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Biomineral-inspired fabrication of semi-permeable calcium phosphate–polysaccharide microcapsules

Ingrid Lévêque, Katja H. Rhodes and Stephen Mann*

School of Chemistry, University of Bristol, Bristol, UK BS8 1TS. E-mail: s.mann@bris.ac.uk

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Mineral–polysaccharide microcapsules are produced by a one-step method in which the deposition of a semi-permeable alginate/chitosan membrane around droplets of sodium alginate is coupled with *in situ* precipitation of amorphous calcium phosphate. Nucleation of calcium phosphate occurs within the membrane by counter-diffusion of Ca^{2+} and HPO_4^{2-} ions and can be controlled by the phosphate concentration in the alginate droplets to produce hybrid microcapsules with enhanced mechanical strength and reduced permeability to encapsulated molecules such as haemoglobin and ibuprofen.

Polysaccharide-based microspheres and microcapsules are of widespread importance for the bioencapsulation and controlled release of cells,[1] enzymes,[2] proteins[3] and drugs[4] in applications such as drug delivery and cell transplantation. The bioactive components are usually entrapped within millimetre-sized gel beads of Ca-alginate (a copolymer of 1,4-linked α-L-guluronic and β-D-mannuronic acids, p$K_a \approx 5$), and coated with polycationic polyelectrolytes, such as poly-L-lysine or chitosan (deacetylated chitin, p$K_a \approx 9$), to improve the microcapsule stability and controlled permeability.[5–7] The coating process is readily achieved for example by immersing droplets of Ca-alginate into chitosan solutions or sodium alginate into Ca-containing solutions of chitosan; in both cases, interfacial charge matching at neutral pH between the anionic and cationic polysaccharides produces a semi-permeable membrane around the alginate droplet.[8] The gel-like interior can be subsequently liquified by removing the Ca^{2+} cross-links using a complexing agent such as citrate to produce hollow microcapsules.[9]

Previous studies have shown that the mechanical strength and permeability of the alginate–chitosan membrane are influenced by changes in the polyelectrolyte molar masses and concentrations.[6] In recent investigations, Livage and co-workers[9] demonstrated that a thin external coating of amorphous silica can be deposited on preformed alginate/poly-L-lysine microspheres, and that the composite structure had improved mechanical strength and controlled release behaviour. Because inorganic–organic hybrid materials often exhibit complementary properties they are likely to be of generic importance in the design of biocompatible microcapsules, and in this regard, synthetic approaches based on mimicking natural processes such as biomineralization should offer much promise. Biomineralization often involves the diffusion-controlled deposition of inorganic minerals within porous organic polymeric matrices,[10] and here we simulate this strategy to prepare semi-permeable mineral–polysaccharide microshells for bioencapsulation. Specifically, we describe a one-step method in which the deposition of a porous alginate/chitosan membrane around droplets of sodium alginate is coupled with the controlled precipitation of calcium phosphate arising from counter-diffusion of ions across the polysaccharide interface (see graphical abstract). Nucleation of calcium phosphate occurs within the membrane rather than as an external coating, and can be controlled by the phosphate concentration in the alginate droplets. The resulting hybrid microspheres show enhanced mechanical strength and reduced permeability to encapsulated molecules such as haemoglobin and ibuprofen.

Millimetre-sized gel beads of Ca-alginate/chitosan were prepared by adding droplets of sodium alginate to 50 mM Ca^{2+}-containing aqueous solutions of chitosan at near neutral pH.† A thin white membrane was observed immediately around the alginate droplet due to interfacial complexation of the oppositely charged polyelectrolytes. The membrane hardened with time but remained permeable to Ca^{2+} ions, such that within 1 hour the droplets were stabilized as gel-like beads due to cross-linking of the alginate network with Ca^{2+} ions. The microspheres could be handled with tweezers, stored in distilled water for several months or oven-dried without loss of structure. Samples prepared for FTIR spectroscopy showed broad bands corresponding to organic groups at 3400 cm^{-1} (OH, NH str), 1620 cm^{-1} (δNH_2, CO, H_2O), 1600 cm^{-1} (CH_2 bend), 1300–1000 cm^{-1} (CN, COC) and 700–400 cm^{-1} (δCH). XRD data indicated that the polyelectrolytes were disordered in the presence of Ca^{2+} but crystallized as lamellar mesostructures in the absence of the divalent cation. SEM images of the polysaccharide microspheres often showed collapsed structures with an intact surface membrane that contained high levels of Ca, Na and Cl (EDX analysis).

