Multi-scale microporous silica microcapsules from gas-in-water-in-oil emulsions

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Controlling the surface area, pore size and pore volume of microcapsules is crucial for modulating their activity in applications including catalytic reactions, delivery strategies or even cell culture assays, yet remains challenging to achieve using conventional bulk techniques. Here we describe a microfluidics-based approach for the formation of monodisperse silica-coated micron-scale porous capsules of controllable sizes. Our method involves the generation of gas-in-water-in-oil emulsions, and the subsequent rapid precipitation of silica which forms around the encapsulated gas bubbles resulting in hollow silica capsules with tunable pore sizes. We demonstrate that by varying the gas phase pressure, we can control both the diameter of the bubbles formed and the number of internal bubbles enclosed within the silica microcapsule. Moreover, we further demonstrate, using optical and electron microscopy, that these silica capsules remain stable under particle drying. Such a systematic manner of producing silica-coated microbubbles and porous microparticles thus represents an attractive class of biocompatible material for biomedical and pharmaceutical related applications.

1 Introduction

Nature has optimised the formation of organic–inorganic hybrid materials for functional purposes over millions of years of evolution. One remarkable example is the formation of silica encasing diatoms where the deposition of the inorganic components is directed by an organic scaffold. These natural hybrid materials have inspired a wide range of studies for mimicking these structures by using strategies derived from the fields of engineering, chemistry and biology.1–3 The interest in bimimetic engineering of silica is motivated not only by the interest to produce nanostructured materials with unique properties, but also the possibility to obtain a low-cost source of natural material that requires minimal processing.4 Synthetic bulk approaches of producing mesoporous silica structures by means of using surfactant micelles as templates for silica precipitation, have resulted in restricting the pore size down to 10 nm,5,6 while triblock copolymers with anionic surfactants7 and butanol8 have also been employed in the formation of mesoporous silica structures with different pore sizes. Moreover, colloids which have a larger size variety can similarly be used as templates to vary pore diameters.7 Additionally, bulk-emulsification techniques for the production of spherical porous silica particles have been employed.10–12 However, systematic control over internal pore dimensions on the micrometer scale (~1–20) is challenging to achieve.

The encapsulation of microbubbles has recently been demonstrated in the context of modulating the porosity of microcapsules for a wide range of biomedical applications13,14 such as targeted drug delivery,15,16 gene therapy,17 delivery of biocatalysts18 and as tumour/thrombus-destruction materials.19 Moreover, hollow particles have also attracted attention in the field of energy-storage20 and in cell culture assays.13,14 Furthermore, bubbles form the basis of generating foamed porous materials which are essential in the cosmetics, pharmaceutical and food industries.15,21 Such porous materials may offer orthogonal routes for controlled release of chemicals and are thus quintessential in these industries. There are numerous ways of producing such multi-phase systems.14 Typically bulk processing techniques or ultrasonic approaches allow for the encapsulation of microbubbles, however, such routes do not offer an easy way to control accurately the number of encapsulated bubbles and their relative size.22,23 To that effect, microfluidic strategies, and in particular droplet-based microfluidics, offer significant advantages.24–30 These strategies afford a high
level of control over the monodispersity and composition of the droplets, and by forming emulsions drop-by-drop, polymeric and protein based particles of various shapes and sizes have been generated.\textsuperscript{24,28,31,32} In particular, however, the use of silica for the generation of such microporous structures offers advantageous routes due to its biocompatibility and ease of production.\textsuperscript{33} In fact, silica nano and micro-particle effects on cells and organs through multiple exposure routes such as dermal, oral, pulmonary and intravenous, indicate the high level of biocompatibility offered by this inorganic material.\textsuperscript{34}

Here, we demonstrate that by using a non-planar microfluidic device, monodisperse gas-in water-in oil emulsions can be generated in a robust and accurate manner. We further show that the number of internal gas bubbles within the external aqueous droplet can be specifically controlled by varying the pressure at which gas is injected, while keeping the liquid phase flow rates constant. Moreover, by using a silicic acid/buffer system as the aqueous phase, the precipitation of silica occurred almost instantaneously around the encapsulated gas bubbles, allowing for the formation of hollow silica capsules with controllable pore sizes on the micrometer scale. We show that using this method, controlling the internal (bubble size as well as number) can easily be modulated by changing the flow rates and gas pressure. These parameters can be altered within seconds, meaning that fabrication of core–shell silica capsules with varying dimensions can be made without the need of making new masters and PDMS stamps, as is the case in previous approaches involving gas encapsulated materials.\textsuperscript{35} Furthermore, electron microscopy was conducted in order to investigate the morphology of the silica beads. Finally, SEM micrographs revealed that the microcapsules remain stable when dried and cross-sectional images indicate that the internal structure of the particles is full of cavities which correspond to the number and size of previously encapsulated gas bubbles.

