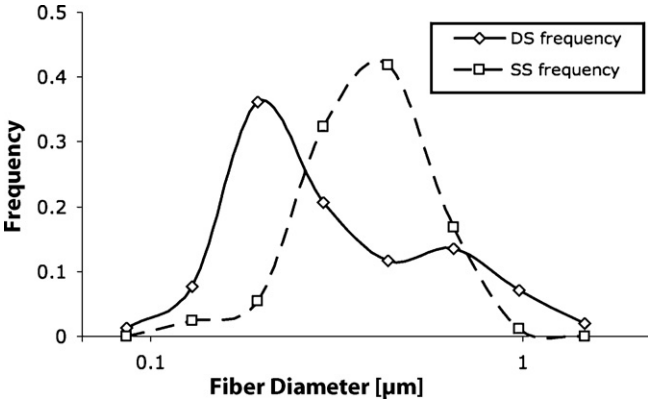

Fig. 4. Distribution of fiber diameter in dual-spinneret scaffold versus single spinneret scaffold, note the use of a logarithmic axis.

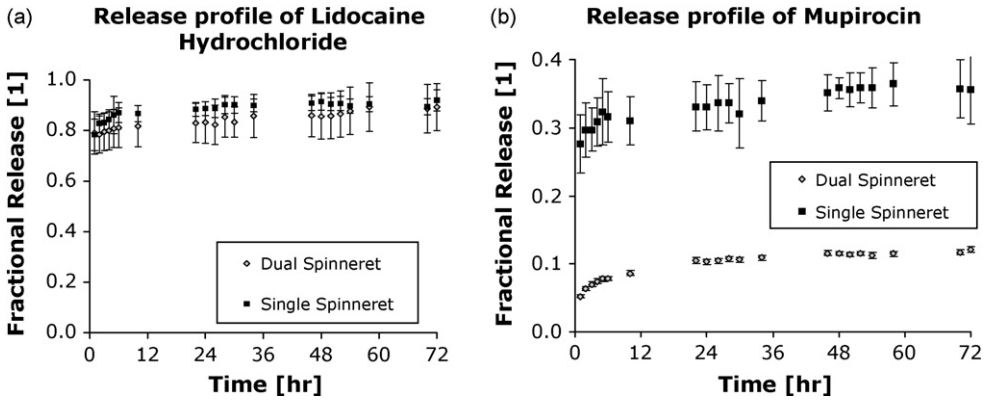

Fig. 5. Release profiles of lidocaine hydrochloride and mupirocin incorporated in PLLA and electrospun by the DS or SS technique. (a) LH eluted from DS (♦) and SS (■) scaffolds demonstrate a burst release followed by a plateau. (b) Mupirocin eluted from DS (♦) and SS (■) scaffolds demonstrate different magnitudes of burst release followed by a slow release. Error bars indicate 95% confidence interval, $n = 5$.

DS and SS scaffolds, which had nearly identical release profiles as the PLLA swelled with water and the drug diffused into the buffer. The release profiles of the four curves from 1 to 72 h were similar to that predicted by Siepmann et al. (1999) for diffusion from a cylindrical construct (Siepmann and Peppas, 2000).

3.3. Differential scanning calorimetry and mechanism of release

DSC of fiber scaffolds produced by the DS and SS techniques provides insight into the causation of these release profiles. Fig. 6 depicts the heat flow into fiber scaffolds as they were heated through the glass transition of the polymer and the melting points of both mupirocin (77–78 °C) and LH (74–79 °C). The electrospinning procedure causes partial alignment of the polymer chains. Hence, after an endotherm associated with the glass transition, an exotherm was observed; this was due to a decrease in the alignment of the PLLA chains and an increase in polymer crystallinity. This effect is clearly depicted in the DSC of fibers with only mupirocin (solution A, solid line). An endothermic peak for the melting of mupirocin crystals was not observed, so the mupirocin is thought to be uniformly distributed in the PLLA fiber. The DSC trace for the DS electrospinning of solutions A and B, on the other hand, was characterized by a large endotherm at 73 °C, associated with the melting of the LH crystals. As the crystals within the PLLA matrix are not pure, the melting point was lower than the reported range

