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Microfluidics-assisted engineering of polymeric microcapsules with high encapsulation efficiency for protein drug delivery

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ABSTRACT

In this study, microfluidic technology was employed to develop protein formulations. The microcapsules were produced with a biphasic flow to create water–oil–water (W/O/W) double emulsion droplets with ultrathin shells. Optimized microcapsule formulations containing 1% (w/w) bovine serum albumin (BSA) in the inner phase were prepared with poly(vinyl alcohol), polycaprolactone and polyethylene glycol. All the particles were found to be intact and with a particle size of 23–47 μm. Furthermore, the particles were monodisperse, non-porous and stable up to 4 weeks. The encapsulation efficiency of BSA in the microcapsules was 84%. The microcapsules released 30% of their content within 168 h. This study demonstrates that microfluidics is a powerful technique for engineering formulations for therapeutic proteins.

1. Introduction

Protein and peptide therapeutics often have poor oral bioavailability. Increasing the bioavailability is challenging, since the gastrointestinal tract has various barriers for the protein drugs to overcome before reaching the bloodstream (Zhou, 1994). Proteins and peptides are very sensitive to enzymatic degradation, aggregation, adsorption, and denaturation (Fix, 1996; Saffran et al., 1986). Other physical barriers for proteins and peptides absorption are the size, charge and solubility constraints (Cox et al., 2002). Crossing the epithelial intestinal cell layer is possible via diffusion through the hydrophobic tight junctions of the cells by passive transport, via facilitated transcellular diffusion through the lipophilic absorptive cells, or via active carrier-mediated transport systems or transcytosis (Ingemann et al., 2000). Thus, drug carrier systems for oral delivery of proteins have an important role in the development of protein-based formulations (Langer, 1998).

Polymeric microcapsules hold great potential as delivery systems for oral protein delivery (Freiberg and Zhu, 2004). Polymeric microcapsules can be widely applied to many situations where continuous and controlled drug administration is essential, and the use of microcapsules for drug delivery is not limited to any specific illness. For example, polycaprolactone (PCL), a semi-crystalline, hydrophobic, (Chandra and Rustgi, 1998) biocompatible and biodegradable polymer (Pitt, 1990; Chen et al., 2000) has been widely used for the preparation of microcapsules for drug delivery applications (Jeong et al., 2003; Natarajan et al., 2011; Scala-Bertola et al., 2012; Somavarapu et al., 2005).

Microfluidic technology has various advantages for the preparation of polymeric microcapsules, (Utada et al., 2005) because it allows precise control over the fabrication process (Umbanhowar et al., 2000). With microfluidic devices it is possible to mix immiscible liquids in a tunable manner by using three-dimensional flows (Squires and Quake, 2005). This makes the precise manufacturing process possible and enables choosing the chemical compositions and structures of the prepared particles independently

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(Duncanson et al., 2012b). More importantly, double emulsion droplets with ultra-thin shells for preparing microcapsules can be created by using a biphasic flow in a glass capillary device that combines co-flow and flow-focusing without additional energy input that affects protein structures (Kim et al., 2011, 2013).

In this study, the microfluidic technique was employed as a tool for templating and fabricating biocompatible polymeric microcapsules for protein drug delivery. The microcapsules were loaded with bovine serum albumin (BSA) and the properties of the formed microcapsules were evaluated.

2. Methods

2.1. Chemicals

The model protein used in this study, BSA, was purchased from Sigma–Aldrich, Germany. The polymers used were poly(vinyl alcohol) (PVA, 87–89% hydrolyzed, Mw 13,000–23,000; Sigma–Aldrich, USA), polyethylene glycol 6000 (PEG6000, Mw 5000–7000; Fluka Analytical, Germany) and polycaprolactone (PCL, Mw 70,000–90,0000; Sigma–Aldrich, USA). Phosphate buffers (100 mM) at pH 7.2 used in the dissolution tests were prepared according to the European Pharmacopoeia (Ph. Eur. 7th edition). All the other reagents were used as received and were of analytical grade.

