Synthesis of Monodisperse Biodegradable Microgels in Microfluidic Devices

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Microgels are promising materials in drug delivery and biomedicine. Although monodisperse microgels would offer considerable advantages, most microgels investigated and used today are polydisperse in size. We report on the fabrication of 10 μm sized monodisperse microgels by emulsifying an aqueous dextran-hydroxyethyl methacrylate (dex-HEMA) phase within an oil phase at the junction of microfluidic channels. Dex-HEMA microgels are biodegradable and are ideally suited for the controlled delivery of proteins.

Hydrogels are three-dimensional polymer networks, connected chemically or physically, which are able to hold a large amount of water due to their hydrophilic nature. Spherical hydrogel microparticles, or microgels, have attracted large interest in drug delivery due to their biocompatibility and the possibility to package drug molecules within the polymer network. When the microgels are fabricated from (bio)degradable polymers, the degradation of these hydrogels regulates the delivery of the encapsulated drug molecules when introduced into biological systems.[1] Stimuli responsive microgels may also be designed using physicochemical motifs, which are able to respond to pH,[2a] temperature,[2b] electric field,[2c] or glucose[2d] changes, and provide an intelligent release of drugs. By tailoring the properties of the microgels, the release-rate and release-profile of the drug molecules can be optimized to suit the specific application. Until now, there is no general method available for the synthesis of monodisperse microgels. The use of monodisperse microgels for use in drug delivery systems or sensing applications should offer considerable advantages compared to polydisperse ones with respect to monitoring, predicting, and modeling of their behavior as they exhibit a constant and predictable response to external stimuli.

"Microparticles" can be prepared in various ways. Microparticles have been prepared in bulk emulsions by mechanically shearing monomer into a continuous, immiscible phase, followed by subsequent polymerization of the emulsifed droplets. However, the resulting particle size distribution is highly polydisperse.[3,4] Various approaches have been proposed to fabricate monodisperse microparticles. Recently, membrane emulsification techniques, in which the discontinuous phase is forced through a porous membrane into the continuous phase, have been reported to produce monodisperse droplets.[5] Sugiura et al.[6] synthesized monodisperse polymeric divinylbenzene beads (both large (>50 μm) as well as smaller (3−10 μm) ones) in microchannels. Thorsen et al.[7] used microfluidic devices to make monodisperse emulsion droplets, with the resulting droplet size controlled by the relative driving pressures of the two immiscible fluids and the geometry of the microchannels. Various groups used this approach to fabricate colloidal assemblies,[8] spherical and nonspherical microparticles,[9] and photonic balls.[10] Nisisako et al.[11] reported on >30 μm sized monodisperse polymeric 1,6-hexanediol diacrylate microparticles obtained by emulsifying a monomer-containing phase in a nonsolvent phase in a cross-flow setup where the two liquid phases meet perpendicular in a T-shaped junction. The droplets were subsequently photochemically cured to form solid microspheres.

The aim of the present research was to synthesize biodegradable monodisperse microgels by photopolymerization of monodisperse pre-polymer droplets formed