2 Experimental methods

2.1 Device fabrication

A two-step photolithographic process was utilised to fabricate the master used for casting PDMS devices as has been previously reported.\textsuperscript{32} A 25 μm thick negative photo-resist (SU-8 3025, MicroChem) was spin-coated onto a silicon wafer. This in turn, was soft-baked for 15 minutes at 95 °C. The photomask in Fig. 1b was then placed onto the wafer, exposed under UV light in order to induce polymerisation and then post-baked at 95 °C for 3 minutes. A second 25 μm thick layer (SU-8 3025, MicroChem) was then spin-coated onto the wafer and soft-baked for 30 minutes at 95 °C. The second mask (shown in Fig. 1a) was aligned with respect to the patterns formed from the first mask. This was in turn exposed to UV light and post-baked for 5 minutes at 95 °C. Finally, to remove uncross-linked photo-resist, the master was developed in propylene glycol methyl ether acetate (PGMEA, Sigma-Aldrich).

A 10:1 ratio of elastomer PDMS to curing agent (Sylgard 184, DowCorning, Midland, MI) was used to fabricate microfluidic devices. The mixture was cured for 3 hours at 65 °C. The hardened PDMS was cut and peeled off the master, while holes of 0.75 mm were punched on the PDMS. This was then bonded onto a glass slide by treating with a plasma bonder (Diener Electronic, Ebhausen, Germany).

2.2 Droplet formation

The flow rates within the channels were controlled using neMESYS syringe pumps (Cetoni, Korbussen, Germany). For droplet formation two aqueous phases were used. One of the phases consisted of silicic acid, while the other was comprised of 50 mM sodium phosphate buffer with 2% Tween 20. Furthermore fluorinated oil (Fluorinert FC-40, Sigma Aldrich) containing 2% w/w fluorosurfactant (RAN biotechnologies) was used as the continuous phase. Nitrogen and carbon monoxide gases were used as the inner phase. The formation of droplets was monitored on-chip using a Mikrotron High Speed Camera.

2.3 Scanning electron microscopy

The silica-coated microbubble samples were mounted onto a glass slide and left to dry for 24 hours. This was then placed onto a multi-pin specimen mount. A 5 nm platinum layer was subsequently sputter coated onto the sample and images were obtained using a TESCAN MIRA3 FEG-SEM operating at 5 kV.

3 Results and discussion

In order to generate microcapsules with variable pore sizes, we first generated gas-in water-in oil emulsions (g/w/o). This was done using a non-planar microfluidic device, where the inner phase contained the gas, while the middle and outer phases consisted of silicic acid (with buffer) and oil respectively. Once the silicic acid comes into contact with the sodium phosphate buffer (Fig. 1), the precipitation of silica occurred rapidly\textsuperscript{1}.

**Fig. 1** (a–c) Design of the microfluidic device used. A two-step lithographic process was used in order to fabricate the device shown in (c). (a) Mask 1: outer oil phase inlet with its respective channels and outlet. (b) Mask 2: middle and inner phase inlets. The aqueous phase consists of two inlets. Inlet 1: silicic acid. Inlet 2: sodium phosphate buffer with Tween 20. Once these two solutions, intersect, a long channel allows for successful mixing before they reach the gas channel. (c) Schematic representation of the device used to generate gas-in-water-in oil droplets. The third junction is the non-planar (3-D) junction.
around the encapsulated gas bubbles resulting in the formation of microporous silica capsules. It was determined that by varying the pressure at which gas was injected through the device, while keeping the flow rates of the liquid phases constant, both bubble size but also the number of internal bubbles could be specifically controlled. The bubbles suspended in the aqueous phase, were then encapsulated by the oil phase resulting in the formation of an bubble loaded aqueous droplet in oil. This gas-water-oil (g/w/o) three-phase system was the basis for all subsequent experiments. The silica microparticles were then de-emulsified and re-injected into an aqueous medium, before being imaged using optical and electron microscopy. The latter of which revealed the cavities within the porous microparticles.

3.1 Device design

The silica-coated microcapsules were synthesised using a g/w/o emulsion strategy that relies on the formation of monodisperse microdroplets.

