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Electrospraying technique for the fabrication of metronidazole contained PLGA particles and their release profile

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ABSTRACT

Advanced engineering of materials for the development of drug delivery devices provides scope for novel and versatile strategies for treatment of various diseases. 'Electrospraying' was used to prepare PLGA microparticles and further encapsulate the drug, metronidazole (Met) within the particles to function as a drug delivery system. Two different solvents were utilized for the preparation of drug loaded PLGA particles, whereby the polymeric solution in dichloromethane (DCM) produced particles of bigger sizes than using trifluoroethanol (TFE). Scanning electron microscopy showed the spherical morphology of the particles, with sizes of 3946 ± 407 nm and 1774 ± 167 nm, respectively for PLGA-Met(DCM) and PLGA-Met(TFE). The FTIR spectroscopy proved the incorporation of metronidazole in the polymer, but without any specific drug–polymer interaction. The release of the drug from the particles was studied in phosphate buffered saline, where a sustained drug release was obtained for at least 41 days. Cytotoxicity evaluation of the drug extract using mesenchymal stem cells (MSCs) showed not hindering the proliferation of MSCs, and the cell phenotype was retained after incubation in the drug containing media. Electrospraying is suggested as a cost-effective and single step process for the preparation of polymeric microparticles for prolonged and controlled release of drug.

1. Introduction

Drug delivery (DD) is the process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals [1]. The routine delivery methods have been replaced by targeted drug delivery and sustained release formulations, such that the DD system can target specific tissues or circulate and release drug over a longer period of time, improving the efficacy of the drugs. Natural polymers such as collagen, gelatin and hyaluronic acid are used in clinical drug delivery applications. However, these materials are derived from animal sources, thus they bear the risk of transfer of viral diseases [2,3].

Alternatively, biodegradable polymers are a good choice, and there are several properties that make them suitable as carriers for DD such as their predictable biodegradation behavior, biocompatibility, ease of fabrication and availability (various molecular weights/copolymer ratios) along with its versatility in properties, allowing the possibility of regulatory approval. Process development and optimization are the most important aspect specifically considered in pharmaceutical research, where the possibility of minimal steps and maximum output (encapsulation efficiency and release profile) is considered relevant. A plethora of techniques have been evolved for the fabrication of drug delivery systems (DDS) including single solvent emulsion, double or multiple emulsion, separation–coacervation, spray drying, phase inversion microencapsulation etc. [4–6]. Among these, multiple emulsion methods have been classified as the most popular method for the production of porous particles, but it could not alleviate the drawbacks such as the low drug loading efficiency in addition to its lengthy preparation methods. Multistep procedures, with low encapsulation efficiency remain a bottleneck for further development of PLGA particles using many of the abovementioned methods. In many cases, the release of drug is quite fast within a period of few hours, which is also a drawback of the existing DDS. Hence, there is a need for the development of DDS that can release drugs over a longer period of time, improving the efficacy of the drugs. Spray drying, though popular in its reproducibility and controlled particle size, involves high capital investment [7]. On the other hand, electrospraying might be a single step procedure for the preparation of drug encapsulated particles, and the process consumes minimum amounts of solvents with no additional wastage is involved.

Electrospraying is an electro-hydrodynamic technique, applied for the generation of nano/microparticles under a high electric field, and it essentially consists of a high voltage supplier, syringe pump and a grounded collector (Fig. 1). Electrospraying involves the application of an electric field, due to which the solution jet at the end of the metallic needle stretches and when the electrostatic force is sufficiently high, the solution forms a conical jet and eventually break into droplets. Once the

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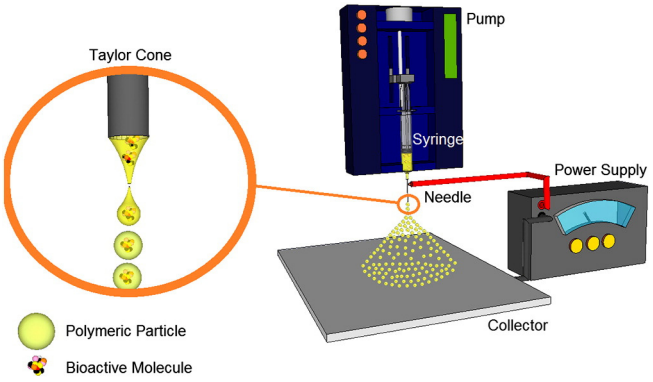

Fig. 1. Schematic representation of particle preparation by electrospraying process.

droplet travels towards the collector, the solvent gets evaporated and solid particles are deposited on the collector. Electrospraying is capable of producing micro/nanometer sized droplets and the self-repelled particles are produced without coalescence from the highly charged droplets. Electrospinning differs from electrospraying process in that a more concentrated solution applied with a much higher voltage results in the production of nano/microfibers instead of particles. Optimization of electrospraying involves not only the selection of an appropriate solvent for dissolving the polymer and the drug for proper electrospraying to take place, but also the factors like the polymer concentration and electrospraying conditions which are critical. Polymers used for electrospinning are explored for their ability to electrospray, such that porous microparticles suitable for drug, protein or peptide delivery can be prepared for disposition to injured or damaged tissue sites. Overall, electrospraying can be considered as a simple, rapid and versatile technique for preparation of drug incorporated particles. Another advantage could be better production efficiency of the process due to the direct collection of particles on the collector in its dried form. However, there are very little reports currently available on the electrospraying of biodegradable polymers or drug encapsulated polymers. Detailed investigations on the release profile of drugs from electrosprayed particles are also missing.

