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Printing particles: A high-throughput technique for the production of uniform, bioresorbable polymer microparticles and encapsulation of therapeutic peptides

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HIGHLIGHTS

- An ink-jet droplet generator allows individual polymer microparticles to be "printed".
- Rapid phase-separation produces highly uniform PLGA and PLA/PLGA blend microparticles.
- Peptides can be encapsulated and produce effective sustained release over several months.
- Using ink-jet nozzle arrays, particle production frequency was increased to >1 MHz.

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ABSTRACT

Bioresorbable polymer microparticles are widely used to provide long-lasting drug delivery in several chronic diseases. Having fine control over the physical properties of such microparticles can improve product reproducibility, performance and process efficiency. By using an ink-jet based droplet generator device, a new approach to microparticle manufacture was explored that uses an extremely rapid phase-separation to produce highly uniform, injectable microparticles from PLGA and a PLA/PLGA blend. To demonstrate the possible pharmaceutical applicability of the microparticle printing technique, the approved peptides ciclosporin A and octreotide were formulated, producing low-density microparticles that showed between sustained release over several months. The facile scale-up of the technique was demonstrated by using an array of 256 ink-jet nozzles, allowing over 1 million discrete particles per second to be produced. The new apparatus and methods described herein could be used across a wide range of biomaterials and therapeutic compounds.

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1. Introduction

Bioresorbable polymer microparticles are a fundamental component in several blockbuster pharmaceutical products, including Sandostatin LAR™ (Grass et al., 1996), Lupron Depot™ (Okada et al., 1989; Okada, 1997), Bydureon™ (Taylor et al., 2013) and Risperdal Consta™ (Knox and Stimmel, 2004). Typically, their function is to control drug release rate and provide a depot effect, enabling several weeks or months of sustained-release therapy from a single injection (Jain et al., 2016; Lee et al., 2016). The patient-experience and patient-compliance benefits this affords are significant (Gilroy et al., 2016; Lasser et al., 2005; Lancranjan et al., 1995; Hamm et al., 2001; Anselmo and Mitragotri, 2014). Though numerous other techniques are available for the production of bioresorbable microparticles, for example spray-drying or super-critical fluid extraction, historically the most widely-used techniques for preparing biopolymer microparticles involve a surfactant-stabilised single or double emulsion and evaporation of a volatile solvent (Jeffery et al., 1991; Cleek et al., 1997; Sah, 2000). Control over particle size and morphology has numerous advantages relating to yield, release kinetics, injectability, suspension homogeneity and stability and overall efficiency of drug encapsulation (Erden and Celebi, 1996; Wang et al., 2015). In recent years, advanced microparticle processing methods such as templated and membrane emulsification have been developed that afford such improvements, most notably control of particle size and of drug release rate (Wang et al., 2015; Wu et al., 2015; Zhu et al., 2015; Qi et al., 2013). Recently, promising microfluidics-based production methods have been demonstrated, which potentially address the limited particle size-control that is often a disadvantage of bulk emulsion processing (Keohane et al., 2014; Perez et al., 2015;

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Fontana et al., 2016; Palmer et al., 2008). Generally, these use segmented flow arrangements to produce uniformly-sized droplets of a dispersed phase, in which a polymer may be dissolved. In our own exploration of multi-channel microfluidic devices for microparticle production (unpublished results), the high viscosity and low surface tension of concentrated polymer solutions (>10% w/v, defined as mass of polymer dissolved in volume of solvent; Mw range 4–15 kDa, i.v. 0.14–0.22 dL/g) produced flow distribution challenges and unacceptable inter-channel variability at all but the lowest droplet production frequencies.

An alternative approach to the production of monodisperse droplets was considered, involving ink-jet-like piezoelectric actuation. In fact, oil/water emulsions involving PLGA have already been demonstrated using a submerged piezoelectric droplet generator (Fletcher et al., 2008). However, at elevated polymer concentrations, this approach may also be frequency limited by low liquid/liquid interfacial surface tension. To try to move away from an emulsion-based approach, it is necessary to rethink the types of solvents used for polymer and drug solvation, and their properties, including biocompatibility and relative miscibility. In 2003, another dual piezo-actuated flow process was described by Park et al., in which the synthesis of microparticles was accomplished via the phase separation of dissolved polymer within a droplet, which was induced by coalescence with another droplet consisting of a miscible anti-solvent (Yeo, 2003). Similarly, polymers of the PLGA and PLA families are highly soluble in DMSO but insoluble in solvents with high hydrogen-bonding potential such as water and alcohols. It was postulated that solid PLGA particles could be manufactured by manipulating this difference in solubility. As DMSO and water are infinitely miscible, a fixed volume of a concentrated PLGA solution in DMSO should rapidly phase separate when exposed to a larger volume of a miscible anti-solvent (such as water) as solvating DMSO molecules diffuse away.

The primary aim of this work was to explore a new, scale-able technique for the production of injectable, sub-50 μm diameter microparticles. Inkjet printing technology has been specifically developed for the facile, highly controllable production of uniform picolitre droplets; and as printing applications have evolved, devices have become compatible with non-conventional ‘inks’ with solvent content and indeed, pharmaceutical payloads (Liu et al., 2017; Kim et al., 2017; Daly et al., 2015; Cheow et al., 2015). It was therefore postulated that a piezoelectric droplet generator could be used to create a semi-continuous stream of DMSO droplets in air. Further, without a liquid/liquid interfacial surface tension limit, significantly higher polymer concentrations could be processed. Herein, a first small-scale apparatus for printing microparticles was developed that utilises a commercially available single-nozzle piezoelectric device to generate a continuous vertical stream of uniform droplets of a DMSO polymer solution; and a transverse anti-solvent jet to capture and separate these droplets before solvent extraction.