2.2. Fabrication of the microfluidic devices

All microcapsule formulations were fabricated using glass microcapillary devices (Chu et al., 2007; Duncanson et al., 2012a; Kim and Weitz, 2011; Shum et al., 2011). Combining co-flow and flow-focusing within the glass capillary device enabled the preparation of microcapsules from more complex and viscous materials (Utada et al., 2005) Cylindrical capillaries were pulled with a Flaming/Brown micropipette puller (Model P-97, Sutter Instrument Co., USA) to obtain tapered tips and to form tips with diameter of 100 and 150 μm. Cylindrical capillaries were coated with hydrophilic or hydrophobic coating, corresponding to whether they contained the water or the oil phase. Hydrophobic and hydrophilic coating agents were trimethoxy(octadecyl) silane (Sigma–Aldrich, USA) and 2-[methoxy(polymethyleneoxy) propyl]-9-12 trimethoxysilane (Gelest Inc., Netherlands).

2.3. Microfluidic encapsulation of BSA

The droplet formation in microfluidic devices is based on jetting to dripping transition and taking advantage of the hydrodynamic instability (Powers et al., 1998). With the jetting to dripping transition the drop formation involves a balance between the viscous drag of the coaxial fluid that pulls on the drop and the surface tension forces (Umbanhowar et al., 2000). The surface energy is decreased as the jet breaks into drops, and thus, the drop formation mechanism can be explained via the Rayleigh–Plateau instability (Squires and Quake 2005; Utada et al., 2007) (Fig. 1).

Because the physics of the process is well understood, microfluidic devices make is possible to mix immiscible liquids with precise control (Squires and Quake, 2005). The microfluidic technology has various advantages, particularly the ability to create actually three-dimensional flows (Utada et al., 2005). This makes the precise manufacturing process possible and enables gaining the control over the immiscible fluids and their dimensions. More specifically, the droplets of desired structure and size can be created by simply adjusting the process and formulation variables.

The microfluidic device employed a biphasic flow to produce microcapsules from double emulsion droplets with ultrathin shells (Fig. 2) (Kim et al., 2011). Emulsion phases of the water–oil–water (W/O/W) emulsion were pumped into the glass capillary devices with syringes using Harvard pumps (Harvard Apparatus Hollston, USA). Syringes were attached to the inlets of the glass capillary device with plastic tubing (PE5 0.86 × 1.32 mm, Scientific Commodities Inc., USA). Microfluidic technology requires specific formulation to successfully produce double emulsion droplets. Various formulations with different flow rates were examined during the formulation optimization process (Table S1). The formulations investigated in the screening study were chosen based on the viscosity and compatibility of the components. The optimized formulation contained 5% (w/w) of PVA in water as the outer phase, 3% (w/w) of PCL in ethyl acetate as the middle phase, and 20% (w/w) of PEG6000 and PVA (1:4) and 1% (w/w) BSA in water as the inner phase. The water-solubility of BSA and increase in viscosity limit the maximum amount of BSA used in this formulation to 1% (w/w). The inner and middle phases flowed at the rate of 1000 μL/h and the flow rate of the outer phase was 3000 μL/h, respectively.

2.4. Particle characterization: particle morphology, particle size, stability and core shell structure

The morphology and surface properties of the microcapsules were examined by electron scanning microscopy (SEM). SEM images were taken with an environmental SEM microscope (Carl Zeiss AG, EVO 55, Germany) with wet stage at chamber pressure of 682 Pa and 26 kV. Samples were placed on wet paper and further preparation was not required. The chamber was cooled down with liquid nitrogen to water vapor state and the electric beam was run through upper and lower aperture of 100 and 500 μm.

