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Microfluidic Synthesis of Reversibly Swelling Porous Polymeric Microcapsules with Controlled Morphology**

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Polymeric microcapsules with controlled morphology and functional properties have attracted considerable interest recently[1] due to their potential utilization as encapsulants for the controlled release of drugs,[2] dyes,[3] enzymes,[4] cosmetics,[5] pesticides,[6] food additives[7] etc. They were also employed as micro reactors for performing confined chemical and biochemical reactions by offering specific environmental conditions.[8] Microcapsules fabricated with biopolymers likewise possess promising applications in the encapsulation of transplanted cells by providing a feasible microenvironment.[9] To satisfy the increasing lack of encapsulation materials, various routes for fabricating microcapsules have emerged.[10] Out of them, some common strategies for producing polymeric microcapsules are the engulfment of templates (particles, droplets or bubbles) by a polymer shell, solidification of droplet shells, interfacial condensation reactions, controlled phase separation, coacervation, construction of colloidosomes, layer-by-layer deposition of poly electrolyte multilayers on a colloidal template etc.[11] However, most of these methods do not give a precise control over dimensions, structure and properties of the resulting capsules. Here we present a multi disciplinary approach to construct multifunctional microcapsules based on polymer-peptide conjugated macromolecules by forming uniform polymer droplets in a symmetrically designed flow-focusing microfluidic device.

Rapid maturation of versatile analytical procedures such as micro-total analysis systems has accelerated the methodology of microcapsule fabrication, which is now the focus of a wide area of research.[1e,12] Intensive studies on microfluidic techniques have enabled the rapid development of microchemical processing plants suitable for the synthesis and fabrication of monodisperse polymeric capsules in the range of several micro-meters.[11j,13] In a microchannel, capsules can be obtained by various physico-chemical processes like polycondensation,[13a,b] photo-polymerization,[13c,d] controlled-release of entrapped solvent from the shell[11j] etc. Recently, we reported a method for producing uniform polymeric microcapsules using a flow-through droplet-based supramolecular self-assembly of the block copolymer in a crossed microchannel.[14] The self-organization of a suitable amphiphilic polymer in the dispersed phase and the hydrodynamic instability arising in the channel between the dispersed and continuous phases were effectively combined for the formation of uniform droplets, which leads to well-defined microcapsules.

The molecular self-assembling behavior of amphiphilic block copolymers generating potential nano-structures has been investigated over the past few decades and continues to be a hot area of research due to their wide applications in various fields.[15] Modern advancements in controlled radical polymerization techniques (CRP) have facilitated the microstructure design and synthesis of block copolymers with various fascinating architectures.[16] Atom transfer radical polymerization (ATRP) is a powerful CRP technique for the synthesis of well-defined polymers with desired properties.[17] Synthetic polymers conjugated with polypeptides results in a new promising class of block co-polymers widely termed as ''molecular chimera''.[18] The synthesis scheme includes the growth of a polypeptide domain from an amino-terminated polymer chain by the ring opening polymerization (ROP) of an appropriate α-aminoacid-*N*-carboxyanhydrides (NCA) monomer.[19] There has been considerable effort to minimize the uninvited side reactions like chain transfer and chain termination, which interrupt the formation of well-defined block co-polymers. A strategy was developed by Deming et al. to minimize these side reactions by enabling the ROP through an amido-amidate metal-acycle intermediate and well-defined polypeptides were obtained with the desired properties.[20] Recently, we reported the synthesis of a star shaped poly(styrene-*b*-L-glutamate) copolymer by combining ATRP and NCA-ROP, intermediated by a similar nickel amido-amidate moiety and investigated its solid phase α–β configurational fluctuations and stability.[21] The linear analogues of this copolymer have also studied in detail by Klok et al.[22]

In this contribution, we describe a straightforward method for producing reversibly swellable microcapsules with high porosity using amphiphilic polystyrene-*b*-polyglutamic acid. The capability of these capsules to encapsulate quantum