Each of the sustained release therapeutics mentioned above comprises an injectable mass of polydisperse polymer microparticles, in which a biologically-active small molecule or peptide is encapsulated. The efficiency of drug encapsulation and drug loading level are key parameters (Iqbal et al., 2015). Both affecting the viability, injectability and cost of a product. To assess the utility of the microsphere production method developed here, the encapsulation of two peptide-based pharmaceuticals was performed. Octreotide acetate, a cyclic 8 amino acid peptide that is indicated in acromegaly and carcinoid syndrome, has been widely studied and is available as a polymer depot formulation (Sandostatin LAR, Novartis). The latter is an once-monthly injection, comprising non-linear PLGA microparticles with a nominal octreotide loading of 4.65% w/w, defined as mass of drug/mass of solid (Mertens et al., 2010).

The cyclic peptide ciclosporin A is widely used as an immunosuppressant. It is administered orally, with 40% bioavailability. In several studies, ciclosporin A has been successfully encapsulated within bioresorbable polymer microparticles (Keohane et al., 2014; Sanchez et al., 1993; Urata et al., 1999). The drug has potential use in the ocular auto-immune disease non-infective uveitis, particularly in patients with posterior segment involvement where very long-acting anti-inflammatory therapeutics are required, and front-of-eye therapeutics are ineffective (Lallemand et al., 2003; Vitale et al., 1996).

The industrial relevance of the microparticle production technique described here is only realised upon achieving a certain throughput level. Modern inkjet printing technology has developed around linear arrays of 256 or 512 piezo-actuated nozzles. These are routinely used to accurately deposit nanolitre and picolitre volumes of various “inks” onto a range of solid substrates (Wijshoff, 2010; Thesen et al., 2014). It was hypothesised that similar devices could be adapted to allow the printing of picolitre volumes of a polymer solution onto a liquid anti-solvent substrate, to enable microparticle production at increased frequency and thus increased product mass/time.

2. Materials and methods

2.1. Chemicals

Poly(D,L)lactide (RG202H, Mw range 10–18 kDa, i.v. 0.16–0.24 dL/g) and Polyl(D,L)lactide-co-glycolide (RG752H Mw range 4–15 kDa, i.v. 0.14–0.22 dL/g) were purchased from Evonik, Germany. Ciclosporin A, dimethyl sulfoxide, phosphate-buffered saline and tert-butanol were purchased from VWR, UK. Sterile water was obtained from an Elga Purelab Option-Q (DV25) (electrical resistivity 18.2 MΩ). Octreotide acetate was obtained from PolyPeptide Laboratories Inc, USA.

2.2. Single-nozzle piezoelectric droplet generation

PLGA (RG752H, 400 mg) was allowed to dissolve in anhydrous DMSO (1 mL) at 37 °C over a period of 12 h. The resulting solution was filtered (PTFE 13 mm syringe filter, 0.45 μm) into a clean, dry glass vial. An MD-K-140 piezoelectric dispenser head (MicroDrop Technologies GmbH, Germany) with internal capillary diameter 70 μm was connected to a MD-K-140 digital pulse-generator. The device fluid feed-tube was submerged in the polymer solution and the vial was screw sealed. Using voltages in the range of 65–90 V, pulse-lengths between 8 and 15 μs and ejection frequencies between 2 and 4 kHz, stable ejection of discrete, satellite-free 180 pL droplets of feed solutions was achieved.

2.3. Anti-solvent fluid jet

To generate a horizontal anti-solvent jet, comprising either water or 15% v/v tert-butanol solution, a MZR 7255 pulseless micro-gear pump (HNP Mikrosysteme GmbH) was connected by HPLC tubing to a stainless-steel nozzle with a circular orifice of internal diameter 500 μm. At a pumping rate of 300 rpm (14.4 mL/min) the fluid jet had exit velocity 1.2 m/s and diameter of approximately 500 μm. The nozzle was located in transverse alignment with the vertical droplet stream, such that the anti-solvent jet coincides and captures the falling droplets. The jet travel distance was 13 mm, before meeting an elbow-shaped stainless-steel collecting tube. For experiments involving the 256 nozzle array, a higher velocity jet was required to prevent droplet coalescence, hence flow rate was increased to 125 mL/min. Here, the jet travel distance was 72 mm. Surface tensions of anti-

solvent fluids were measured using a computer-controlled Nima DST 500 Tensiometer device with disposable Wilhelmy plates (2 cm long).

2.4. Particle isolation

Particles were collected by allowing the fluid jet to deposit onto a stainless-steel filter mesh (~20 μm pore size) laid onto a Büchner funnel. Anti-solvent fluid was removed under reduced pressure to give a dry microparticle cake, which was removed and further dried overnight in a vacuum desiccator over silica gel.

2.5. Drug encapsulation experiments

Octreotide: PLGA (RG752H, 282 mg) was allowed to dissolve in anhydrous DMSO (1 mL) at 37 °C over a period of 12 h. The resulting colourless solution was then used to dissolve a pre-weighed mass of octreotide acetate (43 mg) in a clean glass vial. The mixture was vortexed until homogeneous, briefly centrifuged and then filtered (PTFE 13 mm syringe filter, 0.45 μm) into another clean, dry glass vial. As above, the piezoelectric dispenser head device was connected, the fluid feed-tube submerged in the solution and the vial was then screw sealed. Using voltages in the range of 70–90 V, pulse-lengths between 10 and 12 μs and an ejection frequency of 6 kHz, stable ejection of discrete, satellite-free 180 pL droplets of feed solutions was achieved.