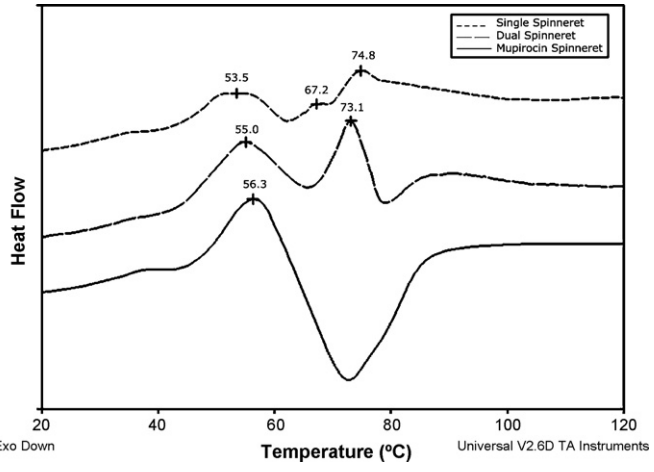

Fig. 6. DSC thermograms of mupirocin only (—) showing no drug crystallization, DS fiber scaffold (– – –) with no mupirocin crystals, and SS fiber scaffold (- - -) indicating lidocaine hydrochloride and mupirocin crystallization in the polymer domains.

of 77–78 °C. Scaffolds produced by SS electrospinning of solution C had two melting points, indicating that both mupirocin and LH crystals existed within the scaffold.

The DSC data demonstrated that the DS electrospinning technique produced one population of fibers with a homogenous distribution of mupirocin throughout the PLLA matrix and a second population of fibers with crystallized LH. In contrast, when both drugs were electrospun by the traditional SS apparatus, there is a possibility that the polymer matrix did not have the capacity to hold both LH and mupirocin homogeneously within its structure, so both drugs crystallized. In drug elution, PLLA quickly absorbs water, and the crystalline drug is released in a burst-release fashion. For this reason, a burst release of LH was observed in both the DS and SS fibers, but the undesirable burst-release of mupirocin was only observed from the SS electrospun fiber scaffold.

Crystallization of drugs in electrospun polymer fibers as a function of polymer content has been observed previously. Verreck et al. (2003) have shown that in the DSC profile of electrospun fibers, the peak associated with crystalline itraconazole disappeared with an increase in the polymer content from which the drug was electrospun. Phase separation is considered the cause of such crystallization. Hydrochloride salts of drugs have been known to crystallize out of electrospun fibers. Tetracycline hydrochloride was observed to have crystallized out of PLA as evidenced by SEM, even with only a 5-wt% drug loading (Kenawy et al., 2003). Likewise, 1.6 wt% doxorubicin hydrochloride crystallized out of PLLA due to poor solution compatibility (Zeng et al., 2005). In our case, LH also seems to have separated out in a similar manner leading to the burst release profile.

Lipophilic drugs, on the other hand, have not been observed to crystallize out of lipophilic polymers. As seen by Zeng et al. (2005), 15 wt % paclitaxel, a lipophilic drug, did not crystallize out of PLLA. Likewise in our studies, mupirocin, with a log P value of 3.44 ± 0.48 (calculated by log P DB software, ACD labs, Toronto, Canada), is a lipophilic drug and remains confined to the PLLA with no burst release, even at a drug loading of 7.5 wt% in the DS fiber scaffolds. In comparison, DSC analysis of SS fibers with half the mupirocin loading (3.75 wt%) demonstrated crystallization of the drug in PLLA. This could be due to displacement from the PLLA matrix with a high LH loading. Thus, the presence of a hydrophilic salt probably enabled a burst release of a lipophilic component from a lipophilic domain.