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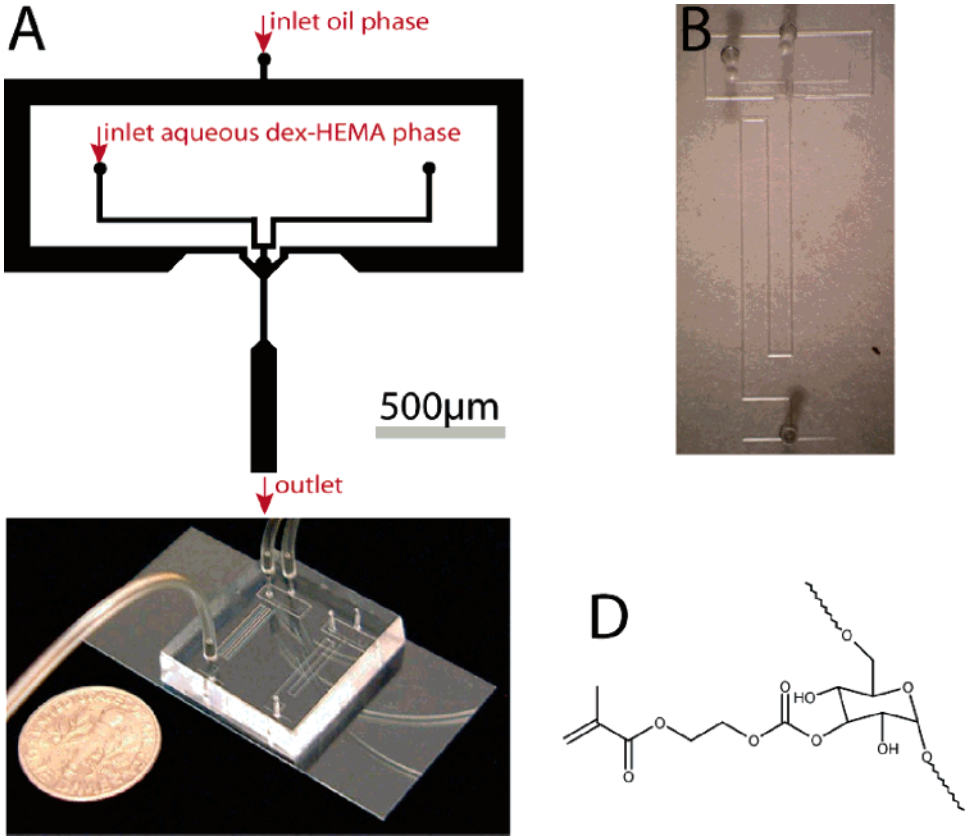

Figure 1. (A) Schematic representation of the PDMS microfluidic device with an in-line droplet generating nozzle. (B) Light microscopy image of the microfluidic channels illustrating channel layout and connection ports. (C) Image of the microfluidic device with the Tygon tubing attached to the PDMS-device with steel pins. (D) Molecular structure of the dex-HEMA polymer (the M_w of the dex-HEMA is 19 kDa).

in a microfluidic device. The biodegradable monodisperse microgels were made from dextran-hydroxyethyl methacrylate (dex-HEMA; Figure 1D). The synthesis and characterization of dex-HEMA were previously reported.[12] Unlike previously reported microfluidic devices in which the droplet formation is performed at a T-shaped junction, an in-line droplet generating channel geometry is utilized (Figure 1A).

The microfluidic devices were fabricated by soft lithography. In soft lithography, elastomeric materials such as poly(dimethyl siloxane) (PDMS) are used as building blocks.[13] PDMS are fluid polymers with a low glass transition temperature that form solid elastomers by cross-linking. Silicon wafer molds containing the microfluidic channels in positive relief were fabricated by spin-coating a positive photoresist (AZ 4620; Clariant) on the silicon wafers followed by the UV-irradiation of the spin-coated wafer through the transparency printed with the microfluidic channels. After a development step, to remove the irradiated material, only the nonirradiated pattern of the microfluidic channels remained on the silicon wafer. By placing the patterned wafer on a hotplate at 150 °C for 5 min, the positive relief rectangular mold profiles were rounded by reflowing the photoresist. While rectangular shaped walls caused the droplets to move along the walls of the channels, round shaped walls kept the droplets centered. The PDMS microfluidic chips were fabricated by casting a 5 mm thick layer of PDMS (Sylgard 184; Dow Corning) on the molds pretreated with chloro-trimethyl-silane (Aldrich). The chips were peeled from the molds, interconnection ports punched, and sealed to glass coverslips precoated with a thin layer of partially cured PDMS. Finally, the microfluidic chips were sealed by overnight curing at 80 °C. The channels were approximately 100 μm wide × 20 μm high, tapering to 10 μm × 20 μm in the region where the oil and aqueous phase merged. Figure 1B shows a microscopy image of the microfluidic channels; Figure 1C shows the PDMS-device connected to the fluid reservoirs, containing respectively the dex-HEMA solution (30% w/w; kinematic viscosity of 44 mm^2/s at 25 °C) and the mineral oil (kinematic viscosity of 28 mm^2/s at 25 °C), by 30 cm long Tygon tubing (500 μm internal diameter, ~30 cm long) and steel pins (New England Small Tube Corp.). Compressed air was used to pressurize the reservoirs. Air pressure regulators (Control Air, Inc.) equipped with digital pressure gages (Dwyer Instruments, Inc.) were used to individually set the air pressure applied to the input reservoirs. To recover the droplets after manufacturing, a collection tube was connected to the outlet of the device. The inlet of the oil phases was split into two channels which met in the nozzle. The viscous oil stream impinged the flow of the aqueous dex-HEMA solution through the central cavity and created droplets via tip-streaming. Repeated contraction and expansion of the water-tip sheared a single droplet at each cycle and the stream was focused toward the outlet port for collection.