PLGA is a biocompatible polymer, approved by FDA as a drug delivery vehicle and it has been extensively used for the development of scaffolds and matrices for drug delivery and tissue engineering [8,9]. It is also used for the delivery of low molecular weight compounds including nucleic acids, peptides and drugs [10,11]. Due to the potential classification of PLGA as a safe material, and considering its world-wide marketing approval, we utilized PLGA as a carrier for incorporation of drug during this study. On the other hand, metronidazole (Met) is an antibiotic highly effective against anaerobic bacteria, either used alone or in combination for the treatment of different bacterial infections, such as the urinary tract infections and periodontitis [12]. It is also a drug of low cost and is predominantly used in the developing countries as an antimicrobial drug. However the oral administration of a single high dose of Met causes nausea and vomiting to patients, and concerns on its carcinogenicity have been raised [13]. Therefore, slow and continuous releasing DDS containing Met are appreciated over the traditional drug consumption, such that they can avoid adverse drug reactions associated with systemic Met therapy. During this study, we utilized 'Met' for encapsulation in PLGA matrices, and the technique of electrospraying was used to obtain the drug containing particles. A better antimicrobial eradication would mean high localized drug concentration or prolonged residence time of the DDS [14]. The simplest method of mixing the drug with polymer in an organic solvent makes it a very attractive option for formulation purposes, and it is the most highly used method for encapsulation. However, the process variables especially the solvent used for the electrospraying process might significantly influence the size of the particles generated, ultimately influencing the release of the drug from the developed particles. During this study we prepared Met containing PLGA particles by electrospraying process, but using two different kinds of solvents and further studied the release profile of the drug from the developed particles.

2. Materials and methods

2.1. Materials

PLGA or poly(lactic-co-glycolic acid), 90:10, Mw 10,000 was purchased from Polysciences (Warrington, USA). Metronidazole (Met), dichloromethane (DCM) and trifluoroethanol (TFE) were procured from Sigma-Aldrich (Singapore) and used without any purification. The human mesenchymal stem cells were obtained from Lonza (Singapore). The media for culturing of cells included Dulbecco's modified Eagle's medium with low glucose (DMEM), fetal bovine serum (FBS) and antibiotics, purchased from GIBCO (Invitrogen, Carlsbad, CA, USA). The other reagents such as the trypsin–ethylenediaminetetraacetic acid and 10× phosphate buffered saline (PBS) were also purchased from Invitrogen. The CellTiter 96 Aqueous one solution was purchased from Promega, Singapore.

2.2. Conductivity and viscosity measurement

The conductivity of the solvents namely, pure DCM and pure TFE was measured using a Jenway conductivity meter (Singapore).

2.3. Electrospraying of particles

PLGA was dissolved in DCM at 10% (w/v) to prepare pure PLGA particles. For preparation of drug containing PLGA particles, the polymer was dissolved in either DCM or TFE, stirred overnight at room temperature and further added with Met before electrospraying, while the stirring was continued for another 1 h. The ratio of drug:polymer (w:w) was always maintained at a ratio of 1:20. Electrospraying of the polymeric solution with or without drug was individually conducted, where a high voltage supplier from Gamma High Voltage Research (USA) was used. The solution was taken in a plastic syringe (BD Biosciences, Singapore) and fitted with a blunted 27 G needle, where the flow rate of the solution was controlled using a syringe pump (KD100, KD Scientific, Holliston, MA). The collector consisted of a flat platform covered with aluminium foil, and it was placed at a distance of 10 cm from the tip of the needle. Experiments were optimized with respect to the solution concentration, in parallel to electrospraying conditions. The experiments were always performed at a high voltage of 14 kV, while a 27 G needle was utilized to maintain uniformity in needle size (constant spray needle conditions) during the electrospraying process. Polymer concentrations in the range of 9% (w/v) to 11% (w/v) were prepared for particle initiation, while uniform and spherically shaped particles were aimed during this study.

2.4. Particle morphology and size

The microstructure of the particles prepared by electrospraying were observed under scanning electron microscope (SEM; JEOL, JSM 5600LV, Tokyo, Japan; FEI-Quanta 200F, the Netherlands). The samples were sputter coated with either gold or platinum before observation under the SEM. Both low and high magnification images were obtained to confirm the uniformity of the particle sizes and to determine the exact size of the particle, respectively. The high magnification SEM images were interpreted by ImageJ software to determine the size of the particles. In short, the size of around 40–50 particles were measured and the average particle diameter (D_p) was determined, with standard deviation (SD).

2.5. Chemical characterization

The chemical composition of the particles generated after electrospraying was evaluated by Fourier Transform InfraRed (FTIR) spectrometer (Avatar 380, Thermo Nicolet, Waltham, MA). The particles were scraped from the collector and used for the analysis. To do so, the particles were mixed with KBr to prepare a pellet, and it was placed in the sample holder of the IR machine and scanned for obtaining the spectra. The pure drug (Met) was also analyzed in the same manner. The samples were scanned for 64 times in the range of 400–3500 cm^{-1} at a resolution of 4 cm^{-1}.

2.6. AFM analysis

Atomic Force Microscopic (AFM) measurement of the electrosprayed particles (collected on coverslips of 15 mm diameter) were carried out using Nanowizard 3 AFM machine (JPK, Germany). Measurements were done using tapping mode, and silicon cantilever with nominal stiffness of 5 N/m was used for all the measurements (Tap150Al-G, Budget Sensors). JPK data processing software was used to process the AFM images and the surface roughness values of the electrosprayed particles were obtained from an area of 20 × 20 μm.

2.7. Degradation behavior of the particles

The electrosprayed particles were collected on coverslips with 15 mm diameter and incubated with phosphate buffered saline (1× PBS) and kept in a shaker at 37 °C for a designated period of time. The buffer was changed every alternative day, until the end of the test period. After the designated time period, the particles were washed with DI water and dried in a vacuum oven at room temperature. Further the particles were sputter coated with gold and observed under the SEM (Zeiss Supra 55VP, MA, Germany) for morphology evaluation. The degradation of the electrosprayed particles were carried out at two time points: day 4 and day 8.