3.4. Bacterial susceptibility tests

Fabrication and sterilization processes can affect the bioactivity of a compound. The modified Kirby–Bauer method (Boyle

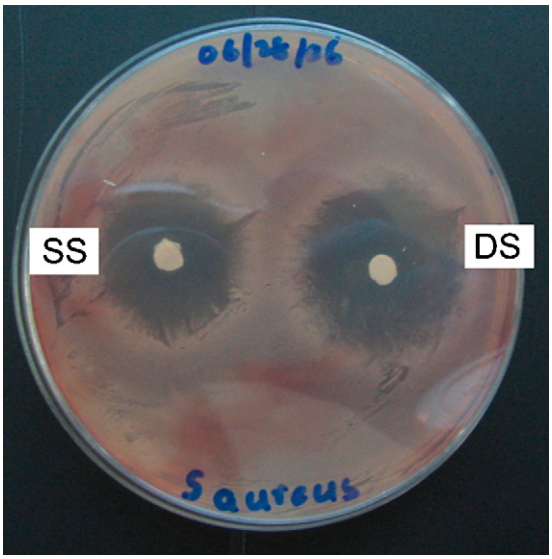

Fig. 7. Bacterial growth inhibition by modified Kirby–Bauer disc method. Scaffold sections of SS and DS were incubated at 37 °C for 6 h on agar plates cultured with *Staphylococcus aureus* and sprayed with MTS reagent. Zones of inhibitions were 22 mm and 26 mm for SS and DS, respectively.

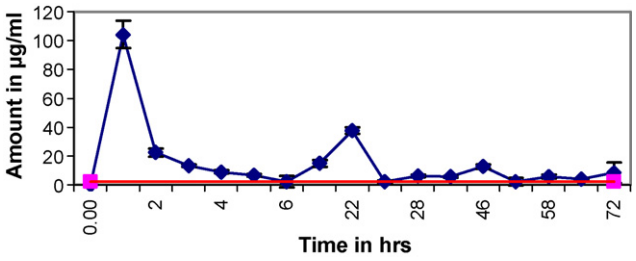

Fig. 8. Amount of mupirocin released in Franz cell receptor between each time point as compared to the MIC (2 µg/mL). A scaffold electrospun by the DS technique of area 4.5 cm^2 was used.

et al., 1973) was used for determining bacterial susceptibility to mupirocin eluted from ethylene oxide sterilized electrospun scaffolds. The use of an MTS reagent enabled rapid and clear delineation of the zone of inhibition (Fig. 7). A 26-mm diameter zone was observed for *Staphylococcus aureus* isolates for the DS scaffold and a 22-mm diameter zone for the SS scaffold over 6 h; a zone diameter of 22–27 mm is considered acceptable for a 5-µg mupirocin disc (Finlay et al., 1997). In our case, the DS and SS scaffolds released approximately 8 µg of mupirocin within 6 h, according to the release profiles and drug content from NMR results. The zone diameters obtained for these scaffolds imply that the bacterial colony is susceptible to mupirocin released from the scaffold. The zones were maintained for at least 6 days after inoculation, proving that the scaffolds release significant amounts of drug throughout the course of therapy. Neither electrospinning nor ethylene oxide sterilization seem to have affected the antibiotic activity of mupirocin. Ethylene oxide sterilization was chosen for this study, for previous work from our group (Hooper et al., 1997) which indicates that gamma-irradiation dramatically reduces the molecular weight and increases the degradation rate of PLLA films. In contrast, samples sterilized by ethylene oxide retained 97% of their MW, had no detectable changes in surface chemistry, yield strength, percent strain at break or degradation rate.

The minimum inhibitory concentrations (MIC) for all the strains of mupirocin-sensitive bacteria range from 0.06 to 0.5 µg/mL (Sutherland et al., 1985; Finlay et al., 1997). The amount released at each time point in our DS scaffold was significantly higher than the MIC for the entire sampling period (Fig. 8). Mupirocin does not form a deposit in the skin and is metabolized into inactive monic acid. Considering that the amount of drug released by the scaffold exceeds the MIC and that mupirocin does not accumulate in the skin, it is safe to assume that the dressing will be able to maintain tissue levels of mupirocin sufficient to prevent infections in the wound for at least 3 days.