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ADVANCED MATERIALS

dots (QDs) and stimuli assisted release is demonstrated. Poly(styrene-*b*-L-glutamate) (~ PS100-*b*-PGlu50, $M_n = 17300$ g mol^{-1}, PDI = 1.15) copolymers possessing three arms were synthesized according to the reported procedure.[21]

The benzyl-glutamate moieties of these star polymers were subjected to hydrolysis in order to form polyglutamic acid blocks. The molecular self-organization of these conjugated macromolecules was studied prior to the fabrication of microcapsules by preparing a stable micelle solution in aqueous medium (DMF–H_2O).The polymer solution in DMF (20 mg mL^{-1}) was mixed with the twice amount of water/DMF (1:1) and was heated to 110 °C for 2 hours. It was then allowed to cool down slowly at a rate of 1 °C min^{-1} in an automated thermal-cycler (Bio-Rad®).[23] This process facilitates slow and controlled precipitation of polymer, thus induce the molecular self-assembly resulting in a stable micelle solution. This solution was then transferred in to a dialysis tube and was then dialyzed against deionized water for 2 days. A trace amount of precipitated polymer was removed by centrifugation. As clearly visible in the TEM images, uniform spherical micelles with diameter in the range of 50 to 75 nm were formed with hydrophobic PS core and hydrophilic PGA shell (Fig. 1).

The microcapsules of this polymer-peptide conjugated block copolymer were fabricated in a PDMS based flow-focusing microfluidic device (Fig. 2). Neat PDMS based micro-channel was prepared according to the reported procedures.[24] The sketch of the microstructure was made using a computer-aided design (CAD) program. This CAD-generated pattern was printed on transparencies, further processed and used as photomask for UV-photolithography to generate the master. A thin layer of photo resist (photo curable epoxy SU-8) was spin coated onto a silicon wafer and was exposed to UV light through this photomask. The unexposed regions were removed by dissolving in a developing reagent. This imprinted silicon wafer serves as the master for the fabrication of micro channel. Initially this silicon/photoresist master was treated with fluorinated silanes for preventing irreversible bonding with PDMS.

Furthermore, the mixture of the silicon-polymer and curing agent (1:10) was poured onto it. Then it was cured at 70 °C for 6 h to produce the final replica of the designed microstructure by peeling off the master. This stamp was then irreversibly sealed to a flat silicon surface by plasma oxidation prior to their mutual attach. Lastly holes were drilled at proper positions to attach infusion tubes and sealed with epoxy.

Polymer microcapsules were fabricated by using the droplet-phase flow and by enabling the supramolecular self-assembly of the block copolymer at the immiscible fluid interface in the flow-focusing microchannel. The microscopic lamellar flow-focusing technique for the controlled production of micro droplets was introduced in the last decade and received much attention due to its applications in diverse fields ranging from material science to lab-on-chip applications and also in nano-bio-technology.[25] Recently, studies were also carried out to interpret the dynamics of the breakup of multi-compound jets by a numerical approach.[26] Here the solution of PS-PGA block copolymer in dichloromethane serves as the dispersed phase and it was infused through the middle inlet of the microchannel. The continuous phase used was deionized water with 5% polyvinyl alcohol (PVA) and it was introduced into the two side channels using an automated syringe pump (Harvard PhD 2000). PVA was used as droplet stabilizer, which strengthens the fluid-fluid interface of droplets. The flow rate of continuous phase was fixed with 15 μL min^{-1} and the infusion rate of polymer solution was adjusted to obtain independent droplets with a stable periodic droplet generation at the narrow orifice (Fig. 2). The polymer solution was injected at a rate of 15 μL min^{-1} and produced a continuous stream as seen in Figure 2. The infusion rate of the polymer solution was decreased slowly and stable droplets were generated when a rate of 5 μL min^{-1} was obtained. This process can be optimized by adjusting the infuse rate of both solutions. At this point, we observed droplets with uniform size and considerable space which eliminated clinging between two droplets in the channel. This continuous droplet phase flow was visualized using a high sensitive fluorescent microscope and instant images were captured using a CCD camera as visible in Figure 2. A slight change in the level of generation of consecutive droplets is observed as different color contrast, which may occurs due to the intermixing effect of liquid streams at the orifice, also helps to avoid droplet clinging. The separation between independent droplets increases upon forward motion due to the large flow rate of the continuous phase.