2.8. Drug release and kinetics

In order to calculate the amount of drug released from the particles, it was necessary to perform a standard curve after dissolving the pure drug in PBS. For this, pure Met was dissolved in 1× PBS at concentrations ranging from 5–30 μg/ml (serial dilutions made) and the UV absorbance was measured at 320 nm (λ max) using a Shimadzu UV-3600 UV–vis NIR spectrophotometer (Japan). Standard curve was plotted from the obtained absorbance values and a high value of R^2 demonstrated the accuracy of measurement of the standard curve plot. For release test of the samples, exactly 10 mg of the drug containing particles was incubated in PBS (at pH 7.4) at a temperature of 37 °C, under constant shaking (150 rpm). At the designated time period, the samples were centrifuged at 10,000 rpm for 10 min and the supernatant was used for UV measurement. Further, the cumulative release of the drug from the particles was determined based on the standard curve obtained from pure drug. The experiments were performed in triplicate.

The Korsmeyer–Peppas model was used to analyze the drug release kinetics obtained from the drug containing particles. This was performed by analyzing the data of drug release obtained at different time points and using the equation $M_t/M_\alpha = Kt^n$, where M_t and M_α are the amounts of drug release at time t and infinite time (maximum release amount), respectively, while K represents the release rate constant and n is the release exponent (diffusional coefficient). Fitting to the Korsmeyer–Peppas model, the value of n was determined from the slope of the curve. Following the above equation, Fickian diffusion followed at $n \leq 0.45$, while an anomalous non-Fickian transport followed at $0.50 < n < 1.0$.

2.9. Cell culture and biocompatibility studies

The biocompatibility of cells in the presence of the drug containing extract was determined by MTS assay. For this, human mesenchymal stem cells (MSCs) were used as the representative cells. The cell proliferation study was performed using the drug containing extract, where the extract at 2.5 h release was chosen for this study. In order to obtain the drug containing extract, approximately 10 mg each of the particles was accurately weighed and incubated with plain DMEM for 2.5 h period at 37 °C. Further the mixture was centrifuged and the clear extract was used for cell proliferation studies.

Cells were cultured in a flask using DMEM supplemented with 10% FBS and 1% antibiotic–antimycotic mixture. The cells were grown till confluency, trypsinzed, counted using a hemocytometer and used for cell proliferation studies. To perform the proliferation test, MSCs were seeded at a count of 10,000 cells in each well, and cells were allowed to attach and grow for 24 h. Further the cells were incubated with the drug extract and allowed to proliferate for 2 days, and the cell proliferation test was carried out. The principle of the assay is that the reduction of yellow tetrazolium salt [3-(4,5-dimethyl thiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4 sulfophenyl)-2H-tetrazolium] in MTS by metabolically active cells forms purple formazan crystals. This formazan dye can be measured at an absorbance of 490 nm, whereby the quantity of formazan crystals is directly proportional to the number of active cells. To perform this assay, after the designated time period (2 days), the cells are incubated with DMEM containing 20% of MTS reagent, for a period of 3 h at 37 °C in the CO_2 incubator. Thereafter, the content is pipetted into a 96-well plate and the absorbance measured by a microplate reader (Varioskan™ Flash Multimode Reader, Thermo Fisher Scientific, USA).

2.10. Statistical analysis

Results were presented as mean ± standard deviation. Statistical analysis was performed by one way ANOVA and the significance was determined at $p \leq 0.05$.

3. Results

3.1. Morphology and surface properties of the particles

Preliminary experiments were carried out to check the dissolution of the polymer, using a variety of solvents including pure dichloromethane (DCM), trifluoroethanol (TFE), acetonitrile and methanol. From among the abovementioned solvents, PLGA and the drug were found soluble in either DCM or TFE. The conductivity of these two solvents was also measured at ambient temperature, and it showed huge differences in their conductivity profile. The conductivity of DCM was measured as zero, while TFE gave a conductivity of 1.07 μs/cm. Electrospraying of PLGA in DCM was carried out, and until up to 9% w/v of PLGA in DCM produced dripping of the solution, and ultimately 10% w/v of PLGA in DCM displayed a stable jet producing particles. The SEM micrographs clearly demonstrated that the developed particles were spherical in shape (Fig. 2), with uniform sizes of particles obtained. The electrosprayed particles of PLGA in DCM displayed smooth surfaces, as observed from the SEM images. Fig. 2A shows the morphology of PLGA particles prepared using pure DCM, with an average size of 4952 ± 550 nm. Secondly, the Met incorporated PLGA particles were also successfully prepared using pure DCM as the solvent. The Met encapsulated PLGA particles prepared using DCM as the solvent [PLGA-Met(DCM)] showed a particle size of 3946 ± 407 nm. The incorporation of the drug was found to reduce the size of the PLGA particles. Attempts to fabricate Met containing PLGA particles at a concentration of 10% w/v using TFE as the solvent were not successful and this did not produce spherical particles. Further, the Met encapsulated PLGA particles in TFE solvent [PLGA-Met(TFE)] was possible using 11% w/v solution.

