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MICROFLUIDIC FORMATION OF HYDROGEL MICROPARTICLES FOR DRUG DELIVERY

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ABSTRACT

Creating monodispersed hydrogel microparticles is advantageous for drug delivery applications. We explore microfluidic flow focusing as a method for generating such particles. Our hydrogel has a unique composition that makes it biodegradable and mechanically strong. We designed and manufactured a polymer microfluidic chip that mixes three viscous precursor solutions and generates a steady stream of microparticles from that solution. We found that microfluidic flow focusing produce particle with a coefficient of variance around 9 %; this was a four times improvement over traditional methods.

INTRODUCTION

In this work we show that we can create monodispersed hydrogel microparticles (MPs) using flow focusing in a microfluidic chip. Currently, there is no general method available for the formulation of monodispersed MPs. Conventional methods to formulate MPs typically involve bulk emulsion formations by mechanically shearing of monomer solutions or precursors into a discontinuous, immiscible phase, followed by the polymerization of the emulsified droplets.

Hydrogels are hydrophilic three-dimensional polymer networks, cross-linked chemically and/or physically, capable of retaining a large amount of water [1]. Hydrogel micro particles have applications in drug delivery systems and can be used for biological sensing[2-5]. The hydrogel particles in this work have a unique composition that makes them both biodegradable and mechanically strong. The MPs are composed of three precursor solutions: partially oxidized dextran (Odex), Teleostean and *N*-carboxyethyl chitosan (CEC). One interesting attribute of the precursor solutions that needs to be considered in design is their high viscosities: 0.347, 0.01 and 0.558 Pas, respectively.

Microfluidic flow focusing has been used to generate MPs with single precursor based (e.g., Ca-alginate), photo-polymerization or one-step reaction systems requiring potentially cytotoxic extraneous cross-linkers or photoinitiator [6-9]. The MPs produced generally have poor mechanical properties [7, 10] rendering them less appealing for certain biomedical applications. Flow focusing using high viscosity fluids has also been demonstrated [11] with a single polymer for the dispersed phase. We designed and tested a microfluidic chip that mixes three viscous precursors and uses flow focusing to generate microparticles that are biodegradable and mechanically strong. Mechanically strong hydrogel are capable of surviving delivery methods such as injection and ultrasound [3-4]. They will also retain their shape longer to allow continuous drug release [3-4]. The flow rate of the dispersed and the continuous phase were varied to create different size particles. For each trial the particles are characterized by the average size and standard deviation.

SYNTHESIS AND PROPERTIES OF THE 3XN HYDROGEL

CEC is ampiphilic with both -NH_2 and -COOH; Odex through its -CHO functionalities serves as a macromolecular cross-linker for materials with free -NH_2. Besides, Odex, Teleostean and CEC are all very abundant in -OH, -COOH and -NH_2 groups capable of forming highly interactive secondary and tertiary structures [5, 12]. Blending of solutions of Odex, Teleostean and CEC formed a transparent hydrogel quickly; this rapid gelatinization property was attributable to the physical interactions of the secondary/tertiary structures, in concert with the chemical cross-linking via Schiff base formation between the -CHO on Odex and the -NH_2 on both CEC and Teleostean. The disparity in the reaction times and the modes of interaction between the three components resulted in the formation of multiple and interpenetrating networks, illustrated in Figure 1.