Ciclosporin A: Here, the drug and mixed polymer solution was prepared according to the procedure above with PLA (RG202H, 150 mg), the PLGA copolymer (75:25 lactide/glycolide) (RG752H, 150 mg) and ciclosporin A (75 mg) in DMSO (1 mL). Voltages in the range of 65–90 V, pulse-lengths between 8 and 15 μs and an ejection frequency of 6 kHz was used to generate a stable stream of discrete, satellite-free 180 pL droplets.

Peptide-containing microparticles were produced, collected, washed and dried as above. Drug loading was determined by RP-HPLC and encapsulation efficiency was calculated by the formula EE% = Drug loading/Theoretical maximum drug loading × 100%.

2.6. Microparticle analysis and characterisation

Representative samples of microspheres were studied under a Motic-SFC-28 series optical microscope with 40× and 100× objectives using normal light. Particles diameters were measured using Motic Images 2.0 software. For ovoid microparticles, size was determined by carefully measuring circumference at at the widest point. After sputter coating with Au/Pt, morphology and surface properties of the microspheres were analysed by scanning electron microscopy (FEG-XL30, FEI, USA; beam energy 20 kV). Residual DMSO and tert-butanol content within microspheres was measured by GCMS using an HP 6890/5932 gas chromatograph - mass selective detector. Samples were analysed in duplicate with mass spectrometry using current reference standards. Acetonitrile was used as a solvent throughout.

2.7. HPLC analysis

2.7.1. Drug loading analysis

Product microspheres were analysed using an Agilent 1100 RP-HPLC; gradient elution; Aeris Peptide XB-C18 Octadecyl phase column (100 mm × 2.1 mm, 3.6 μm; Phenomenex). The gradient elution system (A: 0.1% trifluoroacetic acid in water; B: 0.1% trifluoroacetic acid in acetonitrile) was as follows with respect to B: 0–9 min 15–35%, 9–9.1 min 35–15%. The column oven temperature was 40 °C and flow rate was maintained at 1 ml/min. UV detection of octreotide acetate was carried out at 215 nm. Microsphere samples were dissolved in 0.1% trifluoroacetic acid in DMSO and injected into the HPLC (4 mg in 1 mL, 3 μL injection volume). Octreotide acetate standards were freshly prepared by serial dilution of an octreotide acetate stock solution in 0.1% trifluoroacetic acid in DMSO (calibration curve standards 0, 5, 10, 100, 200, 300, 600 and 1000 μg/mL). Octreotide acetate QC solutions were individually prepared at 50 and 500 μg/mL in DMSO.

2.7.2. In vitro release analysis

Stored receptor solution samples were thawed and briefly vortexed. Using the apparatus, stationary and mobile phases described above, the time course samples were injected directly into the HPLC (10 μL injection volume). Octreotide acetate standards were prepared in PBS (calibration curve standards 0, 1, 2.5, 5, 10, 25, 75, 100 and 150 μg/mL). Octreotide acetate QC solutions were individually prepared at 50 and 125 μg/mL in PBS.

2.8. Drug release measurement

In vitro release of octreotide/ciclosporin A from microspheres was measured as follows. Five milligrams of microspheres were accurately weighed into a 12 mL screw-cap centrifuge tube. PBS (5 mL, pH 7.4, 0.1 M) was added and the suspension was incubated at 37 °C with constant agitation for the duration of the release study. At each sample time point, the tubes were centrifuged at 2000 rpm for 2 min to pellet the microparticles. Samples of the supernatant were then removed as follows. For octreotide-containing spheres, 10% of the supernatant volume was carefully removed via pipette and replaced with fresh PBS. Due to the low solubility of ciclosporin A in PBS, the release medium can quickly become saturated, whereby sink conditions are lost and release rates are affected. Hence, for ciclosporin A-containing spheres, 90% of the supernatant was removed and replenished at each time point. After sampling, tubes were returned to the incubator and agitated as before. Samples were stored in amber vials at −20 °C to await HPLC analysis. All release experiments were performed in triplicate.

2.9. Piezo nozzle-array droplet generation

A KM512-LHX industrial print head array (Konica Minolta, Japan) was connected to a pressure controller (MicroDrop GmbH, Germany) and pre-flushed with DMSO before use. Droplet ejection was controlled by GIS hardware/software (Global Inkjet Systems, UK) and the visualization and in-flight analysis of droplets was controlled by a JetXpert drop watcher device (JetXpert, USA). The head was programmed to run in 'spit' mode (40 ± 3 pL droplet volume; 15.1 V; frequency 2–4 kHz per nozzle; 256 nozzles).

3. Results and discussion

To test the microparticle printing methodology, a droplet-on-demand piezoelectric actuation system was assembled, comprising a single dispenser head (MicroDrop Technologies GmbH, Germany) with an internal glass capillary of diameter 70 μm. The software package Autodrop (Microdrop GmbH) was used to control piezoelectric actuation to produce a medium frequency (2–6 kHz) stream of 180 pL droplets of a PLGA solution in DMSO. Suitable operating conditions for the piezoelectric actuator were identified following some trial and error, eventually producing a stable vertical stream of uniformly sized droplets (Figs. 1 and 1C).