The particle size was determined by optical microscopy and diameter measurements from 5 batches ($n = 100$). Diameter measurements were conducted with software for scientific image analysis (ImageJ freeware, National Institutes of Health, USA) and measured according to 1 mm scale for the optical microscope. Short time stability was examined by monitoring the collapse rate of the particles with optical microscopy. 5 batches of particles were

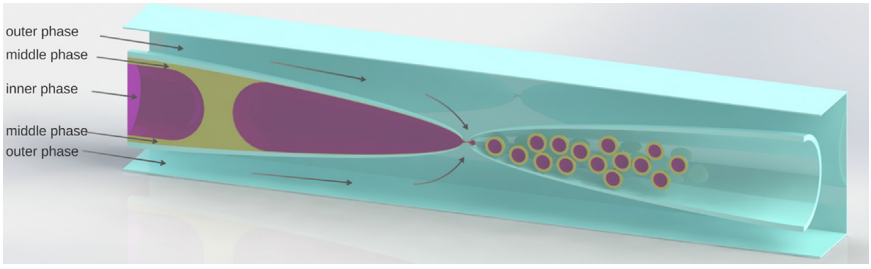

Fig. 1. The inner and middle phases flow in the cylindrical capillary on the left. Stretched capillary is inserted into this cylindrical capillary and the inner phase flows through it, forming large droplets of water phase into the oil phase. This forms droplets with ultrathin shells as the phases move to the collection capillary (on the right) and form a double emulsion with the outer phase flowing from the square capillary.

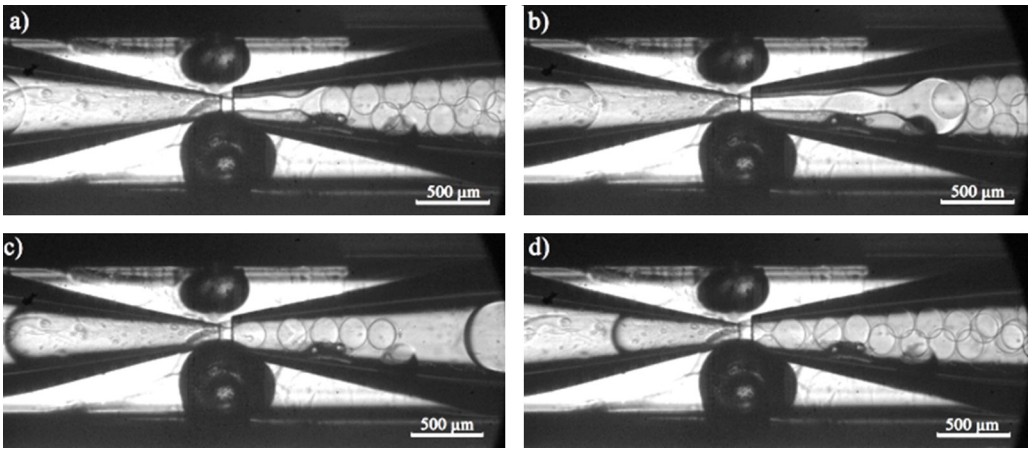

Fig. 2. Preparation process of the microcapsules from double emulsion (W/O/W) in the microfluidic glass capillary device employing a biphasic flow. Optical microscope images show the droplet formation with biphasic flow: (a) oil phase reaching the tip of the collection capillary; (b) oil droplets (O/W) forming; (c) beginning of the formation of double emulsion (W/O/W) droplets; and (d) continuing of the droplet formation from the water drop.

observed and the collapse rate of the particles was determined ($n = 200$). 3 batches (batches #1–3) were monitored for 4 weeks the samples were taken when the time elapsed was 0, 1, 3, 7, 14, 21 and 28 days and 2 batches (batches #4–5) were monitored for 6 weeks and the samples were taken when the time elapsed was 0, 28, 35 and 42 days. Batches for the short time stability tests were stored in the collection media at 8 °C.

2.5. Encapsulation efficiency

The core shell structure of the microcapsules was determined with three parallel tests using confocal microscope (Leica Microsystems CMS GmbH, Germany), ($n = 200$). Two fluorescent dyes were employed: FITC-dextran (Mw 10,000, Molecular Probes, USA) in the inner phase and 3,4,9,10-perylene-tetracarboxylic dianhydride (Sigma–Aldrich, Germany) in the middle phase. The labelling agents were chosen based on their solubility in the phases to be stained; as a very hydrophilic compound FITC-dextran was used in the inner water phase, and perylene, a hydrophobic fluorescent agent, was used for the ethylacetate middle phase. As a result, the fluorescent agents remained in their respective phases after preparation. Thus they remained in their selected phases after preparation. The excitation/emission spectra for FITC-dextran and perylene were 490/525 nm and 410/487 nm, respectively.