As mentioned above, our continuous phase was mineral oil while the emulsified phase was a 30% (w/w) aqueous

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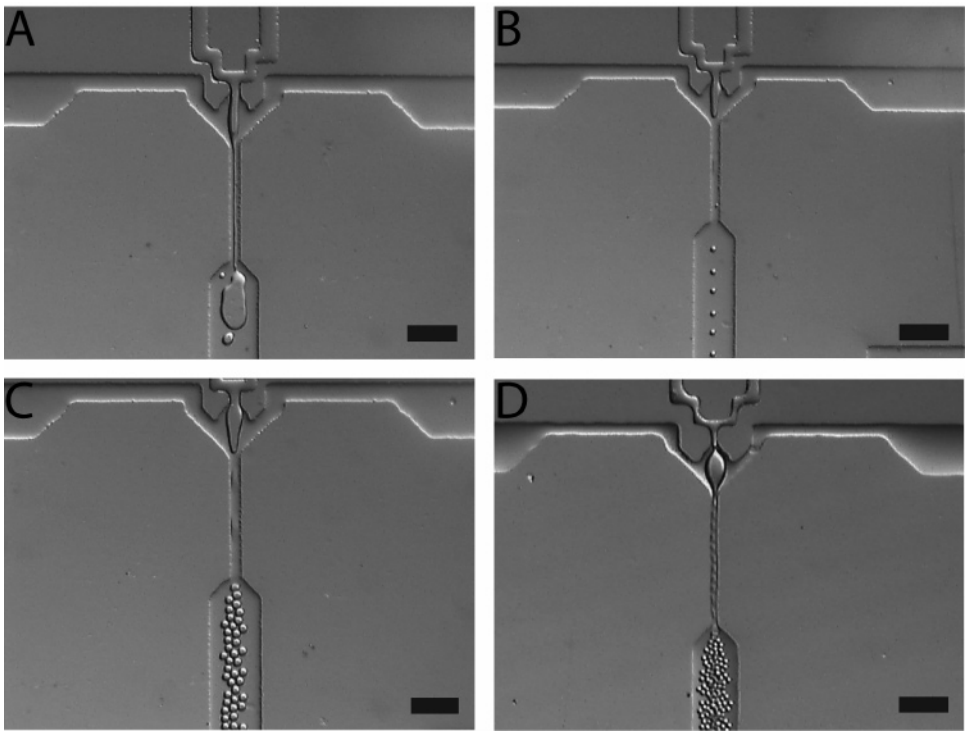

Figure 2. Optical microscopy images of various droplet production modes. (A) Insufficient shear between the aqueous dex-HEMA and oil phases resulted in co-flowing laminar streams, until (B) the addition of surfactant in the continuous oil phase facilitated regular droplet break-off. A single stream of monodisperse droplets may be generated, which travel in an ordered pattern through the channels. Higher operating pressures allows production of monodisperse droplets at (C) medium and (D) high production rates, with a higher volume fraction of droplets in the collected oil. The scale bars represent 100 μm.