3.1. Droplet by droplet processing

To produce discrete polymer particles and avoid uncontrolled bulk-precipitation, care must be taken to keep apart polymer-

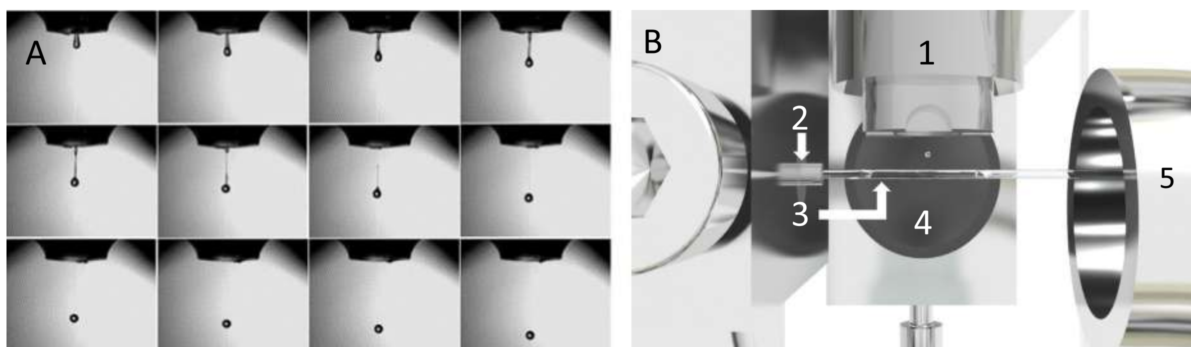

Fig. 1. (A) Piezoelectric droplet ejection from MicroDrop dispenser head with internal diameter 70 μm, aligned between camera and stroboscope. Droplet ejection frequency 6 kHz, voltage 75 V, pulse-length 14 μs. (B) Experimental apparatus comprising (1) Piezoelectric droplet generator. (2) Stainless-steel nozzle. (3) Anti-solvent jet. (4) Camera lens (5) Collection conduit.

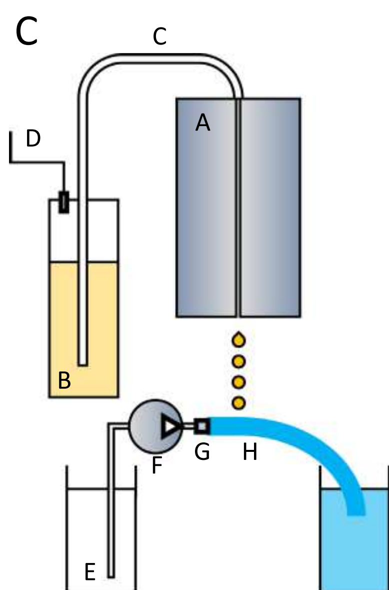

Fig. 1C. Schematic of piezo-actuated droplet generator with anti-solvent jet flow (A) piezo actuator (MicroDrop GmbH), (B) polymer/drug solution, (C) feed-tube, (D) connection to pressure regulator, (E) anti-solvent solution reservoir, (F) pulseless micropump, (G) stainless-steel nozzle (i.d. 500 μm), and (H) anti-solvent jet flow.

containing droplets as they are exposed to the aqueous anti-solvent phase. As the droplets are ejected in a serial fashion from the piezo-actuator, a similar sequential phase separation process was envisaged. In keeping with the concept of flow-processing, the anti-solvent phase was presented as a 500 μm diameter jet, which was aligned to coincide with the droplet stream and thereby capture and separate each falling droplet (Fig. 1B). In initial experiments, distilled water was used as the anti-solvent fluid. Droplet capture and rapid solvent extraction was observed, yielding, after vacuum filtration of the product through a stainless-steel mesh, discrete solid microparticles of lenticular shape (Fig. 2B). This flattened particle morphology was attributed to the deformation the droplets undergo upon contacting the purely aqueous anti-solvent surface, where the surface tension (γ) of water at room temperature was experimentally determined using a Wilhelmy plate-type air/liquid surface tensiometer to be 67.9 mN/m. It was hypothesised that by reducing γ, the droplet deformation effect could be diminished and thus improve the morphology of the resulting particles. Several possible approaches were considered, including increasing temperature, ultrasonic perturbation and inclusion of a second water- and DMSO-miscible liquid with hydrogen-bonding potential. The latter approach proved to be the more readily achievable and several anti-solvents mixtures containing C_{1-4} alcohols were tested. Methanol and ethanol were found to cause rapid plasticisation of PLGA particles and were therefore excluded from further testing. An improved anti-solvent fluid mixture was formulated comprising 15% v/v tert-butanol in water, with a surface tension of 30.4 mN/m at room temperature. Using this anti-solvent mixture, uniformly sized, near-spherical microparticles of mean diameter ~42 μm (standard deviation 2.8 μm) were successfully obtained and analysed by optical and scanning electron microscopy (Fig. 2B). Analysis of the isolated particles showed that their shape and aspect ratio are determined by the extent to which droplets are deformed at the moment they strike the anti-solvent flow. This suggests that the solvent extraction and phase-separation occurred extremely rapidly, i.e. before each droplet is able to fully relax (due to surface tension) to a spherical shape. This is likely due to the very high miscibility of the solvent and anti-solvent solutions, which causes immediate solvent/anti-solvent equilibration.