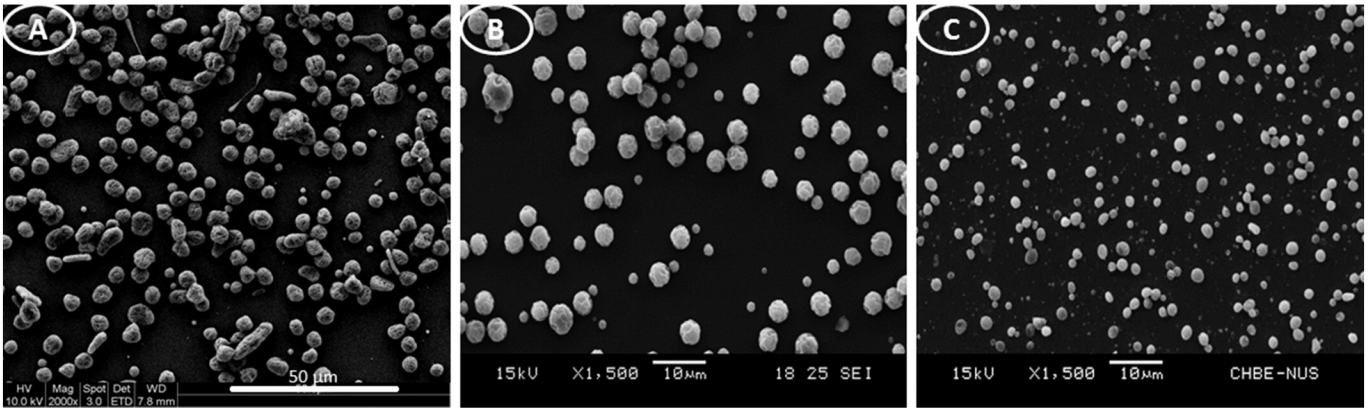

Fig. 2. SEM of electrosprayed (A) PLGA, (B) PLGA-Met(DCM), and (C) PLGA-Met(TFE) particles.

Moreover, the PLGA-Met(TFE) particles had sizes much smaller (1774 ± 167 nm) than that obtained using DCM as the solvent (Fig. 2B–C). The particle size of PLGA-Met(TFE) was significantly different compared to the size of PLGA-Met(DCM) particles ($p \leq 0.05$).

The Fourier transform infrared (FTIR) spectra of PLGA and drug containing PLGA particles were taken by the KBr disc method and further scanned to obtain the spectra (Fig. 3). The typical C–O stretch present in PLGA was observed at 1091 cm^{-1}, while the ester bond (C═O) and the carbonyl peak present in PLGA were visible as a broad band between 1751–1760 cm^{-1}. Additionally the C–O–C ether peak was also visible at 1083 cm^{-1} and 1045 cm^{-1}, along with the methyl group C–H stretching at 1458 cm^{-1}, which was observed in the FTIR spectra of pure PLGA and PLGA-Met particles prepared during this study. The characteristic peaks of Met at 1535 cm^{-1} and 743.58 cm^{-1} were typically observed on the IR spectra of pure drug, while these peaks were slightly visible in the IR spectra of PLGA-Met(DCM) and PLGA-Met(TFE) particles. The absorption peaks of PLGA overlapped with those of the drug around similar wavenumbers, and it was difficult to pick out the peaks separately. Due to the smaller amounts of drug present in the polymer matrix, the intensity of these peaks observed for PLGA-Met particles was lesser than that observed in the IR spectra of pure drug powder.

3.2. Evaluation of the surface roughness of the particles

The surface roughness of the electrosprayed particles was measured by using AFM. The morphology of the particles obtained by AFM is shown in Fig. 4. The average roughness factor of pure PLGA (872 nm) was higher compared to the roughness of Met containing PLGA particles. The roughness factor for PLGA-Met(DCM) and PLGA-Met(TFE) was respectively obtained as 803 nm and 357 nm, which highlights the ability of the solvent TFE in producing smoother particle structures compared to DCM. The reduced roughness can be partially attributed to the densely packed PLGA-Met(TFE) particles, since their sizes were nearly half the size of PLGA-Met(DCM) particles. Very few particles were observed for PLGA-Met(DCM) in the scanned area, compared to the higher number of particles of PLGA-Met(TFE) observed within an equivalent area due to the smaller particle sizes in the latter case. The AFM results shown in Fig. 4 again supported the results of our SEM analysis.

3.3. Degradation behavior of the particles

The morphology of the developed particles was examined by SEM, after incubation in PBS at two different time points (4 and 8 days). Pure PLGA and PLGA-Met(DCM) particles partially retained their morphology on day 4, though they appeared to synergize with the particles next to it (Fig. 5). By day 8, the particles started to loose their morphology from its original spherical shape, moving towards a flattened structure and this was more visible for the PLGA-Met(TFE) particles (Fig. 5).

3.4. In vitro release of the drug from electrosprayed particles

The in vitro cumulative release profile of Met from PLGA-Met(DCM) and PLGA-Met(TFE) was determined, and the results are shown in Fig. 6.

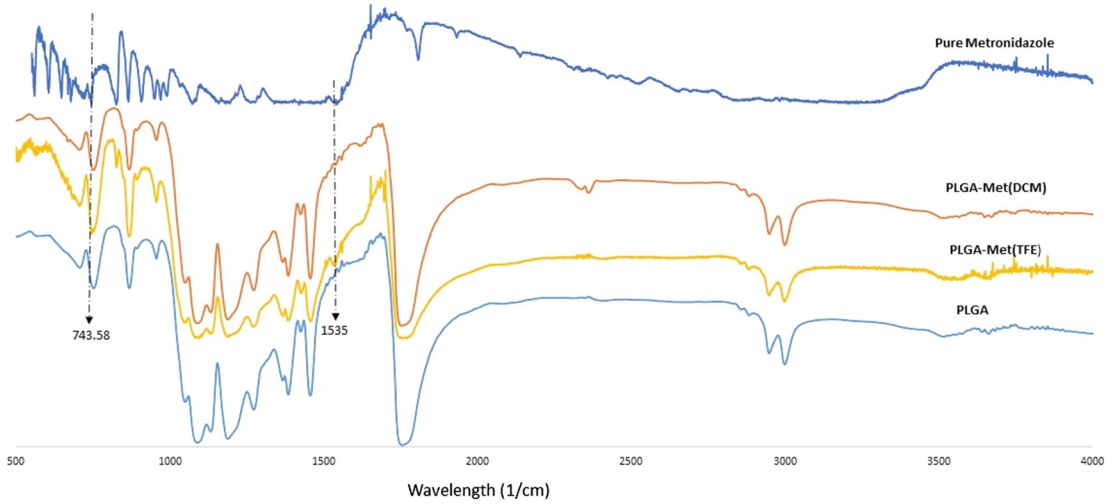

Fig. 3. FTIR spectra of the electrosprayed particles and pure drug.