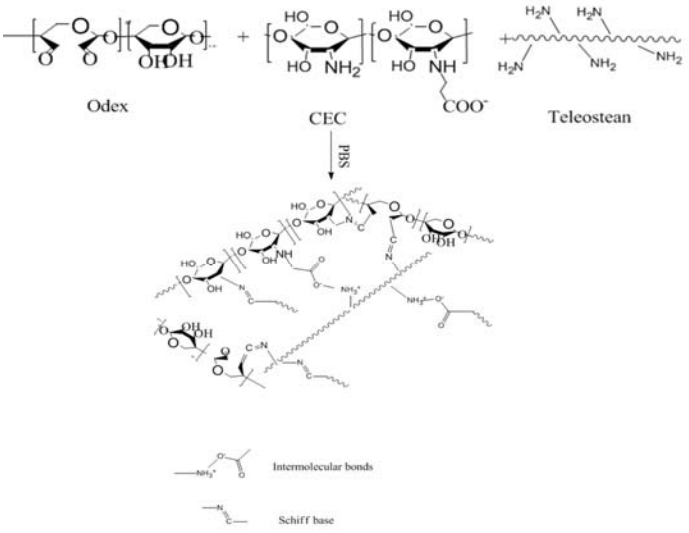

Figure 1. Schematic representation of the formation of the triple-network (3XN) hydrogel from the precursor molecules.

Most hydrogels, particularly those formulated entirely from natural materials, share the common characteristic of a lack of mechanical toughness, thereby, limiting the span of their potential biomedical applications [1]. High mechanical strength is one of the most important physical attributes of the 3XN hydrogel, and thus the MPs formulated. Although the mechanical strength of the MPs produced could be improved by increasing the precursor concentrations, this also requires precursor solutions with higher viscosities, in particular, the CEC solution, and also accelerates the reaction. The results of our studies and other reports have demonstrated that higher viscosity would impede the mixing and flowing of the precursor solutions, increasing the pressure inside the microchannels, rendering it very difficult for the fluid to form droplets [2, 9]. The rapid reaction also increases the likelihood of blockade of the channels in the chip. Collectively, these factors increased the level of difficulty for operation. In our study, the most optimal concentration for Odex/Teleostean/CEC was established as 7.5%, 20% and 2.5% (w/v), making the viscosity 0.347, 0.01 and 0.558 Pas respectively.

DESIGN OF THE MICROFLUIDIC CHIP

Good mixing between the fluids is important to ensure the manufacturing of quality microspheres. Flow in microchannels is usually laminar due to the low Reynolds number; so that mixing is diffusion-limited. As shown in Figure 2, a y-connection and a 33mm serpentine channel was used to mix the fluids Teleostean and Odex.

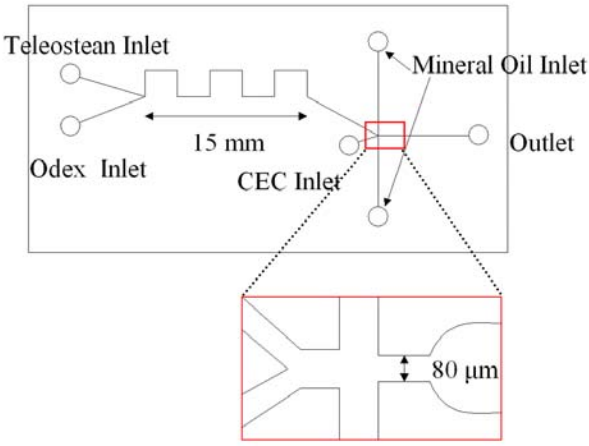

Figure 2. Microfluidic chip geometry

The serpentine channel was used to increase the mixing length while minimizing the area of the microfluidic chip. The height and width of the serpentine channel, h = 100 µm and w = 500 µm, were chosen so that the pressure drop was less than 2 bar at a filling velocity of 17 mm/s. To estimate the pressure drop we assumed a straight channel with the total length of the serpentine channel, 33 mm. It has been shown at Re < 5 that curves and turns in channels do not effect pressure drop [13-14]. To calculate pressure drop, we used the following correlation for channels with rectangular cross-section $\Delta p = 12\mu LQ/(h^3 w \cdot (1\text{-}0.63h/w))$[15], with the measured viscosity of the most viscous of the two fluid, Teleostean, μ = 0.347 Pas. The typical time needed to mix the reagents can be estimated as $\tau = (w/2)^2/D$, the diffusion time of Teleostean and Odex across the channel. Assuming D = 1x10^{-9} m^2/s, corresponding to the diffusion of 2 Angstroms molecules in water, we obtain τ=63s,

corresponding to an operation velocity of 0.5mm/s, corresponding to a flow rate of 1.5 μL/min.