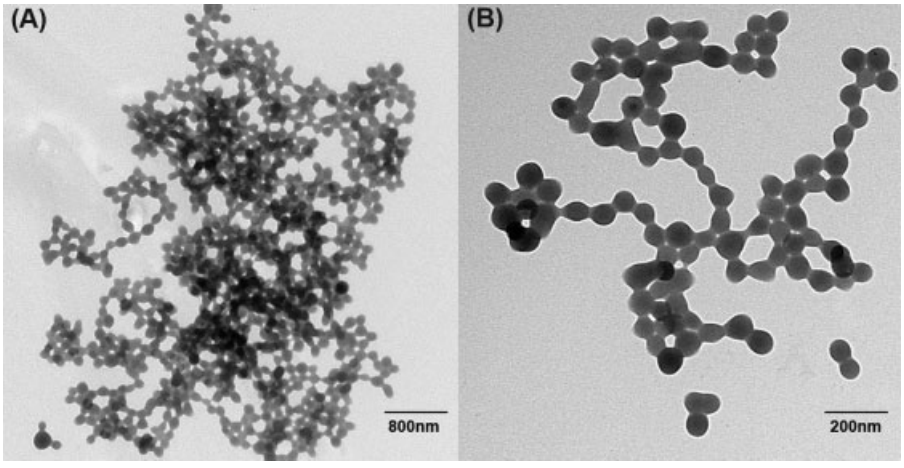

Figure 1. A) TEM image of the micelles of PS-*b*-PGA obtained in a DMF-H_2O solution mixture. B) The high resolution TEM image clearly shows spherical micelles within the range of 65–75 nm. Individual micelles appear to be agglomerated in both images due to drying of the TEM sample grids by solvent evaporation.