3.2. Drug loading

The next objective was to efficiently encapsulate an active pharmaceutical ingredient within the product microspheres. Here, two clinically-relevant cyclic peptide drugs, octreotide and ciclosporin A were tested. DMSO is an excellent solvent for a wide range of peptides, it was therefore straightforward to produce a homogeneous solution of PLGA and octreotide acetate of suitable concentration (28% weight of polymer/volume and 13% weight of octreotide/weight of polymer, respectively), which was loaded as before into the feed reservoir of the piezoelectric apparatus. Again, using the optimised anti-solvent mixture comprising 15% v/v tert-butanol solution, solid microspheres were obtained, which after vacuum filtration were isolated, dried *in vacuo* and analysed. By HPLC, the octreotide content was found to be 1.9% w/w. This surprisingly low drug-loading value was attributed to the partitioning of octreotide acetate into the aqueous anti-solvent phase, in which it is also very soluble. It was postulated that partitioning of octreotide might be reduced if the phase-separation were performed at lower temperatures. By pre-cooling the anti-solvent phase to between 5 and 8 °C, ovoid microparticles of mean diameter 39.3 μm (standard deviation 3.2 μm) were produced (Fig. 3). After drying *in vacuo*, the microsphere sample was analysed for drug content and found to have an octreotide loading of ~7.2% w/w, corresponding to an encapsulation efficiency of 54%. By GCMS analysis, the dried microsphere sample was found to contain 410 ppm DMSO and <100 ppm tert-butanol.

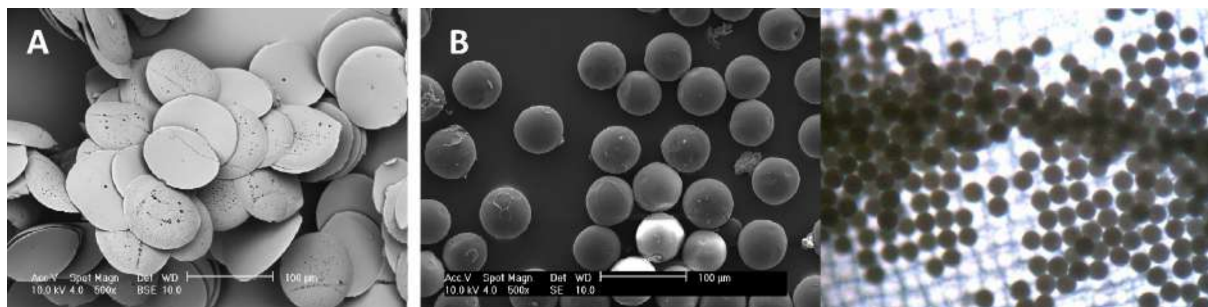

Fig. 2. Microparticle scanning electron micrographs. (A) Flattened PLGA microparticles produced in water anti-solvent at RT. (B) Spherical PLGA microparticles produced in 85:15 v/v water:tert-butanol solution at RT and optical microscope image on stainless-steel filter mesh.

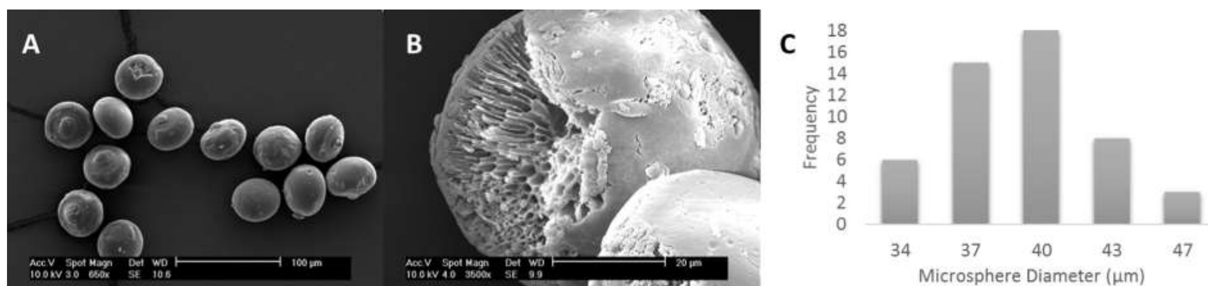

Fig. 3. Solid PLGA (RG7525 H) microparticles containing 7.2% w/w Octreotide: (A and B) scanning electron micrographs. (C) Histogram of particle diameters, measured by optical microscopy and Motic Images 2.0 software.

Ciclosporin A is a highly hydrophobic, cyclic peptide that is used as an orally-administered anti-inflammatory, anti-fungal and immunosuppressant agent (Vitale et al., 1996; Wakeel et al., 2012; Parquet et al., 2000). For some auto-immune conditions such as posterior uveitis, which often necessitates intravitreal injection or implantation, very-long acting formulations of immunosuppressants are desirable. The procedure developed above for producing PLGA particles was modified to enable production of microspheres from a blend of slower-degrading polymers, i.e. poly(D,L)lactide (PLA) and a 75:25 PLGA copolymer. A homogenous solution of PLA and PLGA copolymer (15% w/v RG202H; 15% w/v RG752H) and ciclosporin A (24% weight ciclosporin A/weight of total polymer) was prepared and processed in the single-nozzle droplet ejection apparatus as before. Ciclosporin A is rather more hydrophobic than octreotide (calculated Log P values are 3.35 and 0.77, respectively). It was therefore anticipated that the affinity between ciclosporin A and poly(D,L)lactide would be greater and could result in a higher drug loading value, hence a higher initial peptide concentration was used. Using the previously optimised tert-butanol containing anti-solvent and by pre-cooling this to between 5-8 °C, solid ovoid microparticles were produced with a mean diameter of 59 μm (standard deviation 3.2 μm). The microparticles were analysed for drug content and found to have a ciclosporin A loading of 22.3% w/w, corresponding to a 93% encapsulation efficiency (Fig. 4). By GCMS analysis, the dried microsphere sample was found to contain <100 ppm of both DMSO and tert-butanol.