CHIP MANUFACTURING

The chip was manufactured by milling channels in an acrylic plastic. The chip geometry, shown in Figure 2; was drawn using a computer-aided-design software (Pro-E, Wildfire Inc.), which automatically generated the sequence of operations for the milling process. The channels were machined using a Minitech CNC (computed numerically controlled) milling machine, with a repeatability of 3 μm. Millbits with diameter ranging from 80 to 500 μm were used. All channels were 100 μm deep. Inlet and outlet port were drilled through the chip. The ports were 2.3 mm to provide a leak-free interfacing for pushed-in Tygon tubing. After manufacturing the chip was sonicated and inspected under a stereomicroscope to remove burrs and particulates. The channels were sealed with 80 μm thick transparent tape from Adhesives Research. Flow rates up to 300 μL/min for the precursor fluids could be used to fill the chip without leak at the tape sealing. The tape did not react with the components of the hydrogel, but we found that the mineral oil weakens the tape adhesion if oil is left in the chip for several days.

EXPERIMENTAL SET UP

The oil phase and precursor solutions were independently infused into the microchannels using a digitally controlled syringe pump (PHD 2000, Harvard Apparatus, Inc., Holliston MA). Odex, Teleostean and CEC solutions were injected separately, as shown in Figure 2, they were mixed to form a uniform stream; this steady stream was broken up by the shearing of the mineral oil flow injected bi-directionally, Figure 3, to form polymer droplet. The droplet were collected in a reservoir, Figure 4, containing 50 ml mineral oil with 0.5 ml of Span-80 added as an emulsifier and allowed to auto-cross-link under constant stirring at 250 rpm (LR400 Lab Stirrer, Yamato, Tokyo, Japan). The reservoir was maintained at 37°C overnight to enable partial dehydration of the MPs formed. Subsequently, the MPs were recovered by precipitation; 60 ml of cold isopropanol was added to the mixture at room temperature while stirring, after 5 min, the mixture was centrifuged (5000 rpm) to separate the organic phase and it was discarded. The residual organic phase was extracted by washing the microspheres three times with approximately 20 ml of an acetone/isopropanol (1:1 ratio) co-solvent mixture. The microspheres were recovered by air-drying overnight at room temperature.

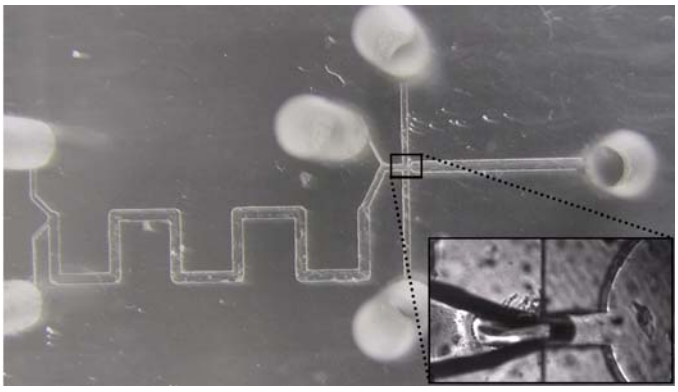

Figure 3. Actual microfluidic chip

For comparison, 3XN MPs were also prepared by a conventional bulk in-emulsion-cross-linking method described in [16]. Aqueous Teleostean and CEC solutions (0.25 ml each) were mixed with 0.5 ml of an aqueous Odex solution. The mixture was added to 50 ml of mineral oil (with 0.5 ml of Span 80 added as an emulsifier), maintained at 37°C. It was kept under rapid agitation at 1000 rpm to form an emulsion and incubated overnight. The MPs formed were recovered following a similar method described above.

