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A single microfluidic chip with dual surface properties for protein drug delivery

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ABSTRACT

Principles of double emulsion generation were incorporated in a glass microfluidic chip fabricated with two different surface properties in order to produce protein loaded polymer microspheres. The microspheres were produced by integrating two microfluidic flow focusing systems and a multi-step droplet splitting and mixing system into one chip. The chip consists of a hydrophobic and a hydrophilic section with two different heights, 12 μm and 45 μm, respectively. As a result, the protein is homogenously distributed throughout the polymer microsphere matrix, not just in its center (which has been studied before). In our work, the inner phase was bovine serum albumin (BSA) in phosphate buffered saline, the disperse phase was poly (lactic acid) in chloroform and the continuous phase was an aqueous solution of poly(vinyl alcohol). After solvent removal, BSA loaded microspheres with an encapsulation efficiency of up to 96% were obtained. Our results show the feasibility of producing microspheres loaded with a hydrophilic drug in a microfluidic system that integrates different microfluidic units into one chip.

1. Introduction

Maintaining a therapeutic drug concentration in the blood stream or target tissue is the basis of drug therapy and is the crucial factor to be considered in drug formulation and determining drug dosage. A therapeutic drug concentration can be achieved by multiple drug administration at predefined intervals, which is the conventional method that has been practiced for a long time, or by a single administration of a long lasting drug formulation, *i.e.*, controlled release. This more recent approach is more convenient for the patient and health care providers as it will reduce the frequency of drug administration.

For protein drugs, the most common approach to achieve a constant release rate over time (zero-order drug release) is to encapsulate the protein in a micro- or nanocarrier (Determan et al., 2004; Pai et al., 2009). After administration, the drug is released from the polymer matrix by diffusion of the drug molecules through the pores of the matrix or by disintegration of the matrix, or by a combination of both (Determan et al., 2004; Freiberg and Zhu, 2004; Yang et al., 2001). The rate of the drug release depends therefore on the molecular weight of the polymer, its hydrophobicity and the size of the particles that entrap the protein (Pai et al., 2009). In order to design a reliable and reproducible formulation with predictable drug release rate precise control of these factors is essential.

The most common polymers used for protein encapsulation are poly(lactic acid), poly(glycolic acid) and their copolymers. They are biocompatible and biodegradable and they are approved by FDA as safe drug excipients (Jain, 2000). The most common protein encapsulation method with the aforementioned polymers is double emulsion-solvent evaporation/extraction that generally results in the production of protein loaded microspheres with broad size distribution.

Due to small channel geometries and controllable shear forces, microfluidic systems can produce homogenous emulsions where the polymer-filled oil-phase droplets can harden into narrowly sized polymer microspheres (Anna and Mayer, 2006; Bokharaei et al., 2016; Häfeli et al., 2010; Martin-Banderas et al., 2005; Martin-Banderas et al., 2011; Nisisako et al., 2004; Schneider et al., 2008; Seiffert et al., 2010; Utada et al., 2007). To allow for the encapsulation of hydrophilic drugs, microfluidic systems have been described in the literature that generate robust double emulsions (Abate and Weitz, 2009; Abbaspourrad et al., 2013; Adams et al., 2012; Chu et al., 2007; Deng et al., 2014; Okushima et al., 2004; Pessi et al., 2014; Utada et al., 2005; Wang et al., 2014). In their system, initial droplets were generated in the co-flowing

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section of the chip and then the droplets passed through a short channel to the flow focusing part where the final water droplet encapsulated in an oil droplet was generated that after solvent removal can lead to the formation of microcapsule (Fig. 1a). They have studied the effects of many different parameters, *i.e.*, geometry of the channels and flow rate of fluids on droplet size and mechanism of droplet generation, yet the production of homogenously loaded microspheres has not been reported (Fig. 1b). In this work, we designed a single chip microfluidic system for the encapsulation of proteins into biocompatible polymer microspheres with homogenous protein distribution. For this purpose, we combined microfluidic droplet generation, droplet splitting and micromixing into one chip. To the best of our knowledge, such a system has not been described yet in the literature. Results will be given for the encapsulation of bovine serum albumin in biocompatible and biodegradable poly (L-lactic acid) microspheres. The emphasis of this work is on the production of monosized microspheres with an average diameter of 20–40 μm and high encapsulation efficiency. The factors affecting particle size and size distribution have been discussed in a separate publication (Bokharaei et al., 2016).

2. Material and methods

2.1. Fabrication of the double emulsion generating microfluidic system

The new microfluidic chip design (Fig. 2b) contained two droplet generation units and a passive droplet break up section. Section 1 contained a flow focusing system and the passive droplet break up unit, referred as FF-PDB section and Section 2 contained a flow focusing droplet generation unit. Each section had been printed on a separate mask labeled mask 1 and mask 2, respectively. The whole system was fabricated on a 100 mm diameter and 2 mm thick borofloat glass wafer with one reference flat and standard transparent finish 60/40 (Valley Design Corp., Shirley, MA, USA).