The encapsulation efficiency of BSA into the formed microcapsules was determined from the supernatant of three different batches immediately after the droplet preparation process was completed. The supernatant sample was withdrawn from the particle-free top of the collection vial whereas all microcapsules were settled at the bottom of the vial due to the phase density difference. The encapsulation efficiency for BSA was examined by comparing the total quantity of BSA with the quantity of BSA in the supernatant. Samples of BSA were analyzed with high performance liquid chromatography (HPLC Thermo System Products, Agilent 1200 Infinity Series, Agilent Technologies, Germany). A Vydac 214MS C4 column (Grace Davison Discovery Science, USA), flow rate of 1 mL/min with a mobile phase consisting of acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA) were used operating at 40 °C. The protein analysis required a gradient of TFA and ACN at ratios of 80:20 (v/v) to 35:65 (v/v) within 12 min and reversing back to 80:20 (v/v) within 8 min, with a total run time of 20 min. The UV detection of BSA was set at 210 nm with a retention time of 8.5 min. A standard curve for BSA quantification was made from BSA concentrations of 5–500 μg/mL ($R^2 = 0.9999$).

2.6. Dissolution experiments

Drug release tests of BSA encapsulated in the microcapsules and of free BSA were conducted in glass vials under heating and stirring (H+P Labortechnik AG, Multitherm, Germany) in pH 7.2 phosphate buffer media. The temperature was monitored during the dissolution tests and kept at 37.0 ± 0.5 °C under magnetic stirring of 400 rpm. The volume of the phosphate buffer media was 20 mL. Each aliquot taken was 1 mL and replaced with the same volume of fresh media. Aliquots were taken at the time points ranging from 30 s to 2 weeks. Drug release tests were conducted in triplicate. The aliquots were analyzed with the HPLC method as described above.

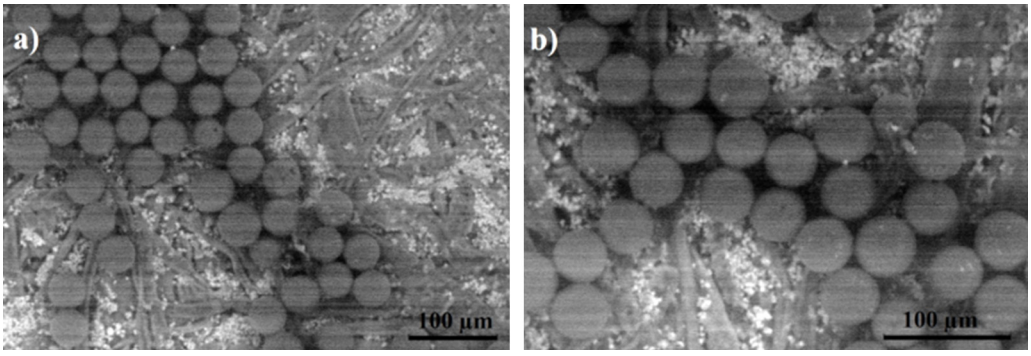

Fig. 3. SEM images of PCL microcapsules: (a) 506× magnification taken at chamber pressure of 682 Pa with 26 kV; and (b) 735× magnification of different particles of the same batch with the same pressure and voltage. The microparticles appeared to have a non-porous structure, smooth surface and were monodisperse in size.