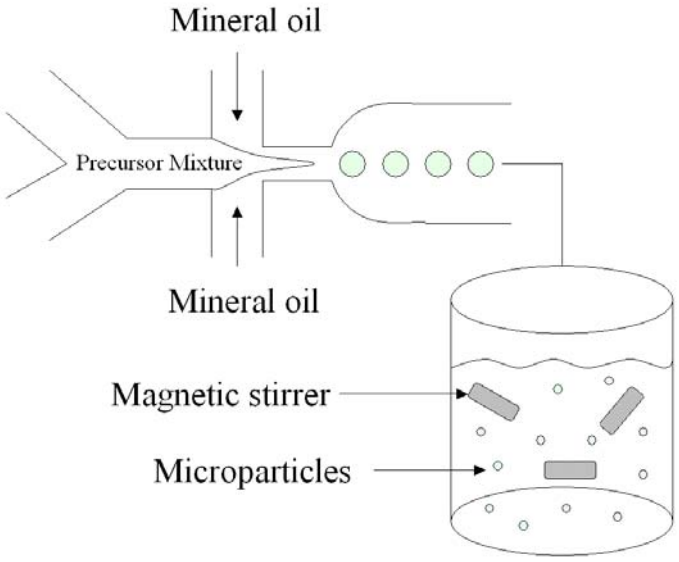

Figure 4. MP generation and collection method

PREPARATION OF SEM SAMPLES

The dried MPs were secured on an aluminum stub with copper tape and sputtered with gold; their morphologies were examined by a scanning electron microscope (SEM) (SFEG Leo 1550, AMO GmbH, Aachen, Germany) at 20 kV [3]. The mean size and size distribution of the fabricated MPs were

characterized by a dynamic light scattering system (DLS), using a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation) at 25 °C with angle detection of 90° for 300 s.

RESULTS

In our experiments the dispersed phase was a mixture of Odex, Teleostean and CEC with a volumetric ratio 2:1:1 respectively. The flow-focusing mechanism generated drops of that precursor fluid in a continuous phase of mineral oil with viscosity μ=0.0276 Pas and density ρ = 840 kg/m^3, with Span 80 as a surfactant. Two syringe pumps were used to drive the dispersed phase and the oil. By optical inspection, we found that the reagents in the droplets generated by the flow focusing process were well mixed.

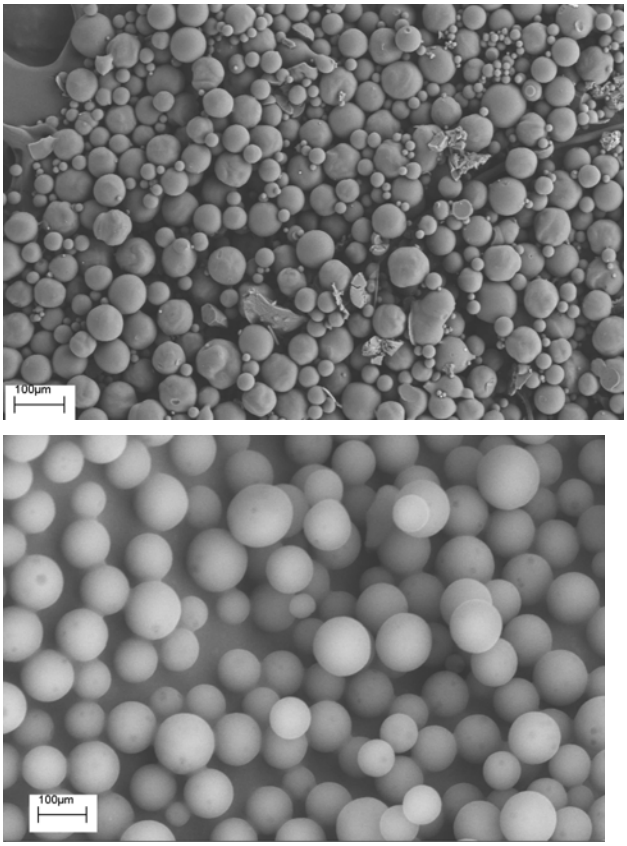

Figure 5. SEM images of microparticles created by Traditional emulsion-cross-linking method (Top) and Microfluidic flow focusing (Bottom)

