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RESEARCH PAPER

Preparation, Characterization, and Release of Amoxicillin from Electrospun Fibrous Wound Dressing Patches

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ABSTRACT

Purpose To produce electrospun polymeric fibrous wound dressing patches that can release the antibiotic drug amoxicillin in a controlled manner.

Methods Poly(D,L-lactide-co-glycolide) acid (PLGA) fibrous dressings with entrapped amoxicillin were produced by electrospinning. The morphology and successful entrapment of amoxicillin in the PLGA fibrous dressings were validated by scanning electron microscopy (SEM) and Fourier Transform Infrared (FTIR) spectroscopy. The rate of drug release from the dressing patches was measured in various media for a period of 21 days using UV spectroscopy.

Results PLGA fibres entrapping amoxicillin were collected for 300 s and then cut to form square patches with an average weight of 55 mg. Each dressing patch contained ~2 mg of amoxicillin. The mean fibre diameter was 2.2 ± 0.4 μm. The drug release from the PLGA dressings was found to be different for each medium during the 21-day release period with the highest and lowest concentration of drug released observed when the dressings were immersed in simulated body fluid (SBF) and phosphate buffered saline (PBS), respectively.

Conclusions The release profiles obtained in this study and the well-established biocompatibility of PLGA indicate that the fibre-based patches with entrapped amoxicillin fabricated in this work are very well suited for applications in wound healing and infection control.

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INTRODUCTION

The biomedical and pharmaceutical industries worldwide are showing a great interest in fabricating advanced wound dressing products that target different aspects of wound healing and tissue regeneration processes (1). The goal of a wound dressing is to protect the wound from environmental threats and penetration of microorganisms whilst simultaneously promoting the process of tissue regeneration (2,3). In the past, traditional dressings such as bandages, gauzes and cotton wool were used to offer protection against bacteria, but when the outer surface of these dressings becomes moistened then protection is lost. Also traditional dressings allow moisture evaporation leading to adhesion of the dressing to the wound, causing pain and discomfort when it is removed (2,4). This has been the stimulus for the development of a wide range of new advanced multifunctional wound dressings made from biocompatible and biodegradable materials with or without embedded bioactive agents (e.g. drugs, growth factors) such as hydrogels, proteins and polymers (5–8).

Complex biodegradable and biocompatible fibre-based wound dressings (2,5), membranes (9) and scaffolds (10) offering antibiotic delivery have been reported recently to promote fast wound healing and tissue regeneration. The fibrous structures have a high surface area-to-volume ratio leading to efficient drug release, absorbency, pliability, high permeability, cell attachment and acceleration of the migration and proliferation phases of wound healing (2,10,11). They also temporarily substitute and mimic the native extracellular matrix and can act as carrier systems of antibacterial agents and other growth factors and cytokines that need to be released in a controlled manner (6,12).

Many techniques have been used to produce fibre-based dressings such as wet and dry spinning (13), melt spinning (14) and centrifugal spinning (15). The high processing costs of these procedures, the time required and the need for solvents and additives that are not fully biocompatible, lead to limited production robustness and to low controllability and biological stability of the bioactive agents (16). In contrast, electrospinning is a simple, inexpensive and well-known electrohydrodynamic (EHD) technique that can produce uniform polymeric multifunctional fibres on the nano/micro scale in a single step, under ambient conditions and with no requirements for surfactants or other additives (17,18).

In electrospinning, a large electrical potential difference charges a flow of viscoelastic liquid, supplied to a nozzle under precise control. A charged droplet initially forms at the nozzle tip. When the surface charges on the droplet interact with the electric field and the viscoelasticity of the liquid is sufficient to prevent droplet break-up a cone-shape jet is formed at the nozzle tip which accelerates towards the collector. The jet is subjected to aerodynamic, inertial, rheological and gravitational forces, as well as solvent evaporation (19,20). Thus, the jet thinning-evaporation phenomenon of the electrospinning process is ideal for the preparation of fibrous products.

The main challenge of producing fibre-based wound dressings with incorporated drugs is to overcome the rapid release of the drug from the dressing. The drug release profile should provide sufficient drug release at the beginning of the wound healing phase in order to respond to the high risk of infection from microorganisms introduced during the initial trauma, followed by effective and controlled release of the drug, over the period required to prevent latent infection (2). To meet this challenge a low molecular weight hydrophilic drug can be entrapped within a biodegradable hydrophobic polymer in order to delay water penetration and outward drug diffusion or boosting of drug bonding to the carrying matrix (21,22). The drug attached to the fibre surface provides an appropriate initial release rate followed by systematic controlled release of the drug from the polymer caused mostly by degradation of its matrix (23).

Poly(D,L-lactide-co-glycolide) acid (PLGA) was chosen for this work because it is a biocompatible and biodegradable polymer that has been used in many previous studies for controlled drug release (22,24). It is safe for use in the human body as it induces a minimal inflammatory response and biodegrades through hydrolysis to lactic and glycolic acids that are also biocompatible (12,25). The degradation process occurs by hydrolysis or biodegradation through cleavage of the backbone ester linkages into oligomers and, finally monomers. Drug release is mainly due to uniform bulk degradation of the matrix where the water penetration into the matrix is higher than the rate of polymer degradation (26). The degradation rate of PLGA depends on various conditions such as the molecular weight and the crystallinity of PLGA, the medium pH and the ratio of glycolic to lactic acid (27,28). Higher concentrations of lactic acid in the PLGA copolymer and higher molecular weight produce slower degradation rates, with the exception of the 50:50 ratio of polylactic acid/polyglycolic acid (PLA/PGA) used for the experiments, which exhibits the fastest degradation. Furthermore, PLGA shows significant mechanical strength and flexibility due to its fairly rigid chain structure, thus it can be used for drug delivery devices (24).

Amoxicillin was used as a drug for this work because it is considered to be one of the most important antibiotics of the penicillin family and has a broad antimicrobial activity, bactericidal effect and a high therapeutic index. It is also relatively cheap, is included on the World Health Organisation's list of essential drugs, and safe if inserted in the human body either by oral, intramuscular or intravenous administration. It is also extensively distributed in body tissues and fluids with acceptable levels of antibacterial activity in most of them and it is approved by the Food and Drug Administration (FDA) for use in drug delivery systems. The disadvantages of amoxicillin are its short biological half-life of 1 h when inserted in serum (almost the same for every form of administration) and also that it is unstable when inserted in aqueous and buffered solutions (29,30). Studies of amoxicillin degradation in dilute aqueous solutions and other media have been carried out by many researchers (31,32). At constant pH, temperature and ionic strength, degradation follows first order or pseudo first order kinetics (30). The degradation rate of amoxicillin in aqueous solutions and buffers can vary within different pH values (1.0–9.0) giving a U-shape curve. Amoxicillin is most stable over the pH range 4.0–7.0, with the optimum pH value for maximum stability being ~6.0 (28,29,31). Degradation of amoxicillin gives amoxicilloates (amoxicillin impurities) and piperazine-2,5-dione as decomposition products (33).