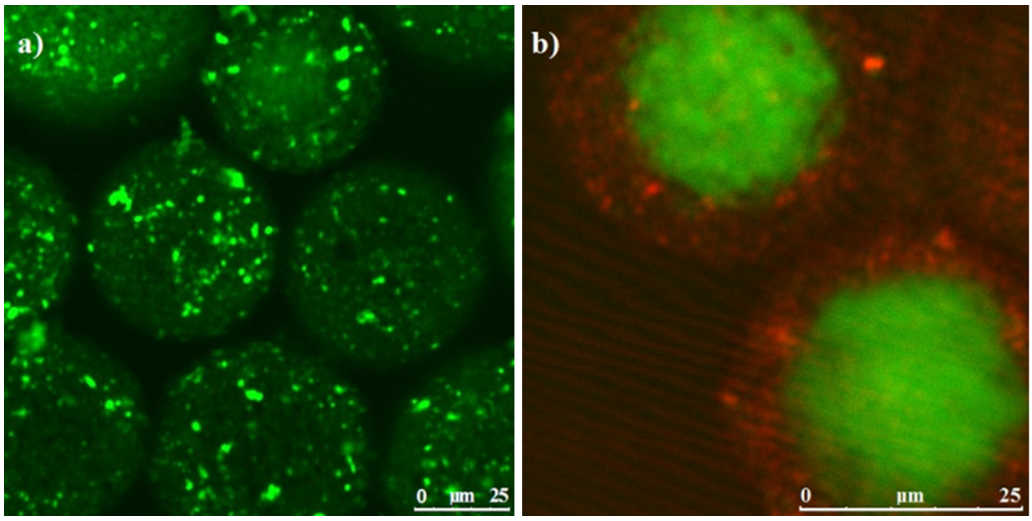

Fig. 4. Confocal fluorescence microscopy images of (a) PCL microcapsules encapsulating FITC-dextran (green); and (b) PCL particles with FITC-dextran (green) in the inner phase and perylene (red) in the middle phase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

For the HPLC analysis the standard curve for BSA quantification from concentrations of 5 to 500 μg/mL corresponding to the dissolution media was determined (R^2 = 0.9999). Furthermore, drug release behavior from a single PCL microcapsule was monitored on a confocal microscope (Leica Microsystems CMS GmbH, Germany), and the fluorescent agent used in the inner phase was FITC-dextran and an excitation/emission spectra 490/525 nm.

3. Results and discussion

3.1. Morphology, size, stability and inner structure of the microcapsules

The morphology studies with SEM indicated that the PCL microcapsules were spherical, intact and monodisperse in size (Fig. 3). Furthermore, the microcapsules appeared to have non-porous structure and smooth surface. Non-porous structure inhibits a fast release of the drug from the microcapsules (Scala-Bertola et al., 2012); PCL particles prepared in other studies also had similar surface properties and intact form (Bolzinger et al., 2007; Hnaien et al., 2011; Jeong et al., 2003; Somavarapu et al., 2005).

The particle size of the microcapsules produced by microfluidics was varied from 23–47 μm depending on the device used for the preparation of the batch. Average diameter of the microcapsules was 39 ± 10 μm (n = 100, 5 batches), particle size variation within each batch was moderate (Table S2). The short time stability tests indicated that the particles were stable up to 4 weeks. Collapse rate of the particles after that was approximately 15% per week.

All the microcapsules produced and collected contained the inner phase and the middle phase. Fig. 4 shows the confocal fluorescence microscopy images of the microcapsules showing the presence of the inner phase stained in green with FITC-dextran and the middle phase stained in red by perylene. By employing two different dyes, the inner phase and the middle phase were clearly distinguished in the confocal images. The confocal images indicate that all the content of the inner phase, FITC-dextran or BSA, is located within the microcapsule structure. The middle phases of the particles were evenly distributed along the inner phases, and the particles prepared were fairly monodisperse. Thus, monodisperse microcapsules were successfully prepared using this methodology and effective formation of a double emulsion structure was also an indication of high encapsulation efficiency (EE) and preciseness of the preparation process.