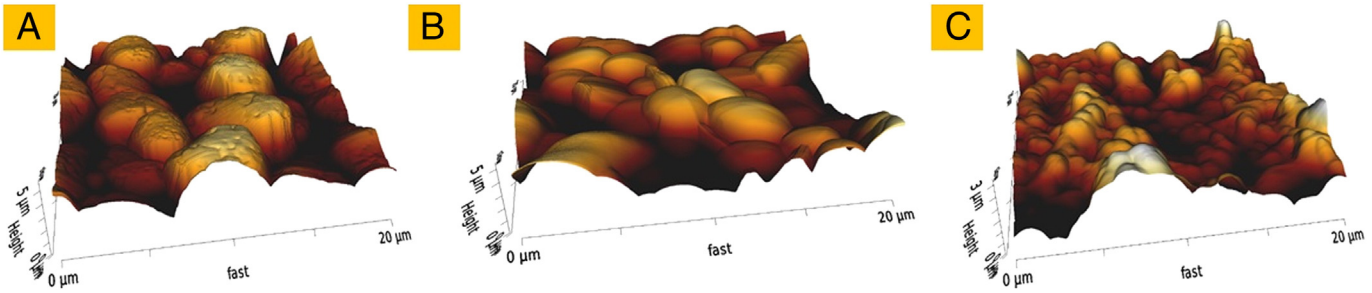

Fig. 4. AFM images of the electrosprayed (A) PLGA, (B) PLGA-Met(DCM), and (C) PLGA-Met(TFE) particles.

Different release profiles of Met were observed for particles prepared from different solvents. An initial burst release of 35.60% was observed from PLGA-Met(DCM) particles, while the release from PLGA-Met(TFE) was 48.33%, after a period of 2.5 h. The drug molecules had to travel a longer distance within the polymer matrix to reach the surface of PLGA-Met(DCM) particles, due to their bigger sizes compared to the release from PLGA-Met(TFE) particles. Due to this reason, the release rate of Met was also less from PLGA-Met(DCM) than from PLGA-Met(TFE). At any particular time point, the release of Met was always higher from PLGA-Met(TFE) than from PLGA-Met(DCM), until at least for the first 26 days period. The release test was extended for a total of 1000 h (~41 days) during this study, with approximately 85% of the drug released from both PLGA-Met(DCM) and PLGA-Met(TFE) particles by this time period.

The release of drug from the particles was fitted with the Korsmeyer–Peppas model, which showed a linear relationship with R^2 values of 0.99 and 0.97 for PLGA-Met(DCM) and PLGA-Met(TFE), respectively. The value of n was calculated to evaluate the release mechanism of the drug, whereby the value of 'n' equal to 0.45 or less suggests the release pattern as Fickian diffusion [15,16]. During this study, the value of n was obtained as 0.1446 ± 0.01 and 0.0855 ± 0.005, respectively for the drug release through PLGA-Met(DCM) and PLGA-Met(TFE) particles. Hence it was clear that the release of drug from the electrosprayed particles followed diffusion controlled phenomena, suggesting the permeation of Met through the PLGA matrix as independent of the solvent type used for its preparation.

3.5. Cellular interaction with drug containing extract from particles

Evaluation of the drug released extract towards cell proliferation was performed using mesenchymal stem cells. The burst release of the drug in 2.5 h period was considered as the sample for evaluation from Met encapsulated PLGA particles, while the extract from pure PLGA particles itself served as the control. Fig. 7 shows the results of the MTS assay performed after a period of 2 days of incubation of the extract with the cells. The cell proliferation for the drug containing particles [PLGA-Met(DCM) and PLGA-Met(TFE)] was not significantly different from that of the cell proliferation observed for pure PLGA particles. The results of cell proliferation assay obtained from MTS assay demonstrate little or no toxicity of the drug released from the particles, suggesting the biocompatibility of the particles.

The SEM evaluation of the cells seeded with the extract was also analyzed after 2 days, and the morphology of the cells is shown in Fig. 8. The morphology of the cells on TCP was used to understand the phenotypic variation of the cells, with regard to the cells seeded with the particle extract. The morphology of the cells appeared fibroblastic with