To study the interior of the microspheres, samples were chopped with a razor blade before sputter coating and SEM analysis. Surprisingly, particles showed a relatively intact, smooth surface, surrounding a highly porous, honeycomb-like network of channels. It is hypothesised that this unusual morphology was caused by rapid solvent egress during the phase-separation process. Theoretically the internal structure and density of microparticles could be modified by reducing the relative miscibility of the solvent and anti-solvent fluids, and thereby the rate of solvent egress.

3.3. Drug release

The release rates of the encapsulated peptides were measured using an *in vitro* release assay. Octreotide-loaded PLGA (RG752H) microparticles showed a mean initial burst release of 2.9%, followed by a steady daily release rate of 0.26% per day until day 21. Thereafter, release rate increased to an average 0.64% per day until day 49, at which time measurements were ceased (Fig. 5). The total quantity of octreotide released into the test system was calculated to be 34.5% of total loading.

Ciclosporin A-loaded PLA (RG202H) microparticles were released in a similar fashion, however the sample volumes were increased to account for the low solubility of the peptide in the receptor medium. A mean initial burst release of 13.8% was measured, and this was followed by a daily release of rate of 0.81 μg/mg (mass ciclosporin A/mass microparticles) per day until day 60 and 0.13 μg/mg per day between day 60 and day 100 (Fig. 6). In this case the total quantity of ciclosporin A released into the receptor fluid was calculated to be 40% of total peptide loading. Interestingly, the plateau phase that is commonly-observed in microsphere-based depot formulations after initial burst release, was not observed in any experiment involving printed microparticles. Instead, a relatively-steady sustained release was recorded from after 24 h.

Theoretically, it may be possible to affect the rate of drug release by seeking to modify the internal structure and/or porosity of the product microparticles. By varying the internal density, the relative effects of surface and bulk-erosion may be affected, thus changing the drug release kinetics.

3.4. Piezo nozzle-array device

Having demonstrated the efficient encapsulation of therapeutic peptides, the throughput of the apparatus was next addressed. To increase the droplet production frequency and thereby the yield of microparticles per unit time, the principle of droplet-by-droplet processing was extended to include multiple nozzles. The single

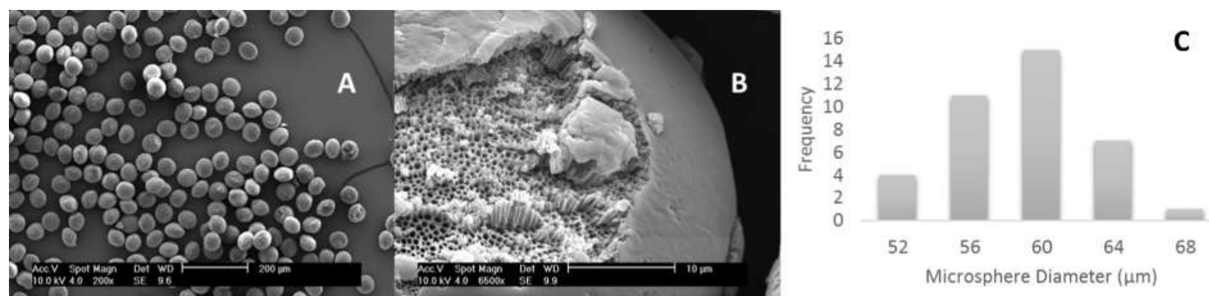

Fig. 4. Solid PLA/PLGA blended microparticles containing 22.3% w/w ciclosporin A: (A and B) scanning electron micrographs. (C) Histogram of particle diameters, measured by optical microscopy and Motic Images 2.0 software.

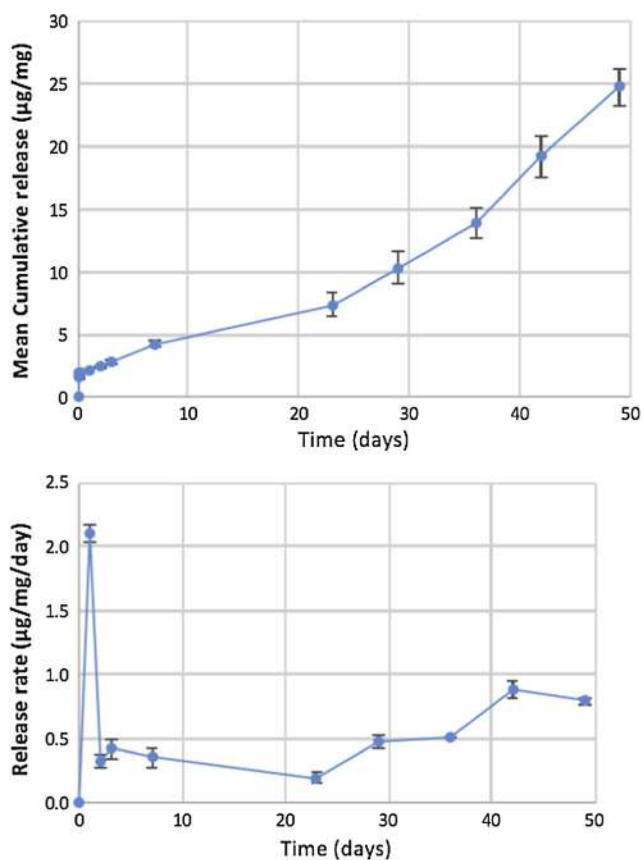

Fig. 5. *In vitro* cumulative octreotide release (1 mg/ml) measured over 49 days, performed in triplicate. Release rate/time plot.