SEM images of MPs formed by the microfluidic and the traditional method can be seen in Figure 5. A summary of results is shown in Table 1. The MPs manufactured in the microfluidic device had a typical CV < 9%. This is about 4 times better than the CV of 35% obtained in this study by emulsion-cross-linking. While this improvement is sensible, even smaller standard deviations of or below 5% have been reported for particle manufacturing in microfluidic devices [11, 17-18]. One difference however between these studies and the study described here is the value of the capillary number. The capillary number scales the importance of viscous forces over surface tension forces, and is defined as Ca = $\mu v/\sigma$ where v is defined as the volumetric flow rate divided by the cross-sectional area of the nozzle. In [11], the capillary number of the continuous and dispersed phase was negligible (Ca≤0.1) so that the drops generated for high viscosity fluids were in squeezing and dripping modes. In our study, assuming an interfacial tension σ = 20 mN/m and a viscosity μ = 0.3 Pas for the dispersed phase, the capillary number for the continuous and dispersed phase is on the order of 1. Less stable generation has been reported for capillary number close to one [19]. The high coefficient of variance could also be an effect of the complex and reactive fluid. As previously mentioned the viscosity and reactivity of the precursors can be varied at the expense of mechanical strength. Further investigation of these parameters could be useful if highly monodispersed particles are required.

Case		A	B	C	D	E
Flow Rate (Oil)	µl/min	250	500	750	500	500
Flow rate (P)	µl/min	200	200	200	100	300
Ca (oil)		0.72	1.44	2.16	1.44	1.44
Ca (Precusor)		6.25	6.25	6.25	3.13	9.38
Particle size	µm	50.4	28.5	18.2	18.1	45.6
STD	µm	4.5	2.0	2.7	1.5	5.0
CV	%	8.9	7.0	14.8	8.3	10.9

Table 1. Summary of particle size and standard deviation based on flow rate and capillary number (precursor flow given is the total prescribed flow rate of the three fluids)

CONCLUSIONS

We have demonstrated that flow focusing can be used to create monodispersed microparticles of a hydrogel. The hydrogel particles generated are biodegradable and mechanically strong making them advantageous for drug delivery applications. In this work we have also explored limiting factors on the capillary number of the dispersed phase. Since our dispersed phase is viscous there is an upper limit on the flow rate we can achieve before the pressure drop becomes prohibitively high. Conversely, since our fluids are reactive there is a minimum limit to the flow rate so they do not gelatinize in the nozzle. The presents a new set of constraints beyond the balance between shear and surface tension forces that normally drive flow focusing.