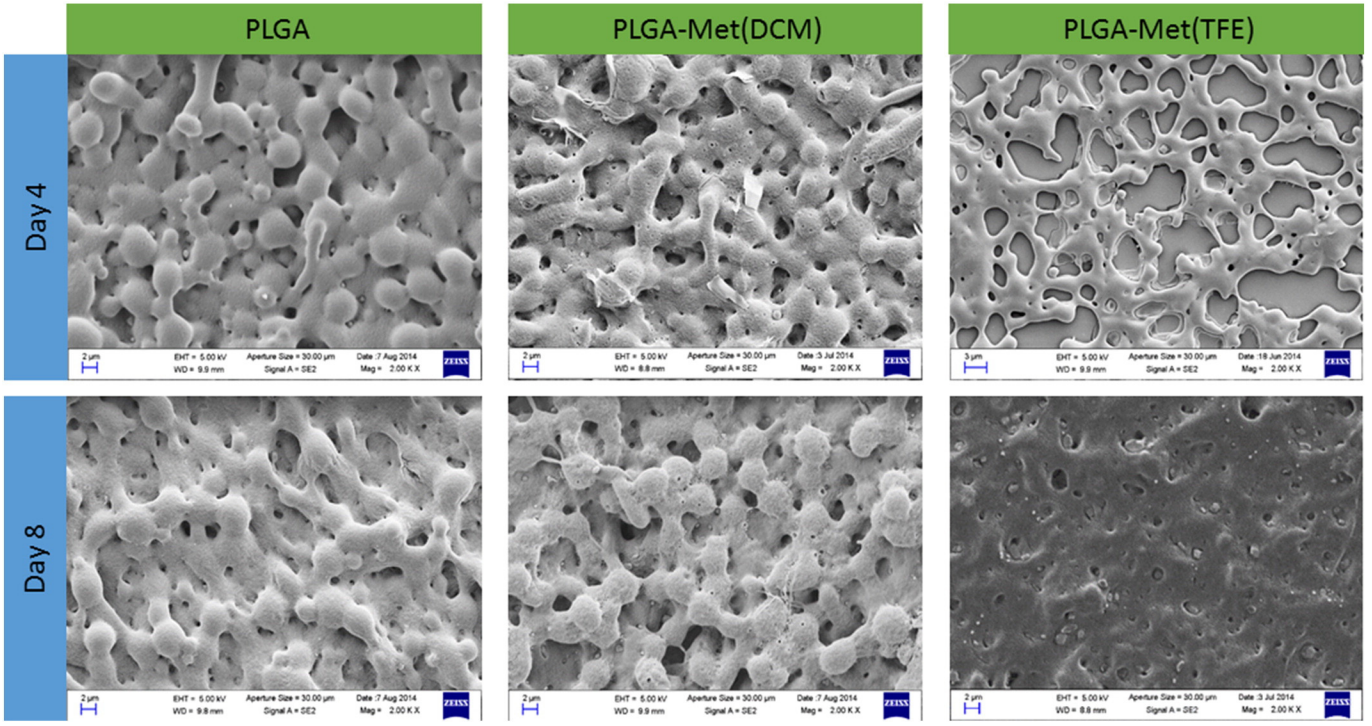

Fig. 5. SEM images of electrosprayed particles after degradation in PBS for 4 and 8 days.

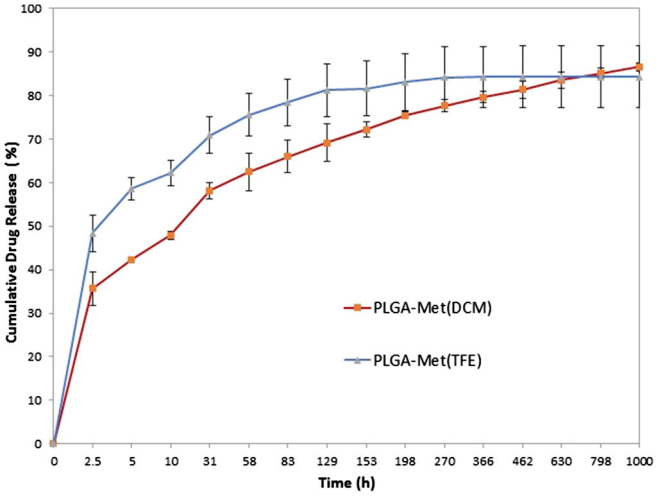

Fig. 6. Cumulative release profile of Met from electrosprayed particles.

elongated shape in every case, demonstrating the non-toxicity of the drug containing extract. Our results suggest the application of electrosprayed PLGA-Met particles to function as a drug delivery vehicle and might serve as a substrate for periodontal regeneration.

4. Discussion

Drug delivery systems are mainly aimed to provide controlled release of drug over an extended period of time, improving the efficacy of the drugs. Dose limiting systemic toxicity is a major issue related to the repeated application of higher doses of drugs, which is carried out on an attempt to achieve the required concentration of drug over a sufficient period of time [17]. By application of controlled DDS, the concerns related to dose limiting systemic toxicity can be avoided. Single dosage formulation or fewer dosage forms are preferred by the patients, while this might improve the patient adherence to medication, in addition to its reduced cost with minimal medication errors. Natural polymers like alginate and gelatin are widely used as drug delivery vehicles, but require crosslinking. Optimization of the crosslinking procedures together with their non-interactive phenomena with drug remains a major drawback of such DDS. Efforts to improve the entrapment efficiency of alginate based DDS was attempted by increasing the amount of the crosslinking agent (Ca^{2+} ions), however such effects were not pronounced by using calcium chloride [18]. The drug loading capacity was studied with respect to the amount of calcium carbonate (i.e., crosslinker) within alginate–pectin beads, and it increased from 68.41% to 81.02% when the calcium carbonate concentration was increased from 0.50 to 1.0% (w/w) [19]. In most cases, where water soluble drugs are incorporated within the DDS, modifications with respect to the method of preparation such as the addition of oil or coating of the beads are required to delay the release of the drugs. Many of the natural polymers require crosslinking, and alternatively synthetic polymers like PLGA offer advantages of not requiring an additional crosslinking step.