These consisted of a gas core, surrounded by an aqueous phase, comprised of silicic acid with sodium phosphate buffer, all of which is encapsulated within a continuous immiscible oil phase. In order to generate such droplets, the use of a non-planar microfluidic device design was employed. This ensured that the aqueous phase did not wet the microfluidic channel surface and allowed for successful droplet generation. The master used for all experiments was fabricated using a two-step soft lithography process. To this effect; 25 µm high structures were formed using a film mask (Fig. 1b) and then aligned with channels fabricated using a second film mask shown in Fig. 1a. This resulted in the generation of a non-planar device which is schematically depicted in Fig. 1c (see Methods for additional details on device fabrication).

Typically, droplet generation involves an aqueous phase intersecting with an immiscible oil phase resulting in the formation of water-in-oil or oil-in-water droplets. However, in order to generate g/w/o emulsions, an additional inlet was introduced. The device architecture thus consists of two main regions, each of which plays a fundamental role in the operation of the device. In the first region, silicic acid is mixed with sodium phosphate buffer and with Tween 20, as shown in the top right inlet of Fig. 1c. A long serpentine channel separating this junction from the second junction allows for the two solutions to mix well before being introduced to the channel containing the gas phase. The channel length separating the first and second junction is crucial in increasing the viscosity of the aqueous phase (as the reduction of silicic acid to silica commences upon mixing with the buffer) which in turn allows for successful bubble formation and encapsulation due to increased solution surface tension. The two aqueous solutions could not be pre-mixed before being pumped through the microfluidic channels due to the speed at which the silicic acid and sodium phosphate/Tween 20 solutions precipitate and gel (see Fig. S1a, ESI†), which is why they were mixed on-chip. In the second junction, the gas intersects with the silicic acid/sodium phosphate/Tween 20 solution resulting in the production of bubbles. This in turn is encapsulated by the oil phase in the third junction. This entire process of g/w/o monodisperse droplet formation is schematically represented in Fig. 1c (bottom right inlet).

3.2 Silica-coated microcapsule formation and characterisation

Next, we explored the generation of micron-sized g/w/o emulsions using nitrogen as a model gas. The relationship between the gas pressure and the number of internal bubbles encapsulated was further investigated. This was determined by keeping the respective aqueous phase flow rates \( (Q_{\text{aq}}) \) constant at 300 µL h\(^{-1}\) and the outer oil phase flow rate \( (Q_{\text{oil}}) \) at 800 µL h\(^{-1}\), while the gas pressure values ranged from 110 up to 200 mbar. As previously mentioned, once the silicic acid comes into contact with the sodium phosphate buffer, the precipitation of silica initiates. If the solutions remain within the microfluidic device for too long, then silica will form in the micro-channels and block the device. To this effect, determining the residence time after which the two aqueous phases (silicic acid and buffer) intersect is crucial for proper droplet generation. It was determined that for aqueous phase flow rates of 300 µL h\(^{-1}\), the residence time for the solutions to reach the junction where the gas bubbles are encapsulated is approximately 0.5 seconds. For the concentration of silicic acid used, it was found that if this residence time was further increased, then silica formed within the micro-channels.

Once formed, the number of encapsulated internal bubbles was determined by high speed imaging and frame by frame analysis of the data. The number of internal bubbles \( (N) \) was found by averaging over a total of 10 subsequent droplets. Fig. 2a shows g/w/o emulsions which are formed at the third junction of the device. Fig. 2a shows g/w/o emulsions with a range of number of internal bubbles, varying from \( N = 1 \) up to \( N = 20 \). The precise and systematic control of this microfluidic setup is demonstrated in each of the optical micrographs, with 1, 2, 3, 8 and 20 internal gas bubbles being encapsulated with increasing pressure. Moreover, an optical image showing \( N = 6 \) internal bubbles is shown in Fig. S1b (ESI†). As expected, the higher the gas pressure, the more internal bubbles that can be encapsulated. Interestingly, a plot of \( N \) against gas pressure, which is shown in Fig. 2b, indicates an almost linear relation, with a gradient close to 0.2. Following formation, droplets were then collected and placed on a cover slide in order to investigate whether the microcapsules remained stable over time. As can be seen in Fig. 3a–d, not only are the particles stable but during silica precipitation, the gas remains trapped within the droplet long enough for a silica shell to be formed around the bubbles. This allows for a precise way of modulating porosity, which suggests that these microcapsules can be used for various biomedical applications where pore sizes are instrumental. Additionally, the high level of monodisperse droplets and encapsulated bubbles formed using this setup can be seen in the micrographs of Fig. 3a–d.