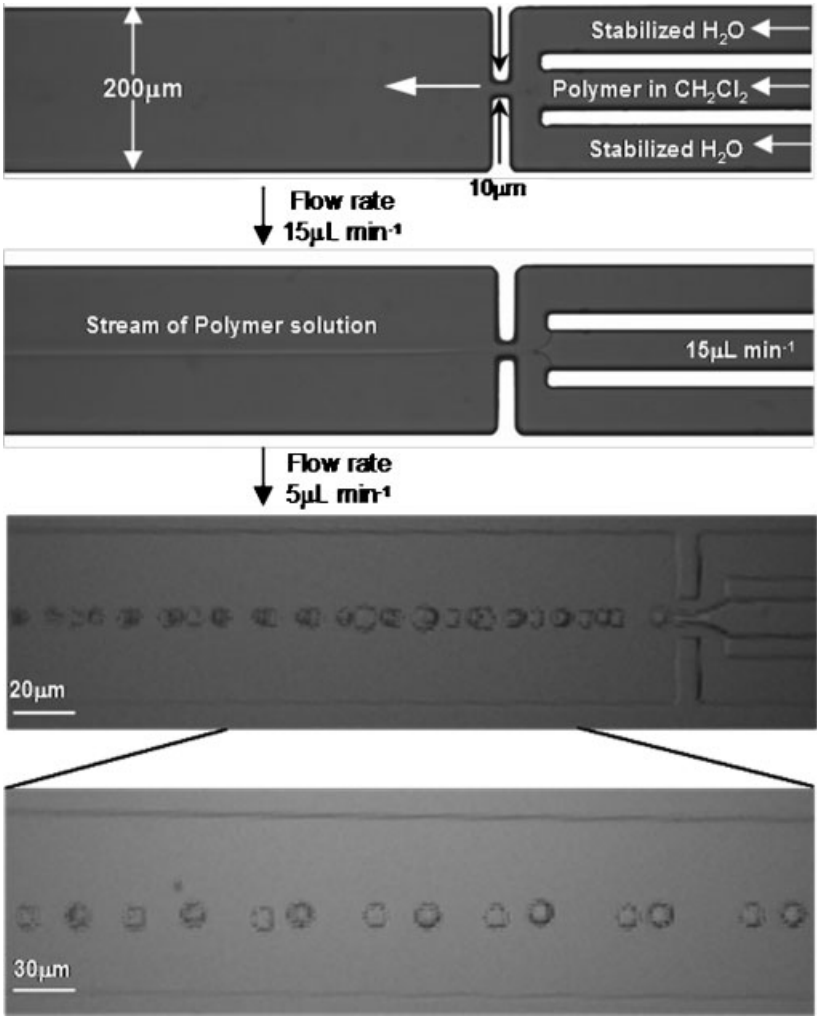

Figure 2. Microscopic images of the PDMS micro-channel containing three inlets and an outlet. Polymer solution was allowed to flow through the middle channel and stabilized water was injected through the side channels. The polymer solution flows like a continuous stream at a flow rate of 15 μL min^{-1}, which changes to individual droplets upon decreasing the flow rate to 5 μL min^{-1}, as clearly visible in the microscopic images.

The droplets were drained out and dispersed on a clean flat glass substrate and was dried by slow evaporation to remove the solvent trapped inside them. Due to this evaporation process and by the molecular self-assembly, microcapsules were formed with inner cavities and porous outer surface. It was assumed that the capsules were formed with hydrophobic cavities and hydrophilic surface due to the solubility induced alignment of individual molecules.

After this drying process, capsules were detached from the glass substrate by dipping into a beaker containing deionized water and further sonication for 5 minutes. The microcapsules were filtered with a copper micro filter with pore size of 10 μm and were further re-dispersed in deionized water for additional purification. It was then sonicated for 30 minutes for the complete removal of PVA layer, thereby clearing the nanopores on its surface. They were filtered out and the residing water and solvent inside the pores were removed by vacuum evaporation at 60 °C. An optical microscopic image of the microcapsules is depicted in Figure 3. The surface morphology and size of the microcapsules were investigated using a field emission scanning electron microscope (Fig. 3).

Microcapsules were also isolated from the channel by directly draining into a beaker containing deionized water. The presence of considerable amount of PVA around the capsules avoids them from merging together. It was kept at this stage by slow shaking for at least one hour to ensure complete solidification by the removal of the solvent inside. A layer of solvent appears over the water surface by this process. The solution was then sonicated for 30 minutes in order to remove the entrapped solvent and further polish off the surface PVA coating. The capsules were filtered using the copper microfilter, re-dispersed in deionized water and additionally sonicated for the complete removal of PVA layer and clean up the surface nanopores. This method offers more easy way to gain capsules from the microfluidic device and these capsules also exhibit comparable morphology and surface properties.

The magnified FESEM images distinctly visualize the surface morphology of the microcapsules. Figure 4A clearly exhibits the panoramic surface of a completely dried microcapsule. It was observed that the surface of microcapsule contains multitude protrudes, in addition to a few pores (Fig. 4A). The pores appeared at the center of some protrudes within 10 nm range. We performed detailed studies regarding this surface morphology by breaking the capsules and investigating the nature of its inner cavity. The surface of the capsule was partially removed by using oxygen plasma ashing[14] and this reveals the inner hollow characteristics and cavities of the capsules. The solvents chosen for generating droplets in the channel offers selective solubility for each block of the polymer-peptide molecule and this forces them to align in such a way that the PS block goes to the interior and PGA block line-up on the surface giving microcapsules with a hydrophobic cavity and hydrophilic surface.