The first aim of this work was to increase the effectiveness of amoxicillin by entrapping it in a PLGA hydrophobic fibrous matrix using the electrospinning process and then studying its stability and controlled release for 21 days in three different media. The second aim of this work was to produce a wound dressing patch which is biocompatible with the human body and to be able to spray it *in situ* on to a patients' wound, in parallel with our novel portable multi-needle spray-gun EHD technology (34).

MATERIALS AND METHODS

Materials

PLGA (co-polymer 50:50, Resomer RG503H, molecular weight: 33,000 g mol^{-1}) was purchased from Boehringer Ingelheim, (Ingelheim, Germany). Acetone, phosphate

buffered saline (PBS) and amoxicillin (molecular weight: 365 g mol^{-1}) were purchased from Sigma Aldrich (Poole, UK). Acetone was used as a solvent to prepare a 25% w/w PLGA solution by dissolving it with a magnetic stirrer in a volumetric flask until full dissolution of the polymer occurred. Then, 10 g of PLGA solution 25% w/w and 0.1 g of amoxicillin were added to a glass vial and stirred for 300 s at low speed with a magnetic stirrer until a homogenous suspension was formed. Simulated body fluid (SBF) was prepared as proposed by Kokubo and Takadama (35).

Characterization of Solutions

The physical properties of the solutions play an important role in EHD processing. The polymer solutions were characterized for their density, surface tension, viscosity, electrical conductivity and pH. Density was measured with a standard 25 ml density bottle. A Kruss tensiometer was used to measure the surface tension (standard Wilhelmy's plate method) and a Brookfield DV-III Ultra Rheometer suitable for small volumes, with an SC4-18 spindle (Brookfield Viscometers Ltd, Harlow, UK) was used to measure the viscosity. The electrical conductivity and pH of the solutions were determined using a handheld pH/conductivity meter PC5000H, pHenomenal (VWR International Ltd, Lutterworth, UK). All the instruments were calibrated before use by following the instruments calibration guide and all experiments were performed at the ambient temperature (22°C), pressure (101.3 kPa), and relative humidity (45–60%). The measured physical properties of the liquids used in the experiments are shown in Table I.

Forming of Microfibres

The experimental setup for producing the PLGA dressing patches with entrapped amoxicillin is shown in Fig. 1. It consists of a single brass needle, a 'PHD ultra' high precision syringe pump (Harvard Apparatus Limited, Edenbridge, UK) to control the flow rate of the suspension to the needle, a high precision voltage generator connected to the needle (Glassman Europe Limited, Bramley, UK) and a high speed camera (JVC TK-C1481BEG, Weinberger AG, Dietikon, Switzerland) to monitor the process. The needle has outer and inner diameters of 1,250 μm and 850 μm, respectively. A 10 ml volume capacity Becton-Dickinson (Becton, Dickinson and Company, Oxford, UK) syringe was loaded to the syringe pump containing the 25% w/w PLGA-amoxicillin suspension and silicone tubes were used to transfer it to the needle.

The processing conditions were optimised in order to give uniform and reproducible fibres. The voltage was set to 14 kV and the flow rate of the suspension was fixed at 300 μl/min. Also 10 ml of 25% w/w PLGA solution (without drug) was used to produce fibre-based dressings as a control. To prepare the dressings without drug the voltage was set to 12 kV and the flow rate to a fixed value of 300 μl/min. The syringe pumps were set to give a continuous solution infusion to the needle for 300 s, thus giving a total output solution volume from the needle tip of 1.5 ml (the infusion time of 300 s was set to start after the solution reached the nozzle tip). The working distance between the needle tip and the collector (aluminium foil) for both experiments was set to 120 mm. The ground electrode was connected through a crocodile clip to the aluminium foil, where the fibre-based dressings were collected. Because the aluminium foil was connected with the ground electrode, it was acting as a fibre attractor; hence the fibres were deposited uniformly on it producing a fibre dressing with a uniform thickness without the need of relative movement between the nozzle and collector.

Fibre Characterization

The fibre diameter and surface morphology of the fibres were studied by optical microscopy (Micropublisher 3.3 RTV, 3.3 megapixel CCD Color-Bayer Mosaic, Real Time Viewing camera, MediaCybernetics, Marlow, UK) and scanning electron microscopy (Hitachi S-3400N and JEOL JSM-6301F field emission scanning electron microscopes, SEM). The fibre-based dressings were collected and left to dry for 48 h. Then they were vacuum-coated with gold for 120 s before obtaining SEM images at an acceleration voltage of 3–5 kV. Analysis of the dressings was carried out using the Image-Pro Insight software (MediaCybernetics Ltd., Marlow, UK).

FTIR Studies

Fourier Transform Infrared (FTIR) spectroscopy was used to confirm the presence of amoxicillin in the PLGA fibres. These studies were performed for the PLGA polymer (dissolved in acetone), amoxicillin powder and PLGA/amoxicillin dressing by mixing 50 mg of each with potassium bromide

Table I Physical Properties of Media Used in Experiments. All % Refer to Weight and the Liquids Property Values Were Calculated in Triplicate

Material or polymer solution	Density (kg m^{-3})	Viscosity (mPa s)	Surface Tension (mN m^{-1})	Electrical Conductivity (μS m^{-1})
PLGA 25%	1210 ± 10	53.8 ± 0.3	27.5 ± 0.7	0.8 ± 0.1
PLGA 25% + Amoxicillin	1230 ± 10	54.4 ± 0.4	26.7 ± 0.8	1.0 ± 0.1

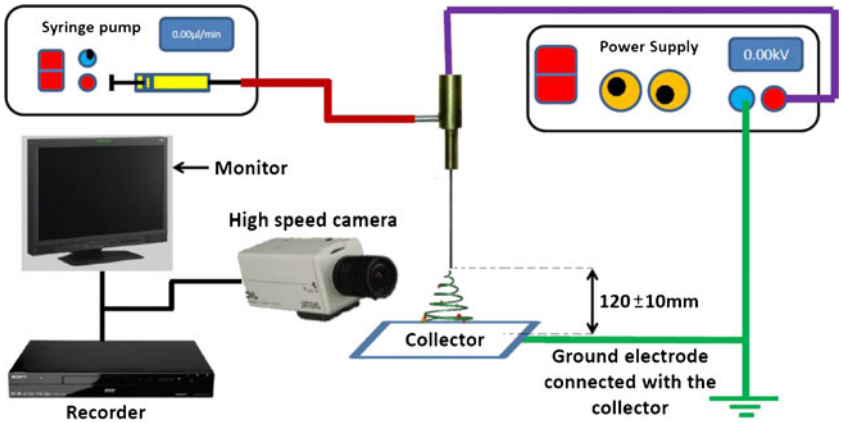

Fig. 1 Schematic of the EHD setup used for the experiments.