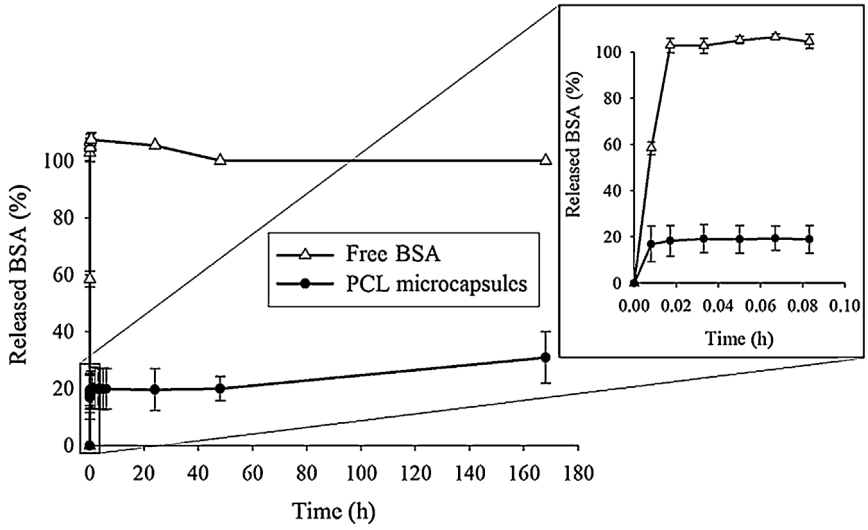

Fig. 5. Release profiles of free BSA and BSA released from the PCL microcapsules in phosphate buffer (pH 7.2) media and 37.0 ± 0.5 °C during the first 0.083 and 168 h of the dissolution tests. Standard deviations are expressed as error bars (n = 3).

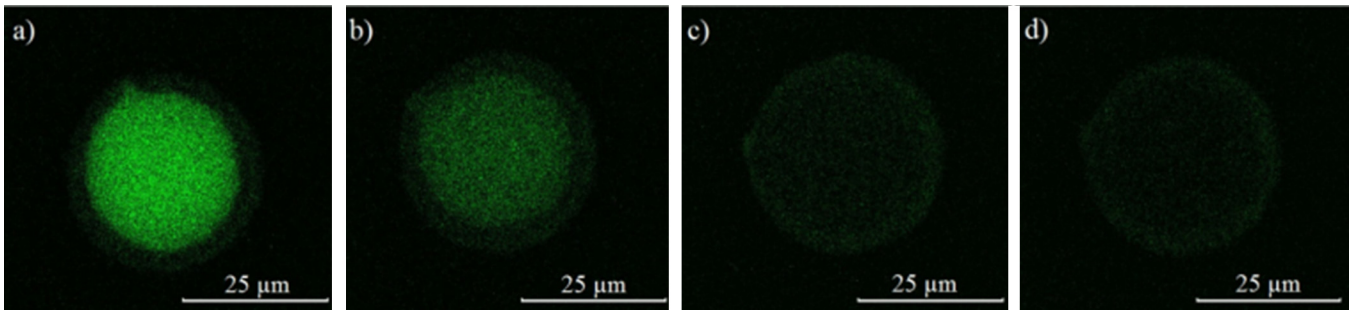

Fig. 6. Confocal fluorescence microscope follow-up images of one PCL microcapsule, showing the release of the FITC-dextran (green) content: (a) the shell rupturing from the thinnest spot; (b) particle releasing FITC-dextran; (c) and (d) particles with no visible FITC-dextran content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Encapsulation efficiency

The average EE for BSA was 84.0 ± 10.5% (n = 3) (Table S3). The results of the EE experiment were reproducible and the variation between the parallel tests was moderate. Encapsulation efficiency is often dependent on the particle size and for the size range of the microcapsules prepared in this study, the EE of 84% is difficult to achieve. In several previous studies the EE for similar or smaller particle size ranges were at the highest approximately ca. 40–60% (Bolzinger et al., 2007; Hnaien et al., 2011; Scala-Bertola et al., 2012; Somavarapu et al., 2005). Other studies indicated poorer EE at the highest of barely ca. 40% (Hnaien et al., 2011). Generally, the EE of 70% was considered high, even for significantly larger particles. For example, PCL particles prepared with flow-focused jetting cell achieved an EE of 42–79% was achieved (Cheng et al., 2010). Thus, PCL microcapsules produced here with microfluidics were superior to previously reported particles in terms of EE.

These results indicate that the microfluidic preparation method is very efficient and additional energy input is not necessary in order to achieve excellent EE. Thus, this method is potentially suitable for protein encapsulation. Furthermore, when using expensive therapeutics, such as protein and peptide drugs, high EE may minimize the material loss during the preparation process and create cost-effective manufacturing methods.