The carboxylic acid moieties (COOH) of PGA can be easily ionized and this ionization-deionization behavior at various pH conditions imparts a swelling nature. At high pH conditions COOH groups ionize to form charged COO– groups which repel each other, leading to this high swelling. The polymeric microcapsules fabricated with the PS-*b*-PGA conjugated copolymer can also be swelled as the carboxylic acid of polyglutamic acid blocks can be ionized in a suitable pH buffer

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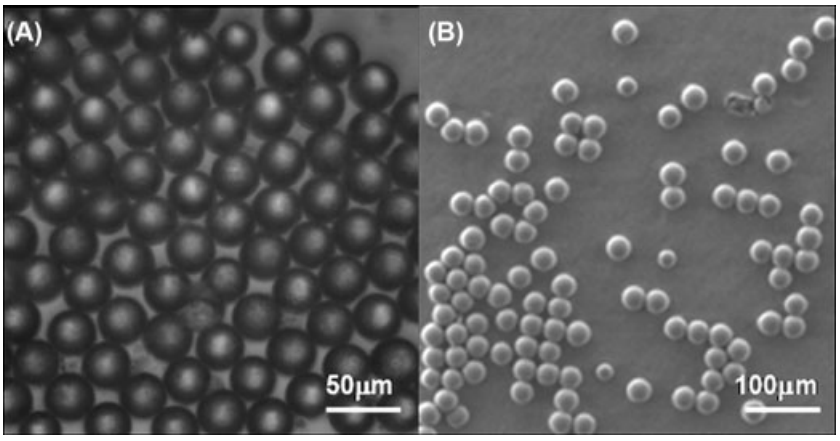

Figure 3. A) Optical microscopic image of the polymeric microcapsules dispersed on a silicon wafer after the filtering and drying process. B) FESEM image of the dried microcapsules are visualized. The microcapsules appear to have perfect spherical shape with controlled morphology and an average diameter of 30 μm.

solution. The previously dried microcapsules were suspended in a KH_2PO_4/NaOH buffer solution of pH 7.8 at a constant temperature of 40 °C for 6 h. They were filtered and characterized without any further heat treatment, including vacuum evaporation. Figure 4B shows the SEM image of an independent microcapsule swelled in the phosphate buffer solution. As a result of the swelling, there is not much considerable change in the size of microcapsules rather by 3–5 μm, the more surprisingly, majority of surface protrudes opens providing a nano-porous surface. The nanoopenings are identical in nature with a diameter in the range of approx.30 nm (Fig. 4B). Simultaneous drying and re-suspending in the pH buffer system helped to evaluate the reversibility of this opening and closing behavior induced by the capsule swelling. Even though complete reversibility was not observed with the entire surface protrudes, majority of them exhibit a reversible opening behavior.

The reversible pH stimulated swelling behavior of these microcapsules brings up its importance in various fields, especially, in the controlled release of the encapsulated molecules. Polymeric capsules embedded with nanoparticles, particularly, quantum dots or magnetic particles, are proved to be smart biosensors which allows external manipulations upon stimulation with suitable energy. But the release of these enclosed metal nanoparticles can be executed only through remote activation methods like laser irradiations and this method may cause damage to the polymer walls of the capsules leading to a triggered release.[8b] During laser irradiation, nanoparticles absorb and convert the electromagnetic waves into thermal energy in a selective manner. Thus causing a sharp increase in the temperature of the nanoparticles and this local heating is responsible for the disintegration of the capsule.

In this aspect, the microcapsules of the polymer-b-polypeptide macromolecules demonstrated here, allows post encapsulation and release of the encapsulated materials by comparatively weak stimulations. Further evaluation studies were carried out by loading the surface cavities with cadmium sulphide (CdS) nanoparticles. The obtained microcapsules ($\sim 2 \times 10^2$ capsules/mL) were immersed in 30 mL phosphate buffer solution with pH 7.8, until it undergoes perfect swelling (over night).