(KBr) and compressing them to form pellets. The pellets were inserted into the FTIR spectrometer (Perkin-Elmer 2000 FTIR spectrometer, UK) and 30 scans with a resolution of 4 cm^{-1} in the 4,000–370 cm^{-1} region were carried out for each sample.

UV Spectroscopy

To determine the rate and amount of drug released from the fibre-based dressing patches, UV spectroscopy measurements were carried out using a Perkin Elmer Lambda 35 UV–vis spectrophotometer (Cambridge, UK), in the 200–500 nm wavelength region for a period of 3 weeks. Calibration curves were prepared for the concentration range 0.001–0.15 mg/ml (1–150 ppm) of amoxicillin dissolved in distilled water, PBS and SBF, respectively. Although amoxicillin absorbs light also at a wavelength of 273 nm, 230 nm was chosen as the reference wavelength for the experiments because it has a higher extinction coefficient and thus a much stronger and clearer absorbance signal could be achieved from the UV spectrophotometer (amoxicillin data sheet was supplied by Sigma Aldrich). Furthermore the UV spectra of distilled water, PBS and SBF were also compensated for in the data analysis. A similar method has been used previously by other researchers to investigate the release mechanism of amoxicillin and other drugs from polymeric matrices (29,36).

Drug Entrapment

To determine the concentration of amoxicillin entrapped in each dressing patch, 3 fibre based patches were added to 100 ml glass vials which were first filled with 10 ml of acetone to dissolve the hydrophobic polymeric patch and release the drug and then with 90 ml of distilled water to dissolve amoxicillin which was released from each patch into the glass vial. The solutions were stirred for 120 s and then 3 ml from each vial were filtered with 0.45 μm cellulose acetate membranes before a UV measurement was taken. A dressing patch without drug was also used for control and correction purposes.

The drug entrapment efficiency, yield and drug loading percentages were calculated using the following equations:

$$Yield\ (\%) = \left[\frac{Weight\ of\ EHD\ product}{Weight\ of\ PLGA + Weight\ of\ amoxicillin}\right] \times 100 \quad (1)$$

$$Drug\ loading\ (\%) = \left[\frac{Weight\ of\ drug\ in\ fibre\ dressing}{Weight\ of\ fibrous\ dressing\ loaded\ with\ drug}\right] \times 100 \quad (2)$$

$$Drug\ entrapment\ efficiency(\%) = \left[\frac{Weight\ of\ drug\ in\ the\ fibres}{Weight\ of\ drug\ used\ in\ the\ formulation}\right] \times 100 \quad (3)$$

In Vitro Amoxicillin Release

To study the drug release kinetics in different media, the fibre-based patches with or without amoxicillin were removed from the aluminium foils and were cut into ~900 mm^2 square shape with an average weight of 55± 5 mg each. Theoretically each cut-dressing was loaded with 2.2±0.1 mg of amoxicillin.

In total 15 patches were prepared and placed in 3 different media: distilled water, PBS and SBF. The twelve patches loaded with amoxicillin were folded (4 fibre-based layers; 2 mm thickness each layer; 225 mm^2 square each layer) so they could be inserted into 12 glass vials of 25 ml volume with sealing caps. To the first 3 of these 12 vials 10 ml of distilled water was added and to the second 3, 10 ml of PBS was added. To the remaining 6 vials, 10 ml of SBF was added.