3.3. Protein release profile

In order to evaluate the efficient encapsulation and release of a protein from the prepared microcapsules, BSA was chosen as a model protein. The release profiles of BSA from the PCL microcapsules and of free BSA in phosphate buffer pH 7.2 are shown in Fig. 5. The cumulative percentages of the released BSA are presented up to 168 h. Free BSA dissolved immediately, within 1 min, into the dissolution media. PCL microcapsules released 30% of their content within 168 h. Similar release kinetics has been observed with PCL particles also in other studies (Coccoli et al., 2008; Hnaien et al., 2011). The release profile shows that 20% of BSA is released immediately and additional 10% of it during the whole experiment. The release of BSA is not directly dependent on the number of microcapsules breaking; it is rather initiated by the contact with the dissolution media. Differences in the osmotic pressure cause the immediate release of BSA, since BSA is pushed out of the capsules. Future work should address the formulation variables that enable tailoring drug release profile.

Fig. 6 shows the release behavior of FITC-dextran from a single PCL microcapsule. The degradation of PCL by autocatalyzed reaction (Pitt, 2009), occurred in the shell evenly, and thus, the thinnest part of the shell broke first. All the microcapsules did not release their content simultaneously. The microcapsules kept the spherical form after the shell ruptured, thus the BSA content was not completely release from all the microcapsules and led to initial release of 20% of protein, followed by a slow release of protein up to 30%.

4. Conclusions

In this study, we have successfully developed a microcapsule formulation template for therapeutic proteins using microfluidic technology. The microfluidic approach enabled to engineer microcapsules with enhanced targeted properties, including high protein EE, monodispersity, low porosity, and high stability. The EE of 84% was the most important feature of these microcapsules. Overall, this research shows that the microfluidic technique has a great potential for engineering and manufacturing drug delivery systems for therapeutic proteins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2014.06.012.

References

Bolzinger, M., Bordes, C., Gauvrit, J., Briançon, S., 2007. Improvement of a bovine serum albumin microencapsulation process by screening design. Int. J. Pharm. 344, 16–25.
Chandra, R., Rustgi, R., 1998. Biodegradable polymers. Prog. Polym. Sci. 23, 1273–1335.
Chen, D., Chen, H., Bei, J., Wang, S., 2000. Morphology and biodegradation of microspheres of polyester–polyether block copolymer based on polycaprolactone/polylactide/poly (ethylene oxide). Polym. Int. 49, 269–276.
Cheng, X., Liu, R., He, Y., 2010. A simple method for the preparation of monodisperse protein-loaded microspheres with high encapsulation efficiencies. Eur. J. Pharm. Biopharm. 76, 336–341.
Chu, L.Y., Utada, A.S., Shah, R.K., Kim, J.W., Weitz, D.A., 2007. Controllable monodisperse multiple emulsions. Angew. Chem. Int. Ed. 46, 8970–8974.
Coccoli, V., Luciana, A., Orsi, S., Guarino, V., Causa, F., Netti, P.A., 2008. Engineering of poly(e-caprolactone) micro-carriers to modulate protein encapsulation capability and release kinetic. J. Mater. Sci. Mater. Med. 19, 1703–1711.
Cox, D.S., Raje, S., Gao, H., Salami, N.N., Eddington, N.D., 2002. Enhanced permeability of molecular weight markers and poorly bioavailable compounds