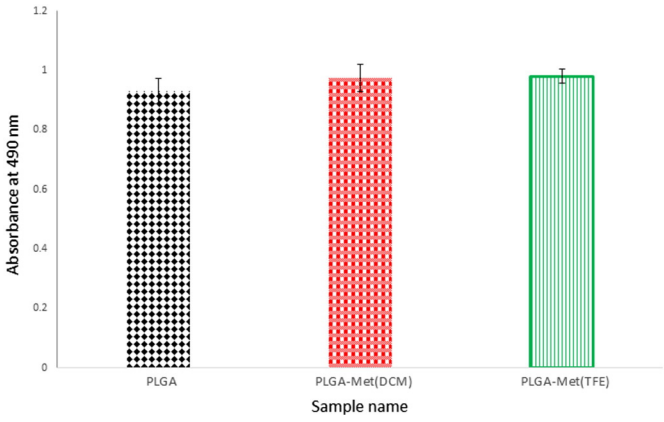

Fig. 7. Cell proliferation studies carried out using the drug containing extract obtained from the electrosprayed particles, studied by MTS assay.

Non-toxicity of the polymer is an important criterion for choosing a polymer for drug incorporation. The advantage of using PLGA as a carrier is that they can resorb harmlessly inside the body, while systemically administering the drug at a controlled rate. Among the different kinds of biomedical devices available in the market, PLGA has been the common material of choice for sutures, implants and prosthetic devices [20]. There is minimal systemic toxicity associated with PLGA based drug delivery carriers. For example; a commercially available PLGA based DDS, namely the Lupron Depot® is currently available for prostate cancer treatment. Distinguished by the biodegradability of PLGA (in the body), which occurs via hydrolysis of the ester linkages in the presence of water to produce lactic acid and glycolic acid, these monomers are also the by-products of various metabolic pathways under normal physiological conditions. The degradation products are easily metabolized in the body via Krebs cycle and are eliminated, avoiding any toxicity effects.

Drugs are commonly loaded via the process of adsorption, covalent attachment or by entrapment within the polymeric matrix [21]. However, many of the particle formulations including those that are at the clinical stage utilize the simple method of mixing the drug with the polymer, to obtain drug loaded polymeric device. Electrosprayed particles as DDS offer advantages over the conventional forms in providing controlled drug release, targeted delivery with possibility of encapsulation of both hydrophilic and hydrophobic drugs and good encapsulation efficiency might be possible. Moreover, electrospraying of particles allows for the drying of the particles without eventual aggregation, a major problem encountered with many of the particle preparation techniques [22]. Electrospraying avoids secondary processing steps such as the centrifugation or freeze drying steps, usually associated with the conventional particle preparation methods, and thus it eliminates the elution of drug molecules associated with the wash-off procedures. The size and morphology of the particles prepared by electrospraying influence the release of drug from the electrosprayed particles. Spherically shaped particles are considered suitable for drug delivery than irregularly shaped particles, mainly because the polymer dissolution and thus the drug release might be inconsistent from within the irregularly shaped particles. Spherical particles were aimed during this study, and two different solvents were used for the preparation of drug containing PLGA particles. Dichloromethane (DCM) is an organic solvent with a boiling point of 40 °C, while the pure PLGA particles prepared using DCM appeared spherical in shape (Fig. 2). Met was soluble in DCM and the PLGA-Met(DCM) particles obtained by electrospraying were also spherically shaped though the surface appeared slightly smoother than the pure PLGA particles. Incorporation of drug within PLGA produced particles with smaller sizes than the pure PLGA itself. However, TFE enabled the preparation of even smaller sizes of PLGA-Met(TFE) particles, which can be attributed to the high conductivity of TFE (1.07 μS/cm) compared to DCM (0 μS/cm). Different kinds of alterations within the electrospin/spraying process have been used to control the radial distribution of drug within the polymer matrices, or to alter their initial burst release. Process variables such as the spraying conditions and related parameters require high precision and encounter difficulties during its translation to large scale production. In this regard, application of two different solvents might be a better option and here we utilized either DCM or TFE for dissolving the drug contained PLGA matrix, for the purpose of electrospraying. The compatibility of the drug within the polymer was confirmed by FTIR analysis. No apparent shift in peaks was observed from the FTIR spectra of pure Met

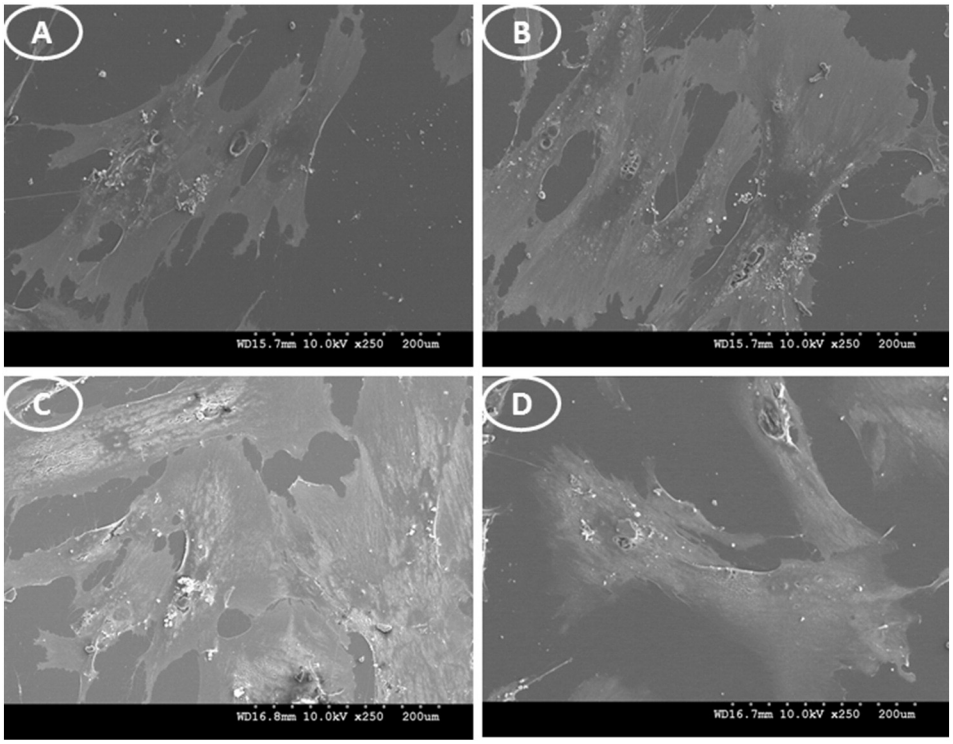

Fig. 8. Morphology of the cells seeded on (A) TCP, compared to cells seeded with the extract obtained from (B) PLGA, (C) PLGA-Met(DCM), and (D) PLGA-Met(TFE) particles.