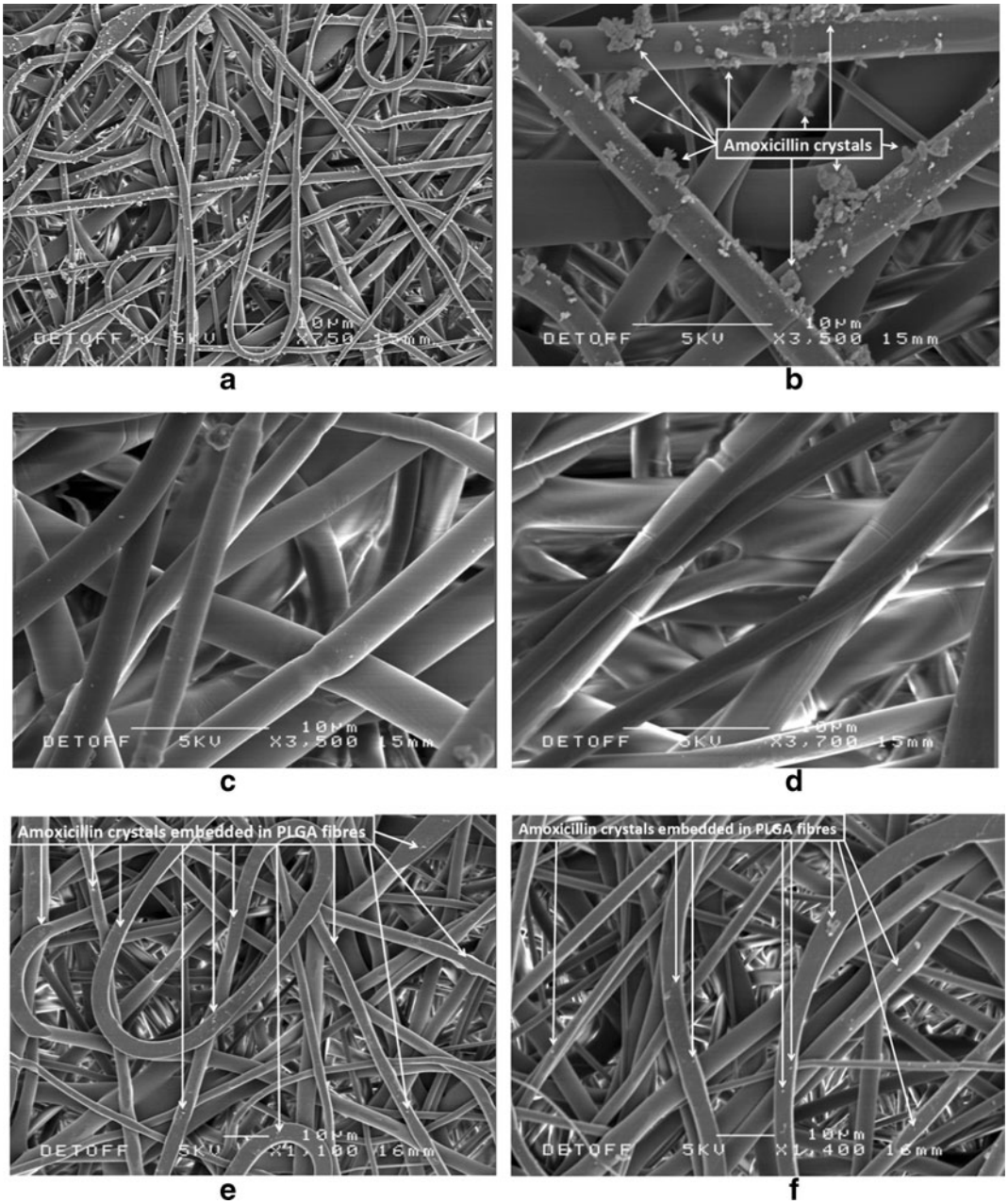

Fig. 2 SEM micrographs of electrospun PLGA microfibres (**a**, **b**) with and (**c**, **d**) without amoxicillin. The crystals attached to the fibre surfaces shown in (**b**) with arrows are amoxicillin. Micrographs (**e**) and (**f**) show sectioned PLGA fibres where the white areas shown with *arrows* indicate the amoxicillin embedded in them.

The 3 patches without drug were added to 3 different vials containing 10 ml of distilled water, PBS and SBF, respectively.

The 15 glass vials containing the patches were kept in an incubator at a constant temperature of 37 ± 1°C (similar to body temperature). At discrete time intervals the samples were removed from the incubator and 3 ml from each glass vial were extracted (and subsequently returned to the same vial after UV measurement) to measure absorbance using the UV spectrophotometer. In order to eliminate excessive unwanted PLGA and amoxicillin degradation products generated in the solutions, 0.45 μm cellulose acetate membranes were used to filter the solutions before a UV measurement was taken. In three of the six glass vials containing SBF, half of the SBF volume (5 ml) was replaced with new SBF solution after each UV measurement, in order to keep the SBF liquid fresh. In the other vials the solutions were not replaced. As mentioned earlier the amount of amoxicillin released in each media from the dressings was determined by interpolation of absorbance values in the calibration curves. These studies were performed in triplicate and the average values and standard deviations

were calculated. The pH value of each solution in the glass vials was also measured before and periodically after the dressing patches were immersed into the solutions.

RESULTS AND DISCUSSION

Morphology of Fibre-Based Dressings

The surface morphology of the fibre-based dressings was studied by SEM. Figure 2 shows SEM micrographs of electrospun PLGA micro-fibres with (Fig. 2a and b) and without (Fig. 2c and d) amoxicillin, taken under different magnifications. Using electrospinning and under the fixed conditions mentioned in the experimental details, PLGA fibres with entrapped amoxicillin were produced having a mean diameter size of 2.2 ± 0.4 μm (mean of 200 fibres). In Fig. 2b amoxicillin crystals can be seen on the smooth surface of the fibres. To check if they are actually amoxicillin crystals, dressing patches without drug were studied by SEM (Fig. 2c and d) and no evidence of crystals were found on the fibre surfaces. To also obtain evidence of drug entrapment within the PLGA, fibres were sectioned along their length with a surgical blade. Figures 2e and 3f show the sectioned fibres, where the white spots indicate the presence of the drug. To study the thickness of the PLGA dressing patches, two samples were positioned vertically in the SEM chamber in order to retrieve cross-sectional images (Fig. 3). The thickness of each fibre-based dressing was found to be ~2 mm.

Morphological modifications of the PLGA fibre-based dressings after incubation in distilled water, PBS and SBF were also noticed during the drug release studies. This is due to the *in vitro* degradation of the PLGA copolymer (23,28). Figure 4 shows micrographs of the changes in the patch morphology after being immersed for 25 days in distilled water (Fig. 4a and b), PBS (Fig. 4c and d) and SBF (Fig. 4e and f). It was observed that the "properties" of the PLGA dressing patches changed after they were immersed in different media. When inserted into PBS they became stiff and brittle and when they were inserted into distilled water or SBF they transformed into a sponge-like structure while their strength decreased and their elasticity increased. Detailed studies on PLGA degradation were not carried out in the present work. However some insight into this can be gained from previous work. Mollo *et al.* (29) studied the degradation rate, mass loss and total drug released from PLGA (50:50 ratio of PGA/PLA) discs with or without amoxicillin for a period of ~40 days when inserted into buffered solutions and showed that the presence of amoxicillin had little impact on the polymer degradation rate. Also according to Shah *et al.'s* (23) results, the mass loss due to degradation of the PLGA for dressing patches after immersion for 21 days into distilled water and PBS, was ~35% (w/w). The mass loss for dressing patches inserted into SBF (replaced with fresh media) solution according to Qi *et al.* (37) was 60% (w/w).

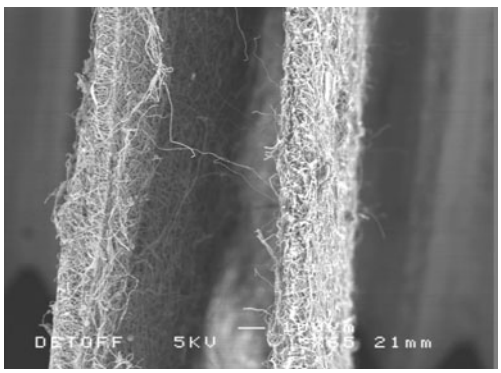

Fig. 3 Cross sectional image of the fibre-based dressings. Scale bar is 500 μm.

Entrapped Amoxicillin

The objective of the FTIR analysis was to verify the existence of entrapped amoxicillin in the fibre-based dressings after their production. In Fig. 5 the FTIR spectra of PLGA, amoxicillin and PLGA fibre-based dressing loaded with amoxicillin are shown.