A pre-synthesized aqueous colloidal solution of CdS nanoparticles[27] (2 mL, conc. $\sim 10^6$ particles/mL) was added to this buffer solution and the mixture was sonicated (~40 kHz) for 2 h at room temperature and an additionally one hour to ensure the maximal degree of encapsulation. Approximate encapsulation efficiency was evaluated using PL spectroscopy by measuring the luminescence intensity of the stock solution before and after encapsulation (Supporting Information). It was observed that about 40% of the CdS nanoparticles from the buffer solution were entrapped in the microcapsules.

The QD loaded microcapsules were filtered and dried by vacuum evaporation at 40 °C for 6 hours. The dried capsules were then re-suspended in distilled water and thoroughly washed. They were then filtered by the copper micro filter and dried by evacuation at room temperature overnight. The release performance of the entrapped quantum dots was investigated by

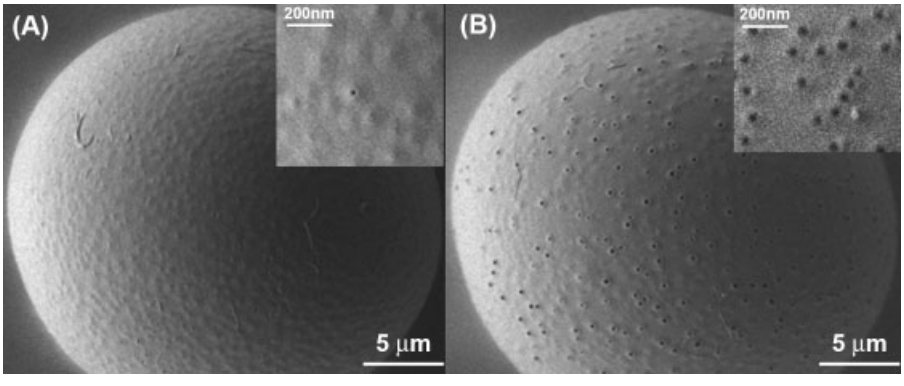

Figure 4. A) FESEM image of a dried microcapsule fabricated using the polymer-peptide conjugated block copolymer. Nano protrudes are visible on the surface due to the closed nanopores attributed by capsule drying. Tiny openings (~10 nm) accompanied with some protrudes is depicted in the inset picture. B) FESEM image of a microcapsule swelled in a phosphate buffer solution of pH 7.8. Porosity of the capsule surface can be clearly visible which are formed by the protrude opening induced by molecular swelling. The magnified view of the surface pores is depicted in the inset. Pores with diameter in the range of 30 nm can be observed.

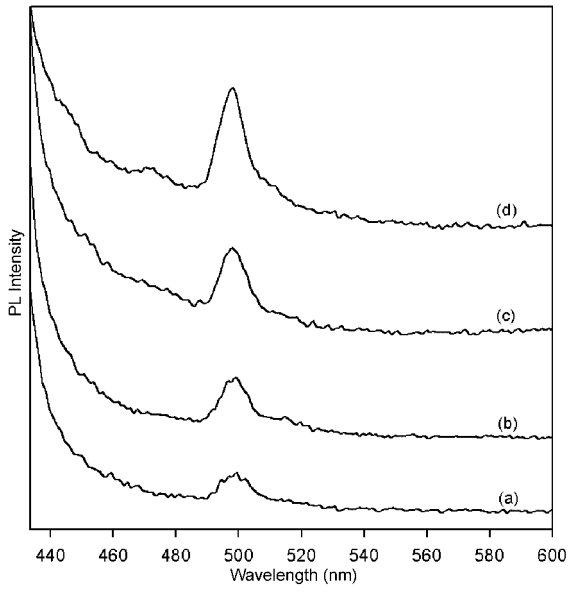

Figure 5. Photoluminescence spectrum of the released CdS quantum dots from the microcapsule cavities. a) PL spectrum monitored after 10 minute swelling and in buffer solution and further 2 minutes sonication. b) After 30 minutes swelling and 2 minute sonication. c) After 2 hour swelling followed by 2 minute sonication. d) After 2 hour swelling followed by 20 minute sonication.