dex-HEMA solution. In the absence of a surfactant, a continuous aqueous stream was formed co-annular with the oil phase, as shown in Figure 2A. A nonionic (cetyl dimethicone copolymer) surfactant, ABIL EM-90 (Degussa), was added to the oil phase at ~4% (v/v) to reduce the surface tension between both phases which facilitated emulsion formation and prevented subsequent coalescence prior to curing. In previous reports by our group[14] and the Hennink group,[3] dex-HEMA microgels were fabricated using a water-in-water emulsion technique based on the immiscibility of an aqueous dextran phase and an aqueous poly(ethylene glycol) (PEG) phase. Initially, we have also tried the use of a PEG solution as continuous phase, but it was impossible to generate aqueous dex-HEMA droplets, due to the lack of shear between the two phases upon mixing. Figures 2B-D illustrate modes of droplet production in the microfluidic device at various pressure balances between the emulsified and continuous phases. Monodisperse droplets were formed at regular time intervals, at rates of $\sim 10^1 - 10^2$ Hz. Monodisperse droplets were formed at a low rate, when a low pressure was applied on the reservoirs and when the pressure on the dex-HEMA reservoir was low compared to the pressure on the oil reservoir (Figure 2B). Because the system remained at low Reynolds numbers, the droplets moved in an ordered way along the microfluidic channel without coalescence. By increasing the pressure on the dex-HEMA reservoir the droplets were individually sheared at a higher rate (Figure 2C−D). Figure 3A illustrates a single-file flow of droplets, while Figure 3B illustrates a self-assembled necklace pattern of droplets that results at higher relative pressures of the aqueous phase. Multiple droplets were formed at the nozzle when the instability generated at the droplet tip propagated faster than the liquid tread can retract. At a droplet frequency of ~80 Hz, ~0.15 mg of monodisperse microgels was collected per hour. The size of the droplets can be slightly altered by changing the production rate, allowing tight control of final product size by modifying the relative operating pressures of the two inputs.

A photoinitiator, Irgacure 2959 (Ciba Chemicals), was added to the aqueous dex-HEMA solution before the emulsification process to allow collected microspheres to be subsequently cured by UV irradiation. When the dex-HEMA droplets containing the photoinitiator left the microfluidic device, they were collected in a separate vial and immediately polymerized by UV irradiation while still suspended within oil. The cured dex-HEMA microgels were then separated from the oil by centrifugation followed by several washing steps with deionized water. The removal of the oil from the surface of the microgels was verified by scanning electron microscopy. Figure 5A is an optical microscopy image of the dex-HEMA microgels suspended in water forming a hexagonal closely packed structure which is typical for microparticles exhibiting excellent size uniformity. Figure 5B shows the size distribution of a population of microspheres ($n = 150$), with an average diameter of 9.9 μm ± 0.3 μm. In contrast to the uniform microgels prepared by microfluidic emulsification, Figure 5C illustrates the polydisperse microgels as synthesized by polymerizing droplets obtained by simply vortexing the same dex-HEMA and oil solutions. The main parameter determining the size of the microgels is the width of the channel in which the dex-HEMA phase is focused when leaving the nozzle. Although very fine channels may be fabricated using soft lithography, with features on the order of a few microns, the operating pressures required to generate sufficient shear for emulsification becomes prohibitively large due to the increase in hydraulic resistance. When the applied pressure becomes too large, the bond between the PDMS and the

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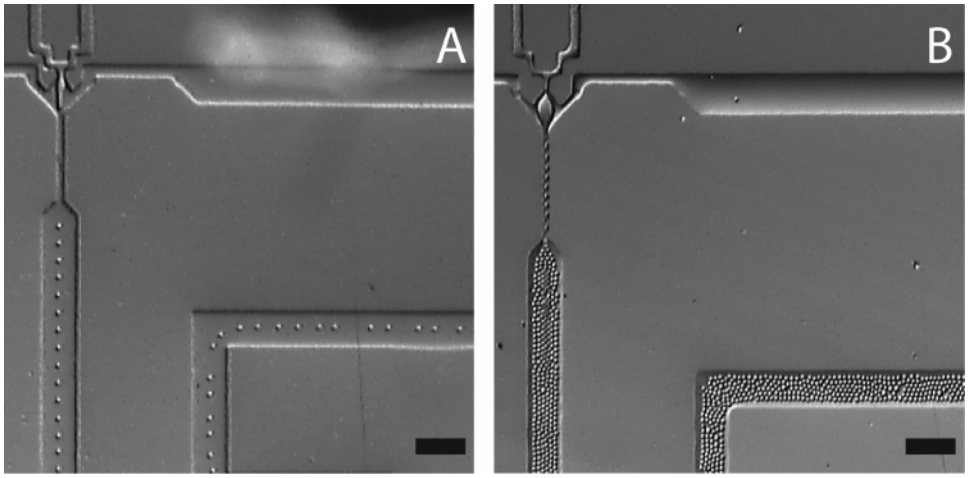