The FTIR spectrum of PLGA/acetone shows a characteristic peak at 1,781 cm^{-1} (C=O stretching vibrations from ester bond), a peak at 1,464 cm^{-1} (wagging vibrations from saturated C-H bond) and a broad peak at 1,238 cm^{-1} (C-O vibrations from ether groups), which are characteristics features of PLGA (38). Major peaks of amoxicillin are observed at 3,175, 3,366 and 3,458 cm^{-1} (amide N-H and phenol OH stretching), 3,000 cm^{-1} (benzene ring C-H stretching), 2,050 cm^{-1} (C≡C and C≡N stretching), 1,789 cm^{-1} (β lactam C=O stretching), 1,692 cm^{-1} (amide I, C=O stretching), 1,520 cm^{-1} (benzene ring C=C stretching) and 1,490 cm^{-1} (N-H bend C-N stretch combination band) (39).

Characteristic peaks of amoxicillin are also present in the FTIR spectrum of the fibre based dressing loaded with amoxicillin with some broadening and reduction in intensity. This indicates that amoxicillin was successfully entrapped in the PLGA polymer.

In Vitro Release of Amoxicillin

For the amoxicillin release studies, calibration curves based on known concentrations of amoxicillin were plotted at 230 nm and for each medium (distilled water, PBS and SBF) in which the patches loaded with amoxicillin were immersed (Fig. 6a, b and c). As shown in Fig. 6d, the calibration curve for each medium was very similar. The yield and drug loading percentage were calculated according to the Eqs. (1) and (2), respectively, and were found to be 52% and 4%, respectively.

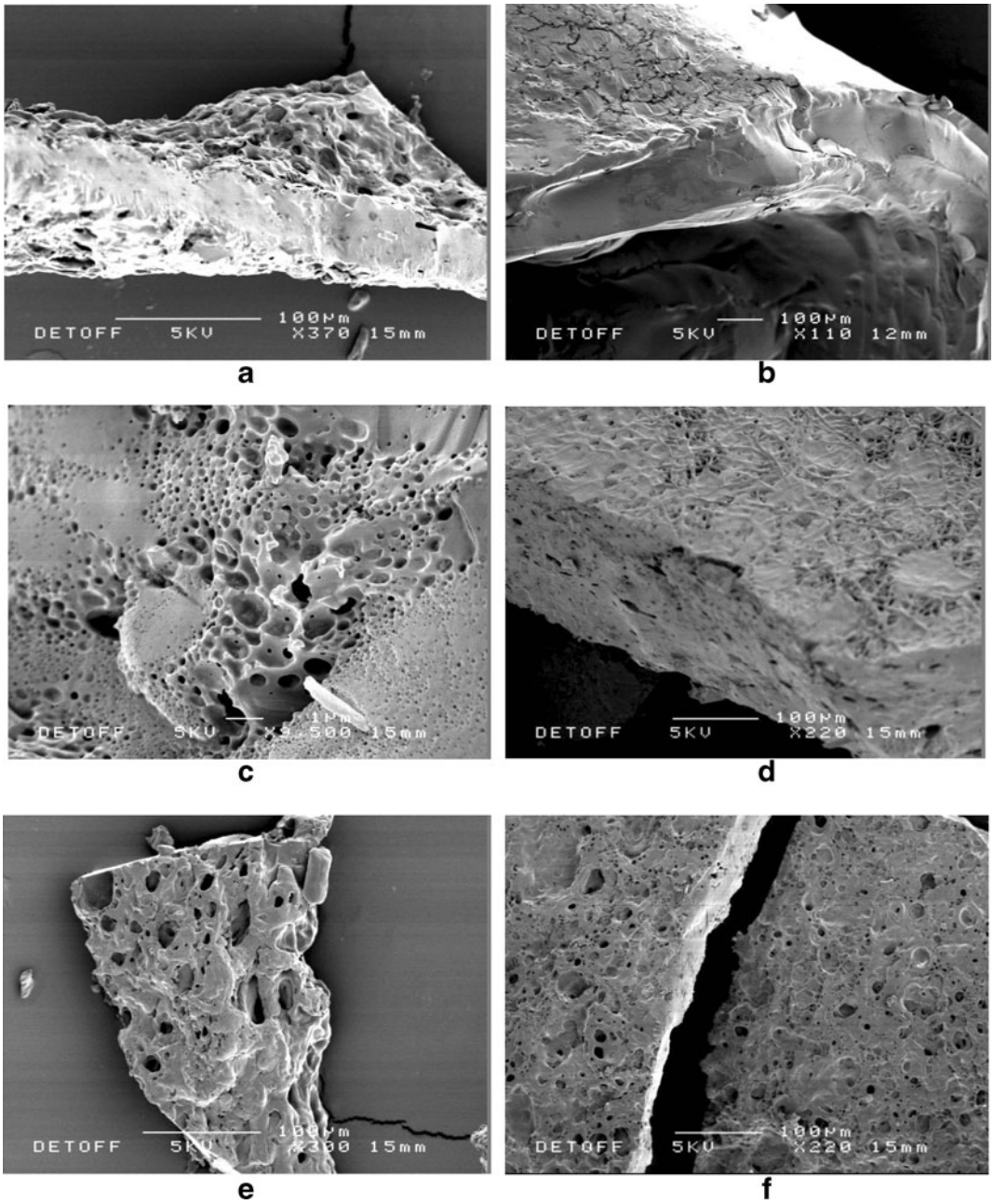

Fig. 4 SEM micrographs of degraded PLGA dressings loaded with amoxicillin and immersed in (**a**, **b**) distilled water, (**c**, **d**) PBS and (**e**, **f**) SBF after 25 days.

The entrapment efficiency (from Eq. 3) was found to be ~90%, with <1% attributed to the amoxicillin attached on the fibres surface. Although the PLGA/amoxicillin suspension, which was added into the syringe, was sprayed, collected and dried on the aluminium foil without any signs of drug loss, it is possible that small quantities of drug were trapped on the inner surfaces of the syringe, silicone tube and needle, hence decreasing the percentage of the drug entrapment efficiency. Also, even though the syringe containing the suspension was rotated periodically while placed in the pump to avoid sedimentation, it is also likely that a small amount of drug remained at the bottom of the syringe. Hence, in each fibre-based patch weighing 55 mg, 1.98±0.18 mg of amoxicillin was entrapped in the PLGA fibres. That is equal to 36 μg of amoxicillin drug in every 1 mg of PLGA fibres.