using photoluminescence spectroscopy. The metal loaded microcapsules were suspended in the buffer solution with same pH and sonicated for 2 min. Depending on the degree of swelling of microcapsules; the entrapped CdS nanoparticles are released and can be monitored by measuring the photo-luminescence intensity. Figure 5 shows the combined photo-luminescence spectra monitored at different swelling level. The increase in the PL intensity reveals the controlled release of entrapped nano-particles from the surface cavities. A maximum release of entrapped nanoparticles was observed after swelling of microcapsules in buffer solution for 2 h followed by 20 minute sonication. The release of encapsulated particles was observed to be about 72% at these experimental conditions. The enlargement of the surface cavities by ionization in a buffer medium and stimulation by sonication cause this controlled release to external surroundings.

In summary, we have demonstrated a versatile strategy for fabricating reversibly swellable porous polymeric microcapsules possessing functional properties. A microfluidic-based approach has been performed to produce the microcapsules with controlled shape and well-tuneable size.

Amphiphilic polymer-peptide (PS-*b*-PGA) conjugated macromolecules used for the preparation of these functional capsules were synthesized by the combination of ATRP and NCA-ROP techniques The molecular self-assembly of this class of block copolymer was also investigated by preparing a stable micelle solution. The surface morphology and shape of these capsules were evaluated by using optical microscopy and FESEM. The hollow inner cavities and reversibly opened nano pores on the surface imparts it applications in various fields. The encapsulation permeability of these microcapsules was evaluated by loading them with CdS nanoparticles and its release performance stimulated by pH was measured by photo-luminescence spectroscopy. The studies regarding the polymer-b-polypeptide conjugates, in addition to its micro-capsule fabrication offers a mean to obtain synergy between the self-organization behavior, micro-fluidics and applications of polypeptides in bio-nanotechnology. The modifications on the microstructure of the polymer, functionalization with bio-molecules and utilization of embedded quantum dots as biosensors are the ongoing research.

Experimental

Hydrolysis of star poly(styrene-block-γ-benzyl-L-glutamate): The benzyl groups of glutamate were removed by hydrolysis using HBr as hydrolysing reagent. The block copolymers (2 g, ~0.1 mmol) were dissolved in CH_2Cl_2 (20 mL) in a Schlenk flask. Trifluoro acetic acid (8 mL) and hydrogen bromide (30% anhydrous hydrogen bromide in glacial acetic acid) (5 mL) were added drop-wise to this polymer solution. The reaction mixture was stirred at room temperature for 6 hours. The hydrolyzed polymer was then precipitated by the addition of diethyl ether, filtered and finally washed with distilled water to remove HBr salts. The obtained polymer was then dried under vacuum and was further characterized. Extend of this hydrolysis was measured by FT-IR spectroscopy. The broad IR absorption peak around 3200 cm^{-1} corresponds to the carboxylic acid groups. ^1H NMR (300 MHz, $CDCl_3$, 293 K, δ): 1.18–1.3 (initiator and leucine proton), 1.59–3.7 (CH_2 of PGA, backbone CH_2 of PS), 3.98–5.18 (backbone CH of PGA and PS), 5.51 and 8.13 (backbone NH of PGA), 6.37, 9.13, and 10.43 (OH of carboxylic acid and NH_2), 6.9 and 7.3 (aromatic Ph-H).

The TEM images of polymer micelles were taken on Philips Morgagni Transmission Electron Microscope operated at 80 kV. The droplet generation in the micro channel was visualized using a Nikon Eclipse (80i) microscope and the images were taken with ProgRes (C10 plus) CCD camera. The SEM images of the microcapsules were taken with a JEOL-FESEM (JSM-6700F). The photoluminescence spectroscopy was monitored using a Hitachi fluorescence spectrophotometer (F-45000).

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