The wafer was coated with 0.02 μm of chromium and 0.25 μm of gold and then spin coated with positive photoresist (Shipley S1813; MicroChem Corp., Newton, MA, USA; Fig. 2a.1). The pattern on mask 2 was transferred using a Canon PLA-501F mask aligner and developed with MF-319 photo developer (Rohm and Hass Electronic Material LLC, Marlborough, MA, USA; Fig. 2a.2). After removing the photoresist (Fig. 2a.3), channels were etched 33–38 μm deep using concentrated HF (49%; Avantor; Fig. 2a.4). The wafer was spin coated with photoresist S1813 one more time (Fig. 2a.5). Pattern on mask 1 was aligned with structure on the wafer and transferred onto the wafer using the Canon mask aligner (Fig. 2a.6). The pattern was developed, the photoresist removed (Fig. 2a.7) and the wafer finally immersed into 49% HF solution to etch the channels.

Etching stopped when the channel depth in the FF-PDB section was between 10 and 12 μm. The chip features were measured by an Alpha step 200 profilometer (Tencore instrument, Mountain View, CA, USA). Gold and chromium coating were removed (Fig. 2a.8) and the wafer was diced into $50 \times 15\ mm^2$ chips. Glass lids of the same size were cut from another wafer and thermally bonded to the microchips (6 h at 650 °C).

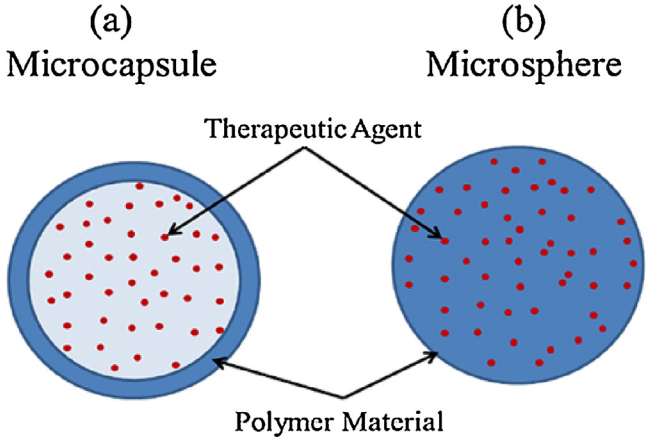

Fig. 1. Schematic of polymeric microcapsule and microsphere structures.

2.2. Coating of the chip

Before bonding the glass slide to the chip, the FF-PDB section of the chip was coated with 1H, 1H, 2H, 2H, perfluorododecyltrichlorosilane (PFDTCS; Santa Cruz Biotechnology Inc., Santa Cruz, CA, U.S.A.) (Fig. 3). This selective coating of the FF-PDB section, but not of the second flow focusing section, was necessary to prevent water droplet adhesion to the glass surface in the FF-PDB section.

Coating was done according to the method explained by Srinivasan (Srinivasan et al., 1998) with slight modification. Specifically, a sealed flask was weighed and a tip of the spatula of the PFDTCS was added to that and weighed again. Immediately, the flask was placed under vacuum for 5–10 min and then filled with argon gas. Iso-octane was added to the system to get to the final concentration of 1 mM PFDTCS in iso-octane, while the solution was kept under argon gas. The chip which had been etched by HF and rinsed thoroughly with water was immersed in a Petri dish filled with 2-propanol. After 5 min it was transferred to another Petri dish filled with iso-octane. Solvent was removed after 5 min, the dish filled with fresh iso-octane and the chip immersed for another 5 min. The chip was then vertically placed into the flask containing the PFDTCS solution, so that the solution only covered the FF-PDB section of the chip. Argon was pushed into the flask and purged from the outlet port for 3 min, the system closed and left for 10 min for the PFDTCS-coating reaction to take place. After 10 min, the chip was removed from the flask and placed in a Petri dish filled with iso-octane for 10 min with a solvent change after 5 min, followed by two more 5 min incubations in a 2-propanol filled dish. Finally, it was submerged into water for 5 min and rinsed and dried thoroughly.

The hydrophobicity of the surface after coating was determined *via* contact angle measurement of a water droplet on the coated surface using the free version of the Analysis Software "First Ten Angstroms Drop" (FTA32 2.0, Portsmouth, VA, USA). Results were compared to the contact angle of an uncoated chip.

2.3. Protein loaded microsphere production

Three different phases are required for the generation of a w/o/w emulsion in the chip. The inner phase (IP) was prepared by dissolving bovine serum albumin (BSA) in PBS. The dispersed phase (DP) was made of 10% poly(L-lactic acid) (PLLA) (2–3 kDa, Resomer L104, Boehringer Ingelheim GmbH, Germany) in chloroform. Different concentrations of Span 80 (Sigma-Aldrich, Canada) were added to the dispersed phase to stabilize the formed water in oil emulsion. The continuous phase (CP) consisted of 2% poly(vinyl alcohol) (PVA; 13–23 kDA and 83–87% hydrolyzed, Sigma Aldrich). Solutions were prepared fresh and filled into glass syringes. Different operational conditions (Table 1) were used to optimize the encapsulation of BSA in PLLA microspheres. The formed droplets left the microfluidic chip as semi-hardened microspheres and were collected and placed under vacuum (60–70 mTorr) for 45 min under stirring for further solvent removal, washed three times and then suspended in distilled water and lyophilized.