REFERENCES

1. A. Nakayama, A. Kakugo, J.P. Gong, Y. Osada, M. Takai, T. Erata, S. Kawano, High mechanical strength double-network hydrogel with bacterial cellulose, *Advanced Functional Materials*, **14**(11) 1124-1128 (2004).
2. C.H. Choi, J.H. Jung, Y.W. Rhee, D.P. Kim, S.E. Shim, C.S. Lee, Generation of monodisperse alginate microbeads and in situ encapsulation of cell in microfluidic device, *Biomedical Microdevices*, **9**(6) 855-862 (2007).
3. H. Epstein-Barash, G. Orbey, B.E. Polat, R.H. Ewoldt, J. Feshitan, R. Langer, M.A. Borden, D.S. Kohane, A microcomposite hydrogel for repeated on-demand ultrasound-triggered drug delivery, *Biomaterials*, **31**(19) 5208-5217 (2010).
4. Q.X. Ji, Q.S. Zhao, J. Deng, R. Lü, A novel injectable chlorhexidine thermosensitive hydrogel for periodontal application: preparation, antibacterial activity and toxicity evaluation, *Journal of Materials Science: Materials in Medicine*, 1-8 (2010).
5. S.R. Van Tomme, W.E. Hennink, Biodegradable dextran hydrogels for protein delivery applications, *Expert Review of Medical Devices*, **4**(2) 147-164 (2007).
6. B.G. De Geest, J.P. Urbanski, T. Thorsen, J. Demeester, S.C. De Smedt, Synthesis of monodisperse biodegradable microgels in microfluidic devices, *Langmuir*, **21**(23) 10275-10279 (2005).
7. E. Rondeau, J.J. Cooper-White, Biopolymer microparticle and nanoparticle formation within a microfluidic device, *Langmuir*, **24**(13) 6937-6945 (2008).
8. M. Seo, Z. Nie, S. Xu, M. Mok, P.C. Lewis, R. Graham, E. Kumacheva, Continuous microfluidic reactors for polymer particles, *Langmuir*, **21**(25) 11614-11622 (2005).
9. C.H. Yang, Y.S. Lin, K.S. Huang, Y.C. Huang, E.C. Wang, J.Y. Jhong, C.Y. Kuo, Microfluidic emulsification and sorting assisted preparation of monodisperse chitosan microparticles, *Lab on a Chip - Miniaturisation for Chemistry and Biology*, **9**(1) 145-150 (2009).
10. H. Zhang, E. Tumarkin, R. Peerani, Z. Nie, R.M.A. Sullan, G.C. Walker, E. Kumacheva, Microfluidic production of biopolymer microcapsules with controlled morphology, *Journal of the American Chemical Society*, **128**(37) 12205-12210 (2006).
11. Z. Nie, M. Seo, S. Xu, P.C. Lewis, M. Mok, E. Kumacheva, G.M. Whitesides, P. Garstecki, H.A. Stone, Emulsification in a microfluidic flow-focusing device: Effect of the viscosities of the liquids, *Microfluidics and Nanofluidics*, **5**(5) 585-594 (2008).
12. S.R. Van Tomme, M.J. Van Steenbergen, S.C. De Smedt, C.F. Van Nostrum, W.E. Hennink, Self-gelling hydrogels based on oppositely charged dextran microspheres, *Biomaterials*, **26**(14) 2129-2135 (2005).
13. N. Rosaguti, laminar flow and heat transfer in a periodic serpentine channel with semi-circular cross section, *International Journal of Heat and Mass Transfer*, **49** 2912-2923 (2006).
14. Y.Z. Liu, B.J. Kim, H.J. Sung, Two-fluid mixing in a microchannel, *International Journal of Heat and Fluid Flow*, **25** 985-995 (2004).
15. H. Bruus, Theoretical Microfluidics, Oxford University Press, New York, 2008.
16. L. Weng, N.D. Ivanova, J. Zakhaleva, W. Chen, In vitro and in vivo suppression of cellular activity by guanidinoethyl disulfide released from hydrogel microspheres composed of partially oxidized hyaluronan and gelatin, *Biomaterials*, **29**(31) 4149-4156 (2008).
17. L. Yobas, S. Martens, W.-L. Ong, N. Ranganathan, High-performance flow-focusing geometry for spontaneous generation of monodispersed droplets, *Lab on a Chip - Miniaturisation for Chemistry and Biology*, **6**(8) 1073-1079 (2006).
18. S.-H. Tan, S.M. Sohel Murshed, N.-T. Nguyen, T.N. Wong, L. Yobas, Thermally controlled droplet formation in flow focusing geometry: Formation regimes and effect of nanoparticle suspension, *Journal of Physics D: Applied Physics*, **41**(16) (2008).
19. A. Utada, A. Fernandez-Nieves, H.A. Stone, D.A. Weitz, Dripping to Jetting Transitions in Coflowing Liquid Streams, *Physical Review Letters*, **99** (2007).