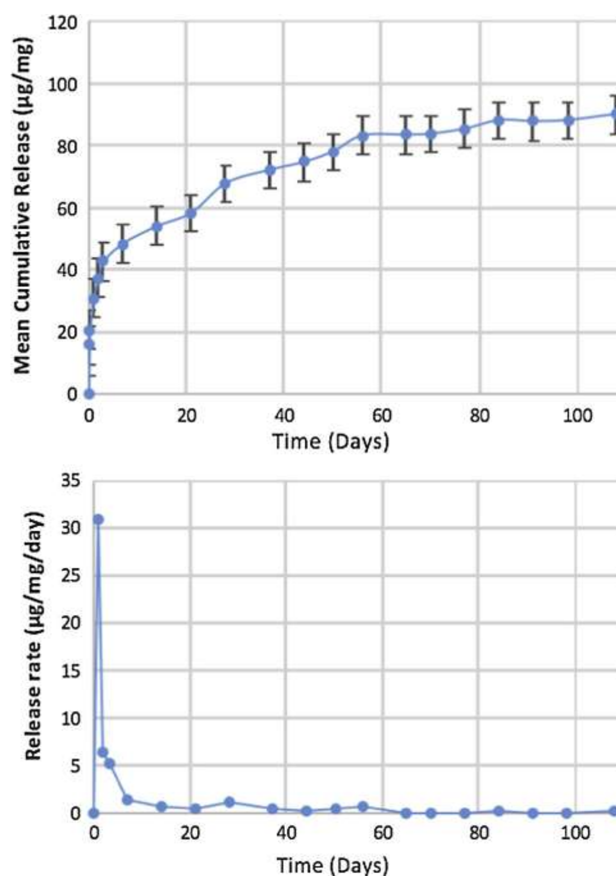

Fig. 6. *In vitro* cumulative ciclosporin A release (1 mg/ml) measured over 110 days, performed in triplicate. Release rate/time plot.

nozzle apparatus was adapted to incorporate an off-the-shelf piezoelectric nozzle array (KM512LH, Konica Minolta, USA), featuring 512 parallel apertures. This ink-jet array device can be similarly software-driven, allowing some or all nozzles to eject droplets at a desired frequency. This particular device ejects droplets of 42 pL volume and was therefore expected to produce substantially smaller solid microparticles. Again, following initial trial-and-error optimisation of voltage and pulse-length parameters, stable ejection conditions were identified, enabling continuous ejection of a PLGA solution in DMSO from 256 nozzles and at frequencies between 2 and 4 kHz.

As before, a transverse anti-solvent jet was employed. A single high velocity fluid jet was aligned along the length of the linear nozzle array to capture droplets as they fell under gravity. The product, a slurry of solid microspheres suspended in anti-solvent, was collected as before into a wide-bore conduit and allowed to continuously flow onto a stainless-steel filter mesh (Fig. 7).

Initial experiments involved filtered solutions of PLGA in DMSO and the preferred tert-butanol-containing anti-solvent solution. Uniform solid microparticles of $\sim$33 µm (standard deviation 2.4 µm) diameter were successfully manufactured at an overall rate of 0.51×10^6/s. These were analysed by SEM and optical microscopy and shown to be discrete and remarkably uniform in size (Fig. 8). As before, the particles were non-spherical in morphology, showing obvious deformation due to surface tension effects and rapid phase-separation.

The experiment was expanded further to include the soluble peptide octreotide acetate. Using the previous drug/polymer formulation and similar droplet ejection conditions, 256 vertical droplet streams were established at an individual nozzle frequency of 4 kHz and hence an overall frequency 1.02×10^6/s. To maximise drug encapsulation efficiency, the anti-solvent fluid was pre-cooled to between 5 and 8 °C. Using the same collection method,

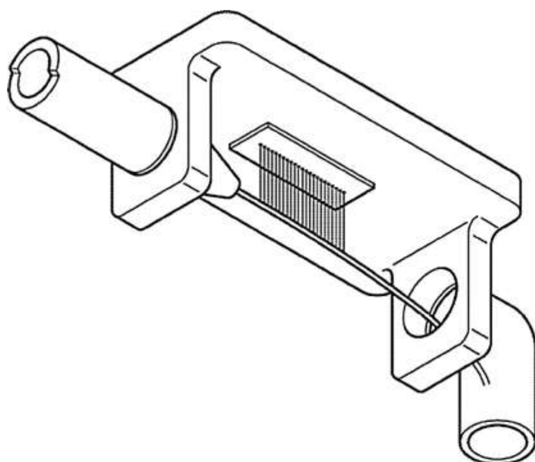

Fig. 7. Schematic of print-head nozzle array apparatus with transverse anti-solvent jet flow and collection conduit.

solid octreotide-containing microparticles of ∼26 μm (standard deviation 2.0 μm) diameter were isolated, dried and analysed as above (Fig. 8). Drug loading analysis by HPLC showed an octreotide loading of 6.9% w/w corresponding to an encapsulation efficiency of 52%.