Scanning electron microscopy (SEM) was used to characterise the microscale morphology of the silica-coated microcapsules. Following emulsion generation, droplets were incubated at room
temperature for 1 hour before washing and de-emulsification. The silica particles were then re-emulsified in deionised water, placed on a glass slide and left to dry for 24 hours. The SEM images in Fig. 4a show monodisperse, spherical particles with a rough surface morphology. Moreover, the microcapsules remain mostly stable and do not seem to collapse upon drying. They do, however, shrink by approximately a factor of 2, which is due to the water diffusing out of the microbeads.

The pores that can be seen on the surface of some silica particles is probably the result of a gas bubble coming too close to the droplet interface during silica formation. However, this can be resolved by increasing the viscosity within the aqueous solution during microfluidic droplet generation in order to restrict gas movement within the emulsion. This can either be done by increasing the silicic acid concentration, or by increasing the serpentine length between the first and second junction. Furthermore, cross-sectional micrographs of the particles were taken by cutting the silica beads in half. The images in Fig. 4b–g reveal the cavities within the capsules where the bubbles were and give an insight into the internal structure of the particle. It is clear that by regulating the number of encapsulated bubbles one can tailor the porosity of microcapsules to the point where molecular release/uptake through the silica network and into the environment can be specifically controlled.

The pore size of the capsules is essential in controlling the release kinetics for drug delivery applications. The structures that we have synthesised in this paper contain two length scales for the pore sizes: a nm scale porosity originating from the silica shell formation and a micron scale porosity controlled through the presence of gas bubbles at the synthesis stage. In order to investigate the nm porosity lengthscale, we used two solutions containing fluorescent dyes but with different molecular sizes.

First, a solution consisting of 500 mM bovine serum albumin (BSA) labelled with fluorescein (FITC) was encapsulated within the silica capsule and the release kinetics were investigated by measuring the fluorescence intensity as a function of time. Conversely, a 500 mM solution of purely FITC molecules was encapsulated within silica capsules and the release kinetics of that system were also investigated. The relative intensity of the two systems was then compared in order to determine the relative amount of molecules that escape from the two different systems. From Fig. 5, it is clear that even though the concentration of dye molecules is the same for both cases, the BSA–FITC system has 10 times less relative intensity than the FITC–alone system. The initial increase in fluorescence from the BSA–FITC system can be attributed to the protein–dye molecules present on the surface of the capsule.

Therefore, it can be concluded that the pore size of the system must lie between the sizes of the two molecules, FITC and BSA–FITC, i.e. between 1–6.8 nm.
4 Conclusions

Controllable generation of monodisperse micro-sized gas-in water-in oil (g/w/o) droplets in a reproducible manner is desirable for next-generation delivery strategies, yet remains challenging. Here, we show that by utilising non-planar microfluidics, a scalable platform for generating silica-coated microcapsules can be developed, mimicking the formation of diatoms in nature. G/w/o droplets were generated on chip, and by mixing silicic acid with sodium phosphate buffer, multiple microbubbles stabilised by a silica shell within the same microcompartment could be formed. We demonstrate that using this approach, control over bubble size and number of encapsulated bubbles within individual capsules can be precisely achieved by modulating the pressure at which the gas-phase is introduced on-chip. Crucially, these important parameters can easily be changed within seconds, allowing for the formation of core–shell silica capsules with varying dimensions. In addition, following droplet generation, optical microscopy reveals that these emulsions are stable and that the gas remains trapped within the microparticles long enough for the precipitation of silica to form around the bubbles. Moreover, scanning electron micrographs further corroborates that these particles are stable when dried and that cavities formed due to the presence of gas bubbles during droplet generation contribute towards the silica capsule microporous morphology. Such silica-based microcapsules represent a class of biocompatible and non-toxic material, and in conjunction with the high level of control over their formation, these multi-scale microporous capsules have favourable characteristics enabling them to serve as a platform to explore various delivery and related biomedical applications.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The research leading to these results has received funding from the European Research Council under the European Union's...
Seventh Framework Programme (FP7/2007-2013) through the ERC grant PhysProt (agreement no. 337969). We are also grateful for financial support from the EU Horizon 2020 programme (Marie Skłodowska-Curie ITN G. A. No. 675007 to T. A. H., G. J. L. B. and T. J. P. K.), the Oppenheimer Early Career Fellowship (A. L.), the BBSRC (T. P. J. K.), the Newman Foundation (T. P. J. K.) and the Cambridge Centre for Misfolding Diseases.

Notes and references