powder compared to the spectra of PLGA-Met particles. Our FTIR results suggest little or no interaction of Met with PLGA polymer (Fig. 3).

The most common feature of release profiles of drugs and proteins through DDS is the burst release observed within the first few hours. Earlier studies by Berkland et al. reported control over the radial distribution of molecules by electrospraying process [23,24]. Burst release is considered a negative effect, and preventing or minimizing the burst release effect is mostly performed by modification of the DDS fabrication technique. For example, an additional coating step or surface extraction of active agents is followed during emulsion particle preparation procedures [25]. However, electrospraying offers advantages such that the process might not require an additional processing step to improve the drug release profile, instead an alteration of the solution concentration or the solvent used for polymer solution preparation is sufficient to cause such an effect. Altering the particle size by utilization of a different solvent could also change the burst release profile of the drug, through the same polymer matrix. In this regard, the release of Met from PLGA-Met(TFE) was found higher than the release of Met from PLGA-Met(DCM) particles. Approximately 12.73% increase in the release of Met was observed from PLGA-Met(TFE) particles than from PLGA-Met(DCM) particles after a period of 2.5 h. Smaller particles have a higher surface area, and the release of drugs from smaller particles is faster than from large sized particles, due to shorter diffusion pathways. The particles being spherically shaped, with differences only in their sizes, the release curve appeared more or less similar with the continuous release of the drug from both types of particles was observed throughout the study period. However, the amount of drug released was always high from PLGA-Met(TFE) particles compared to the other. At the same time, the period up to which the final release of drug from the polyester depends on the co-polymer ratio of the polymer. For example, studies by Cui et al. showed that the melittin release from poly(lactic-co-glycolic) acid with 50:50 monomer ratio was completed in 14 day time period, while the 75:25 monomer ratio of PLGA delivered the peptide for a longer time (21 days) [26]. During our study, PLGA with a monomer ratio of 90:10 (LA:GA) was used and the drug release was extended for a period of 41 days. Apparently, the electrosprayed PLGA-Met particles prepared during this study revealed prolonged drug release properties, which is a key requirement placed on antibiotic release delivery vehicles.

The drug release profile from poly(lactic-co-glycolic acid) was previously described by Bock et al., where these researchers suggested the first phase being dominated by diffusion or passive movement of drug through the polymer matrix and the second phase due to polymer degradation that involves the breakdown of the polymer matrix (disintegration) and the swelling of the polymer [27,28]. During the first phase of release, the agglomeration of particles occurs and it substantially depends on the particle size, further influencing the release of the drug (amount and rate of release) from the particles. The PLGA-Met(TFE) might have a higher tendency to agglomerate than PLGA-Met(DCM) due to their smaller sizes, and the release of drug was also higher from these particles compared to the latter. Among the various factors that affect the drug release, the size of the delivery device has gained particular attention. It is also known that the particles with smaller sizes degrade faster due to the increased surface area to volume ratio, and this might be a reason for the faster release of the drug from PLGA-Met(TFE) particles [29]. Results of our drug release test are also supported by the results of the degradation study (Fig. 5), which showed that the PLGA-Met(TFE) lost their shape more faster than the PLGA-Met(DCM) particles.

Drug release kinetics can help in evaluating the relationship between the drug release pattern and drug dissolution. The release data was followed and supported by the Korsmeyer–Peppas model, with highest fitting of R^2 values obtained during this study. Moreover, the release exponent value (n) obtained was less than 0.45, indicating the Fickian diffusion as the principal release mechanism of Met from the electrosprayed particles. Studies on the release of protein carried out by Okada et al. through PLGA matrices were also defined as diffusion mediated during the initial phase [30].

The biocompatibility of the developed particles was ensured by cell proliferation assay using mesenchymal stem cells, since these cells are

highly acknowledged for tissue regeneration applications, including periodontal tissue engineering [31,32]. The morphology of MSCs seeded with the extract (containing the drug) appeared similar to the morphology of cells on control TCP, with an elongated fibroblastic phenotype. In summary, our study clearly shows that the PLGA microparticle based systems can provide long term release of drugs, and are beneficial for local treatments including the infections of tissues or for the systemic delivery of labile drugs and other bioactive molecules and drugs which might be explored in the future.

5. Conclusion

A controlled release system that involves single step fabrication procedures might be ideal, provided that they allow for the continuous release of drugs with high drug loading. The electrospraying method was utilized for the fabrication of Met containing PLGA particles. Tailoring the drug release properties of the electrosprayed particles was possible by selection of a suitable solvent for electrospraying of the drug containing polymer solution. DCM and TFE were utilized as the solvents for the preparation of PLGA-Met particles, and the drug release properties of the electrosprayed particles were investigated. Our results suggest that the microparticles prepared using DCM as the solvent could reduce the burst release and provide a continuous release over a period of 41 days. The biocompatibility of the particles was also demonstrated by mesenchymal stem cells, elucidating its potential towards periodontal tissue regeneration applications.

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