Figure 3. Optical microscopy images of the droplets moving in an ordered pattern along the microfluidic channels prior to collection. The scale bars represent 100 μm.

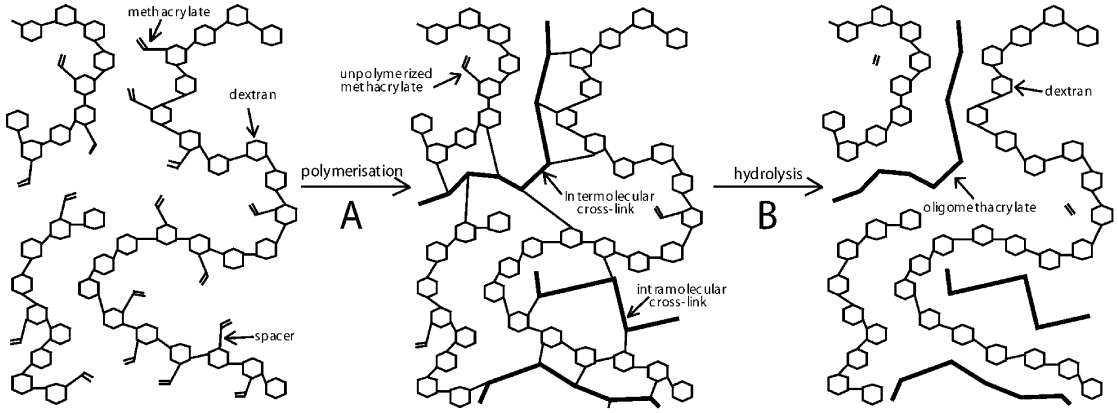

Figure 4. Schematic representation of the polymerization of dex-HEMA (step A), leading to the formation of intra- and intermolecular cross-links which form the three-dimensional hydrogel network, and (step B) the hydrolysis of the dex-HEMA hydrogels leading to the formation of dextran chains and oligomethacrylates as degradation products.

coverslip loses integrity, resulting in the destruction of the device. This tradeoff limits the size of the microgels which can be produced by our soft microfluidic devices at a given operating point.

Dex-HEMA hydrogels are biocompatible[15] and degrade by hydrolysis of the carbonate ester groups which connect the polymerized methacrylate groups and the dextran chains, leading to the formation of both the original dextran chains and low molecular weight oligomethacrylates. This degradation process is schematically represented in step B of Figure 4. The degradation rate of dex-HEMA hydrogels depends on the cross-link density and can be tailored from days to months by varying (i) the number of methacrylate groups per dextran chain and (ii) the initial water content.[12] Dex-HEMA hydrogels are ideally suited for the encapsulation of proteins as the network structure is able to sterically entrap these proteins.[16] Once the hydrogels start to degrade, the size of the pores between the dextran chains increases due to the cleavage of the cross-links. This increase in porosity promotes the diffusion of the entrapped proteins out of the microgel into the bulk environment. To show that the microfluidic based method also allows loading of the microgels with proteins, we added fluorescein-labeled bovine serum albumin (FITC-BSA; 0.1 mg per mg dex-HEMA) to the dex-HEMA phase. It was verified that the FITC-BSA was insoluble in the oil phase thus ensuring total encapsulation efficiency. Moreover, it is known[17] that BSA tends to accumulate in a dextran rich phase. Figure 5D is a confocal microscopy image of a FITC-BSA loaded dex-HEMA microgel produced by microfluidic emulsification. Figure 5E−F shows the FITC-BSA containing dex-HEMA microgels respectively during degradation (Figure 5E) and when completely degraded (Figure 5F). In this experiment we used sodium hydroxide to accelerate the degradation of the microgels, which normally takes several days to several weeks, depending on the cross-link density of the dex-HEMA microgels.[16]