The curves from Fig. 6 were used in preparing the *in vitro* drug release profiles for the dressing patches. The amount of drug released *in vitro* from the fibre-based dressings was determined from the amoxicillin concentration in the release media, plotted as a function of time (Fig. 7). The drug release from the PLGA dressings, which was performed in distilled water, PBS, SBF not replaced with fresh media (SBF (NR)) and SBF replaced with fresh media (SBF (R)), was found to be different for each medium during the 21-

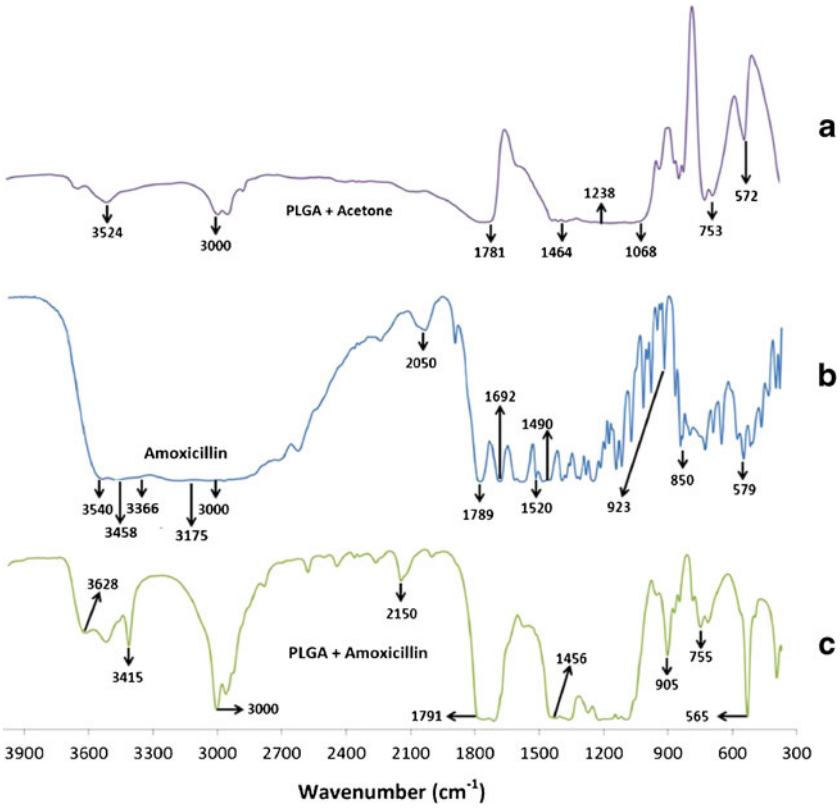

Fig. 5 FTIR spectra of (**a**) PLGA, (**b**) amoxicillin and (**c**) PLGA + amoxicillin fibres.

day release period. After subtracting the noise caused by PLGA degradation products from the UV spectra obtained, it was found that the drug release was higher if the dressing was placed in SBF (R) after every UV measurement was taken. The lowest concentration of amoxicillin release from the PLGA dressings was observed for the PBS solution (Fig. 7a). Figure 7b shows the percentage of amoxicillin released from the dressing patches into the media. After 21 days 68%, 22%, 67% and 81% of the total drug weight, which was entrapped in the dressing, was released into distilled water, PBS, SBF (NR) and SBF (R), respectively. In comparison, most of the studies performed on other similar controlled amoxicillin release products (microspheres, fibrous membranes) do not achieve 100% release, probably due to the chemical instability of amoxicillin in the release media (9,33,40). Although the experiments took place over 25 days, the last 4 days were not included in the experimental analysis because the calibration curves lost their linearity for concentrations higher than 200 ppm (0.2 mg/ml), so the actual amount of amoxicillin released into the media could not be calculated accurately. This was not of great concern however since the major part of the healing process and the highest risk of infection takes place during the first 3 weeks following an injury (1,6).

The drug release from the dressings into the media was characterised by a diphasic drug release, which is due primarily to the properties of PLGA polymer (23). The first release phase (initial burst release during the first day) could be attributed to the drug located on the fibre surface (Figs. 2b and 7a) and that close to the polymer matrix surface. During the second phase, the solution which infiltrated the matrix hydrolysed the polymer into soluble oligomeric and monomeric products thus creating a passage for drug to be released by diffusion and erosion until complete polymer solubilisation (26).

The biodegradation products of PLGA caused a pH reduction in all the solutions because the pH in the case of distilled water and SBF decreased from ~7.5 to ~3.0, so the degradation rate of PLGA was affected. It is well known that an acidic environment accelerates the degradation rate of the PLGA polymer (27). Table II shows the pH values of the solutions used to immerse the dressing patches including also the pH values at the start and at 12 and 25 days after the dressings were immersed in the media. It was observed that in the case of distilled water and SBF the pH values dropped dramatically after 25 days, but, as expected, for the PBS there was only a slight difference. The pH in conjunction with the higher water uptake of PLGA fibres in distilled water and SBF, than in PBS and the hydrophilicity of the amoxicillin drug explain the low drug release from the dressing patch to the PBS in contrast to distilled water and SBF (23): The higher drug release measured from the dressing patch into the SBF was caused by the interactions between SBF and the PLGA ester linkages, thus causing faster hydrolysis and degradation of the PLGA polymer, resulting in higher drug release concentrations than in the other media. Furthermore, when the SBF (R) was replaced with fresh stock after each UV measurement those