Externally, the octreotide-loaded microparticles appeared to have a smooth, dome-shaped front face and a small centrosymmetric tail-like projection on the back face, due to strong deformation of the droplet upon striking the surface of the anti-solvent jet. A further reduction in the surface tension of the anti-solvent phase could minimise this deformation of droplet morphology and thereby produce more-spherical microparticles. Before SEM analysis, the octreotide-containing microparticles were again cross-sectioned with a razor blade to expose the interior structure. As before, a similar low-density honeycomb-like structure was observed.

4. Conclusion

In this study, the suitability of ink-jet printing technology was demonstrated for the production of long-acting microparticulate peptide formulations. In particular, highly uniform microparticles of PLGA and a PLA/PLGA blend were produced with mean diameters ranging between 20 and 60 μm, depending upon ejected droplet volume. Size distributions of microparticle samples were narrow, with standard deviations (on diameter) of between ±2 and ±5 μm. It is envisaged that by further varying the ejected droplet volume, an ever-wider size range could be achievable. The encapsulation of a hydrophilic and a hydrophobic peptide was demonstrated, giving acceptable encapsulation efficiencies, sensible drug-loading levels and very low residual solvent levels. The technique should be further applicable to other peptide and small-molecule therapeutics, where polymer-mediated sustained release is required. The physical properties of product microparticles (including density, hydrophobicity and internal morphology) are affected by the physicochemical nature of an encapsulated drug compound, and as discussed, this can impact drug release kinetics by altering the rates of ongoing processes of surface and bulk erosion.

The flow-process developed here was readily scaled-up to give microparticle production rate of >1 MHz. This approach could be further up-scaled by increasing droplet ejection frequency or simply by using multiple parallel piezo nozzle arrays; thus delivering tens of millions of uniform microparticles per second. The particle printing methodology described here is particularly efficient with minimal wastage of active ingredients. In further testing, continuous multi-nozzle droplet printing and microparticle production have been sustained for periods of >6 h without intervention. The process has recently been used to manufacture microparticles at a 250 g pilot-scale for the clinical trial of a unique formulation of octreotide acetate.

The technique could become widely relevant for printing other biomaterials and pharmaceutical payloads, particularly as the

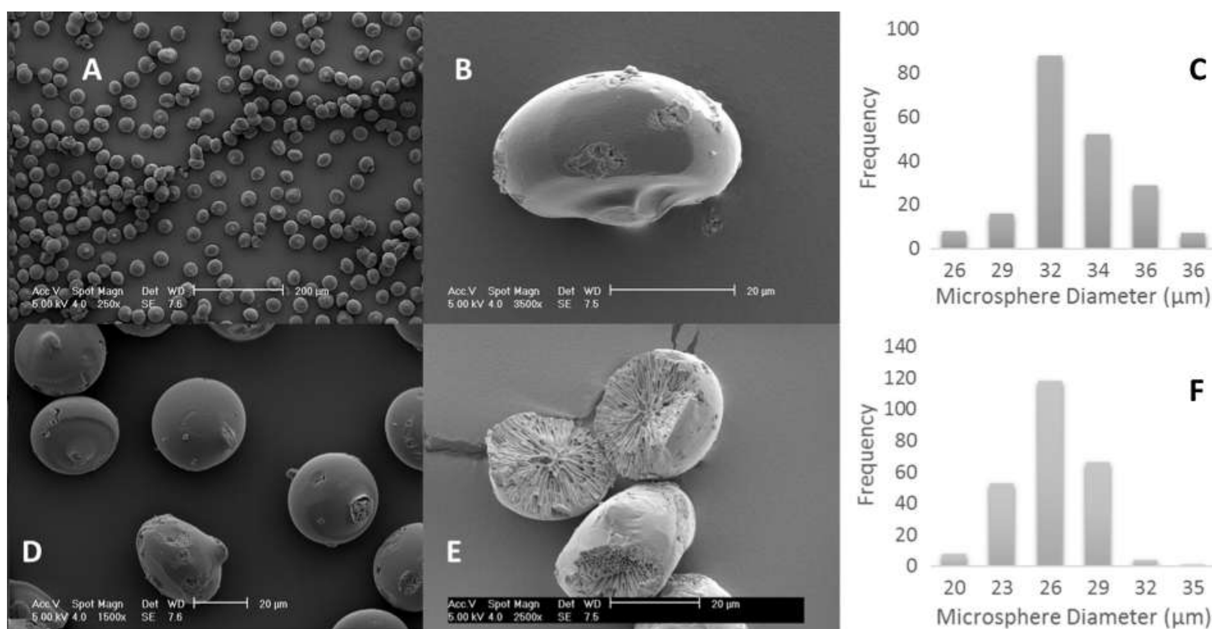

Fig. 8. Nozzle array microparticles (A) Non-spherical 33 μm (standard deviation 2.4 μm) diameter drug-free PLGA microparticles produced from piezo-nozzle array, 256 nozzles, @4.0 kHz. (B) Close-up SEM image of single particle. (C) Particle size distribution histogram (200 individual particles measured by optical microscopy). (D) Non-spherical 26 μm (standard deviation 2.4 μm) diameter Octreotide-loaded PLGA microparticles produced from piezo-nozzle array, 256 nozzles, @4.0 kHz. (E) Close-up and cross-section SEM image of Octreotide-containing microparticles. (F) Particle size distribution histogram (250 individual particles measured by optical microscopy).

pharmaceutical industry continues to move towards continuous manufacturing processes.

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