The slow release of mupirocin from the DS fibers ensured that the drug is released in a fashion able to satisfactorily maintain MIC levels. This prevented dose dumping at any point in the DS fiber release profile, unlike the initial hours for the SS scaffold. This is important, as excess drug can be responsible for developing antibiotic resistance and adverse events following systemic absorption. The polymeric membrane can be used as a wound healing scaffold for more than 3 days if required, for the remaining drug in the scaffold ensures continued mupirocin release and antibiotic activity. Application of commercially available ointment containing mupirocin is recommended for up to 10 days for treatment of skin lesions (Source: Bactroban® Monograph), with a limit of 120 days on usage set by the Health and Recovery Services Administration.

3.5. Cell viability, attachment, and proliferation

Wound-healing scaffolds should be able to support cell proliferation and viability for fast wound healing. Electrospun PLLA has been seen to support the growth of cells such as neural stem cells (Yang et al., 2005) and cardiac myocytes (Zong et al., 2005). It is possible that inclusion of drugs may alter the cell proliferation *in vivo* (Martinsson et al., 1993). However, Drucker et al. (1998) found that the histopathologic appearance of wounded tissues infiltrated with lidocaine did not vary consistently in relation to collagenization, edema, or acute and chronic inflammatory processes. Neither did lidocaine substantially alter wound healing or the breaking strength of the wounds.

We examined the cytocompatibility of electrospun nanofibers and initial cell adhesion and spreading. The dressing was seeded with fibroblasts, and calibrated MTS assays were performed to study adhesion and viability achieved on days 3, 4, and 6. Human dermal fibroblasts showed a significant attachment to the scaffold at day 3 as compared to controls. The number of viable cells attached increased 3.2 times from day 3 to day 4 and 1.3 times between day 4 and day 6. The rate of cell proliferation likely decreased at day 6 because of the reduced area available for spreading and attachment. The SEM micrographs showed fibroblast attachment at each timepoint. The data implies that the drugs in the matrix do not inhibit cell proliferation and the dressing is able to support healing in addition to providing prophylactic action and pain relief.

4. Conclusions

In this study, it was determined that the dual spinneret electrospinning technique facilitated the fabrication of a polymeric dressing with dual drug release kinetics that could have potential application for wound therapy. An anesthetic, LH, crystallized in the PLLA matrix and was eluted through a burst release mechanism. This action would be useful if this mat was used as a wound dressing for immediate relief of pain. Mupirocin, an antibiotic, was released simultaneously with LH through a diffusion-mediated mechanism for extended antibiotic activity. The dual spinneret electrospinning technique was able to achieve the required dual release profiles by allowing LH to crystallize in other PLLA fibers and maintaining mupirocin in the non-crystallized form within the PLLA matrix. The

traditional single spinneret technique could not prevent the crystallization of mupirocin in the presence of 40 wt% LH. Electrospinning and ethylene oxide sterilization did not affect the antibiotic activity of mupirocin, as evidenced by the fact that the scaffold retained its antibacterial activity *in vitro*. However, more comprehensive studies are required to further support this result. In conclusion, we have been able to deliver therapeutic concentrations of two drugs useful in wound healing for a 3-plus day therapy through a dressing. However, these results need to be substantiated by further *in vivo* studies for the real-world application of the scaffold mat as a wound dressing.

If one desires to achieve an immediate release of a lipophilic drug from a lipophilic polymer, our findings suggest that the addition of a hydrophilic salt could be used to manipulate the release profile. This minimizes the restrictions on the choice of polymer for drug delivery; for instance, a burst release of a drug can be obtained from both hydrophilic and lipophilic polymers. In future work, a sustained release coupled with complete drug elution within three days and scaffold degradation within 10 days could be pursued through the use of rapidly degrading, surface-eroding polymers such as polyanhydrides, rather than PLLA. This could alter the kinetics, allowing drugs like mupirocin to be eluted in a linear, rather than logarithmic profile.

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