across Caco-2cell monolayers using the absorption enhancer, zonula occludens toxin. Pharm. Res. 19, 1680–1688.
Duncanson, W.J., Abbaspourrad, A., Shum, H.C., Kim, S., Adams, L.L., Weitz, D.A., 2012a. Monodisperse gas-filled microparticles from reactions in double emulsions. Langmuir 28, 6742–6745.
Duncanson, W.J., Lin, T., Abate, A.R., Seiffert, S., Shah, R.K., Weitz, D.A., 2012b. Microfluidic synthesis of advanced microparticles for encapsulation and controlled release. Lab Chip 12, 2135–2145.
Fix, J.A., 1996. Oral controlled release technology for peptides: status and future prospects. Pharm. Res. 13, 1760–1764.
Freiberg, S., Zhu, X., 2004. Polymer microspheres for controlled drug release. Int. J. Pharm. 282, 1–18.
Hnaien, M., Ruffin, E., Bordes, C., Marcillat, O., Lagarde, F., Jaffrezic-Renault, N., Briançon, S., 2011. Integrity characterization of myoglobin released from poly (ç-caprolactone) microspheres using two analytical methods: UV–vis spectrometry and conductometric bi-enzymatic biosensor. Eur. J. Pharm. Biopharm. 78, 298–305.
Ingemann, M., Frokjaer, S., Hovgaard, L., Bronsted, H., 2000. Peptide and protein drug delivery systems for non-parenteral routes of administration. In: Frokjaer, S., Hovgaard, L. (Eds.), Pharmaceutical Formulation Development of Peptides and Proteins. Taylor & Francis, London, pp. 189–205.
Jeong, J.C., Lee, J., Cho, K., 2003. Effects of crystalline microstructure on drug release behavior of poly(q-caprolactone) microspheres. J. Control. Release 92, 249–258.
Kim, S.H., Kim, J.W., Cho, J., Weitz DA:, 2011. Double-emulsion drops with ultra-thin shells for capsule templates. Lab Chip 11, 3162–3166.
Kim, S.H., Kim, J.W., Kim, D., Han, S., Weitz, D.A., 2013. Polymersomes containing a hydrogel network for high stability and controlled release. Small 9, 124–131.
Kim, S.H., Weitz, D.A., 2011. One-step emulsification of multiple concentric shells with capillary microfluidic devices. Angew. Chem. 123, 8890–8893.
Langer, R., 1998. Drug delivery and targeting. Nature 392, 5–10.
Natarajan, V., Krithica, N., Madhan, B., Sehgal, P.K., 2011. Formulation and evaluation of quercetin polycaprolactone microspheres for the treatment of rheumatoid arthritis. J. Pharm. Sci. 100, 195–205.
Pitt, C.G., 1990. Poly-ε-caprolactone and its copolymers. In: Chasin, M., Langer, R. (Eds.), Biodegradable Polymers as Drug Delivery Systems. Marcel Dekker, New York, pp. 71–120.
Powers, T.R., Zhang, D., Goldstein, R.E., Stone, H.A., 1998. Propagation of a topological transition: the Rayleigh instability. Phys. Fluids 10, 1052.
Scala-Bertola, J., Javot, L., Camargo, J., Bonneaux, F., Lecompte, T., Maincent, P., Sapin, A., 2012. Evaluation of subcutaneous forms in the improvement of pharmaco-kinetic profile of warfarin. Int. J. Pharm. 431, 33–38.
Saffran, M., Kumar, G.S., Savariar, C., Burnham, J.C., Williams, F., Neckers, D.C., 1986. A new approach to the oral administration of insulin and other peptide drugs. Science 233, 1081–1084.
Shum, H.C., Zhao, Y., Kim, S.H., Weitz, D.A., 2011. Multicompartment polymersomes from double emulsions. Angew. Chem. 123, 1686–1689.
Somavarapu, S., Pandit, S., Gradassi, G., Bandera, M., Ravichandran, E., Alpar, O.H., 2005. Effect of vitamin E TPGS on immune response to nasally delivered diphtheria toxoid loaded poly (caprolactone) microparticles. Int. J. Pharm. 298, 344–347.
Squires, T.M., Quake, S.R., 2005. Microfluidics: fluid physics at the nanoliter scale. Rev. Mod. Phys. 77, 977.
Umbanhowar, P., Prasad, V., Weitz, D.A., 2000. Monodisperse emulsion generation via drop break off in a coflowing stream. Langmuir 16, 347–351.
Utada, A., Chu, L.Y., Fernandez-Nieves, A., Link, D., Holtze, C., Weitz, D.A., 2007. Dripping, jetting, drops, and wet-ting: the magic of microfluidics. MRS Bull. 32, 702–708.
Utada, A., Lorenceau, E., Link, D., Kaplan, P., Stone, H., Weitz, D.A., 2005. Monodisperse double emulsions generated from a microcapillary device. Science 308, 537–541.
Zhou, X.H., 1994. Overcoming enzymatic and absorption barriers to non-parenterally administered protein and peptide drugs. J. Control. Release 29, 239–252.