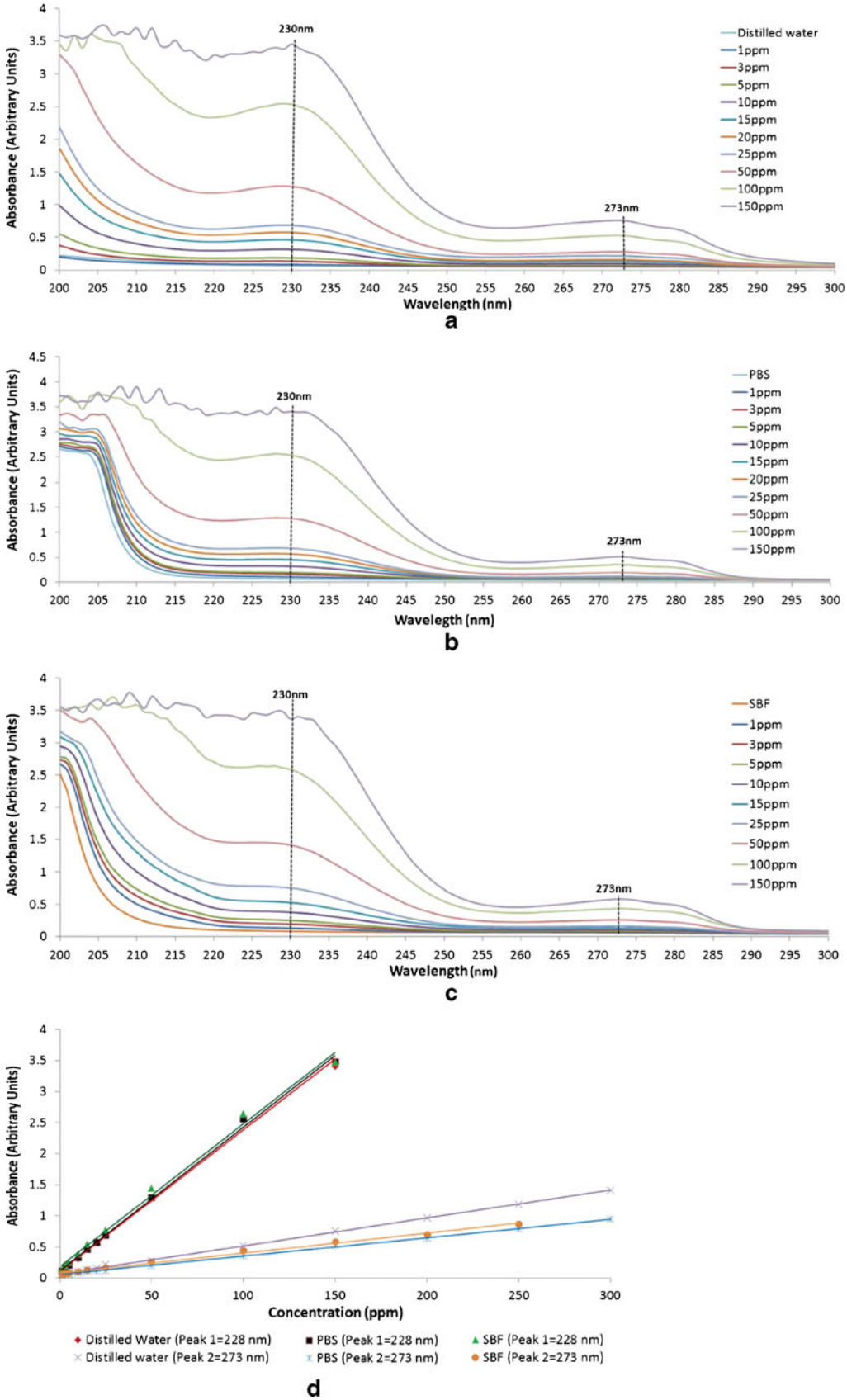

Fig. 6 UV spectra for different amoxicillin concentrations, when dissolved in (**a**) distilled water, (**b**) PBS and (**c**) SBF. The amoxicillin calibration curves for the 3 media are shown in graph (**d**). These *curves* were used in preparing the *in vitro* drug release profiles for the dressing patches and their R^2 values were > 0.99.

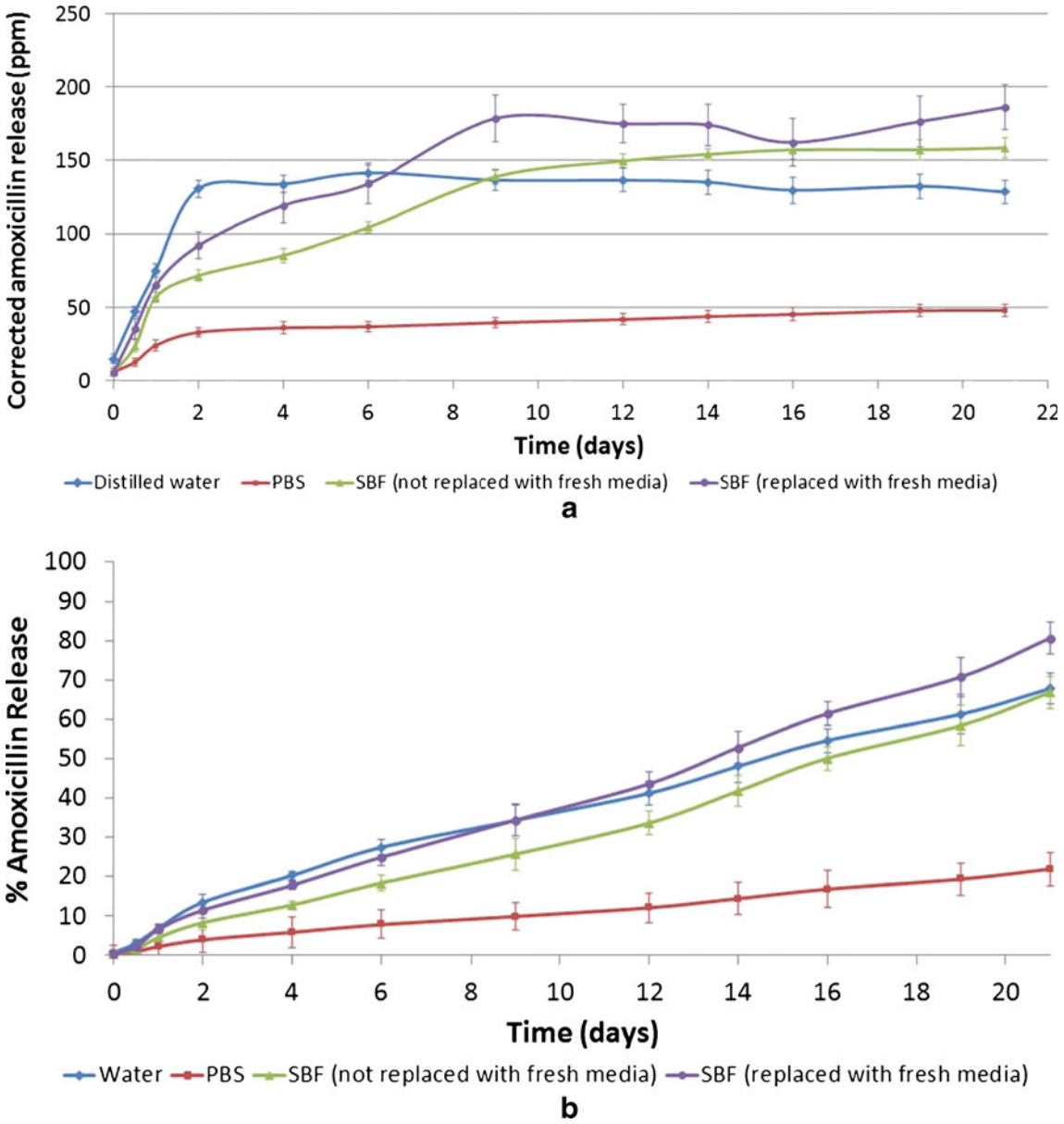

Fig. 7 *In vitro* drug release profiles in different media of amoxicillin encapsulated in the fibre-based dressings showing (**a**) the concentration of amoxicillin released in each media and (**b**) the amoxicillin % released from the dressing into the media. The error bars represent the standard deviation of amoxicillin concentration (3 samples).

interactions were increased compared to the SBF (NR), which caused even larger quantities of drug to be released due to the higher percentage of mass loss (~60% in 21 days).