In conclusion, we have shown that monodisperse biodegradable dex-HEMA microgels can be prepared by the use of a PDMS microfluidic device. Aqueous dex-HEMA droplets were formed by periodic shearing of an aqueous dex-HEMA stream within a co-flowing immiscible oil stream by an in-line nozzle geometry. The droplets were collected and polymerized by UV-irradiation with the formation of dex-HEMA microgels. A high production rate, by the simultaneous formation of monodisperse droplets, could be obtained by applying a high pressure on the dex-HEMA containing reservoir relative to the

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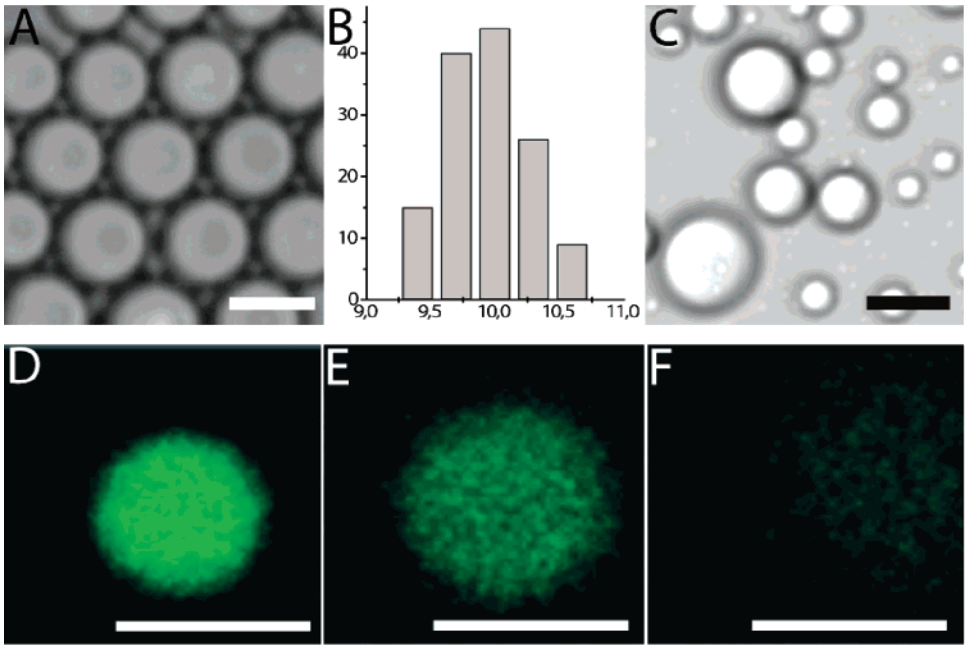

Figure 5. Optical microscopy images of (A) monodisperse dex-HEMA microgels synthesized using the microfluidic device, (B) the size distribution of the monodisperse microgels ($n = 150$) and (C) polydisperse microgels obtained by ordinary emulsification of the equivalent solution in mineral oil. Confocal images of dex-HEMA microgels containing FITC−BSA, respectively before (D), during (E) and after (F) degradation. The time lapse between the images D, E and F is ~30s. The scale bars represent 10 μm.

pressure on the reservoir containing the mineral oil. We also showed that the dex-HEMA microgels prepared by the microfluidic approach could be readily loaded with proteins. Microfluidic emulsification techniques such as those introduced in this paper could be useful for rapid prototyping of drug delivery systems based on microgels with narrow size distributions. Further research will focus on the scale-up[18] of the process and on the evaluation of the monodisperse biodegradable microgels for specific applications in drug delivery.

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Supporting Information Available: Video clips of the droplet formation at respectively low and high production rate are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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