Amoxicillin Stability

Kim *et al.* (33) proved by high–performance liquid chromatography (HPLC) that when amoxicillin is entrapped in a PLGA matrix no degradation products are formed, hence indicating that the drug remained stable inside the PLGA matrix in the solid state and any dissolved drug was released into the medium by diffusion or PLGA degradation. This was supported by the amoxicillin release profiles (Fig. 7a), which showed continuous drug release from the dressings even after 21 days of immersion in the various release media, although the amoxicillin is very unstable when it is released in aqueous or buffered media.

The stability of amoxicillin was studied in 3 different release media, at 37°C, using UV-spectroscopy. From the UV spectra obtained amoxicillin degradation products were detected in the release media at a wavelength of ~ 350 nm. Piperazine-2,5-dione is one of amoxicillin's degradation

Table II pH Values of Each Solution, in which the Fibre-Based Dressings Were Immersed, Taken at 22°C as a Function of Time. The pH Values Were Noted in Triplicate

Media/pH	0 days	12 days	25 days
Distilled water	7.4 ± 0.2	–	–
PBS	6.7 ± 0.2	–	–
SBF	6.4 ± 0.2	–	–
Drug dressing in distilled water	7.3 ± 0.2	5.5 ± 0.2	2.9 ± 0.2
Drug dressing in PBS	6.6 ± 0.2	6.5 ± 0.2	6.2 ± 0.2
Drug dressing in SBF (NR)	6.3 ± 0.2	5.3 ± 0.2	3.6 ± 0.2
Drug dressing in SBF (R)	6.3 ± 0.2	5.8 ± 0.2	3.9 ± 0.2
Dressing without drug in distilled water	7.3 ± 0.2	5.3 ± 0.2	3.0 ± 0.2
Dressing without drug in PBS	6.6 ± 0.2	6.4 ± 0.2	6.1 ± 0.2
Dressing without drug in SBF	6.4 ± 0.2	5.2 ± 0.2	2.8 ± 0.2

products that absorbs light at the wavelength of 340 nm, therefore causing noise in the UV drug release profiles. This is an additional reason why the wavelength of 230 nm was selected for analysis instead of the 273 nm, as it is not affected by the UV signal of the decomposition products (41). The piperazine 2,5-dione concentration increased over time thus giving stronger UV absorbance values (Fig. 8). As shown from the drug release profiles, drug degradation may depend not only on the PLGA degradation rate but also on the release medium and on the pH value. At lower or higher pH values (compared with pH 6), the degradation rate of amoxicillin is much higher. In Fig. 7a, it is shown that the degradation rate of amoxicillin and the drug release concentration from the dressings reached an equilibrium state after 12 days of immersion in the various media (within the limits of the standard deviation). This means that the concentration of the drug released from the dressing patch into the medium is almost the same as the degradation rate of the drug in the medium. However, it should be noted that translation of these results to an *in vivo* scenario needs to take into account of the fact that the media used in our experiments are not fully buffered, in comparison with blood which is buffered within a narrow range of pH (42).

CONCLUSIONS

Novel biodegradable and biocompatible drug-loaded PLGA fibre-based dressings were prepared by electrospinning and shown to release the antibiotic drug amoxicillin in a controlled manner *in vitro* for over 21 days. The release profiles exhibited an initial burst release accompanied by a sustained release of the drug from the dressing. Both diffusion and polymer degradation contributed to the release of amoxicillin from the PLGA dressings. While the entrapment of amoxicillin in the PLGA fibres offered initial protection to the drug, hydrolysis of the polymer due to the media in contact with it caused release of the drug over time and thus decomposition of amoxicillin degradation products were also detected in the release media. The PLGA fibres successfully controlled the release of amoxicillin to extend its stability period and its biological activity for at least up to 21 days, when immersed in distilled water, PBS and SBF solutions. The effect of the type of media on the controlled release of amoxicillin was also studied. The highest concentration of drug released from the dressings into the media was found in the SBF solutions and the lowest in the PBS solutions. FTIR spectroscopy showed that the electrospinning process used for the dressing production did not affect the chemical structure of amoxicillin, thus

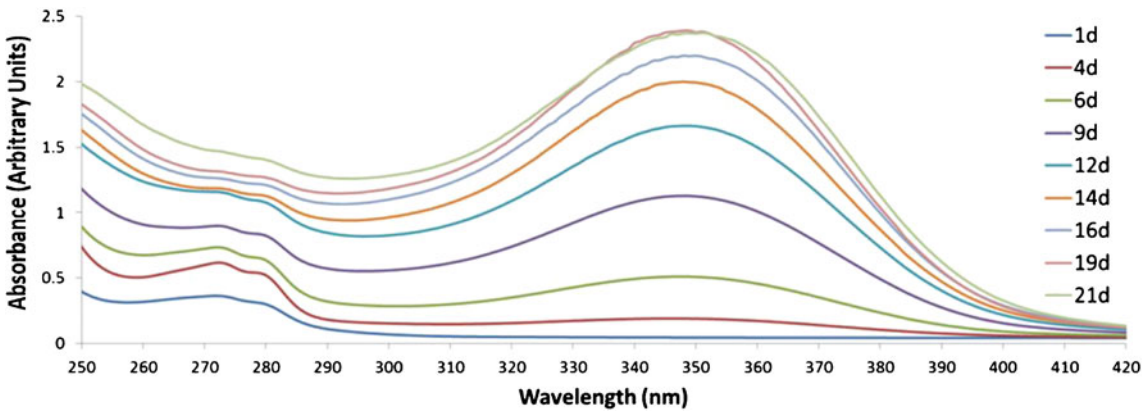

Fig. 8 Graph showing the concentration of the amoxicillin degradation product piperazine 2,5-dione, increasing over the period of 21 days.

preserving its pharmacological and antibacterial properties. It is concluded that the fibre-based dressing loaded with amoxicillin prepared in this work can be successfully considered as a promising product for advanced wound healing applications.

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