In contrast, dropwise addition of a sodium alginate solution containing high concentrations of phosphate (300 mM) to the Ca/chitosan solution produced mineralized polysaccharide microcapsules, 2–3 mm in diameter, that were structurally stable when examined in the SEM (Fig. 1a). High magnification SEM images showed a progressive change in the texture of the outer surface of the microcapsules as the phosphate concentration was increased, due to calcium phosphate nucleation within the alginate/chitosan semi-permeable membrane. With no phosphate or at a concentration of 50 mM, the membrane was soft and continuous and uniformly creased by exposure to the high vacuum of the SEM microscope (Fig. 1b). In comparison, alginate/chitosan shells prepared in the presence of 100 mM phosphate showed a heterogeneous surface texture (Fig. 1c), whereas a continuous granular-like membrane that was relatively unaffected by the high vacuum conditions of the SEM was observed at the highest levels of phosphate (300 mM) studied (Fig. 1d). In each case (except for 0 mM phosphate), the presence of Ca and P within the outer polysaccharide shell was confirmed by EDX analysis. XRD showed no reflections that corresponded to crystalline calcium phosphates suggesting that the mineral phase was amorphous calcium phosphate (ACP). This was confirmed by FTIR spectra recorded from dried samples, which showed only broad P–O bands centred at 1060 and 560 cm^{-1}.

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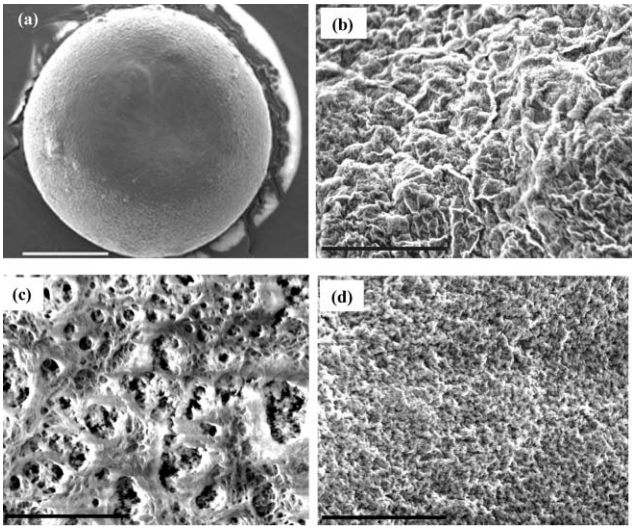

Fig. 1 (a) SEM image of an intact calcium phosphate mineralized polysaccharide microcapsule prepared in the presence of 300 mM phosphate. Scale bar, 400 μm. (b)–(d) High magnification SEM images showing microcapsule surface texture prepared in the presence of (b) 0 mM, (c) 100 mM and (d) 300 mM phosphate; scale bars, 20 μm.

The results indicate that counter-diffusion and co-precipitation of Ca^{2+} and HPO_4^{2-} ions at the droplet/water interface occurs concurrently with the formation of an alginate/chitosan membrane to produce mineralized microcapsules with increased structural stability. The changes in mechanical strength were further demonstrated by a simple shear test in which 100 microcapsules were stirred continuously at 300 rpm in 50 mL Tris-HCl (50 mM, pH 7.2) and the number of fractured capsules obtained after different periods of time recorded. While samples prepared in the presence of 50 mM phosphate showed similar low mechanical resistance to that observed for non-mineralized microspheres (50% were fractured after 400 min), those formed at 100 mM phosphate were significantly strengthened such that only 10% of the population were fractured even after 1400 min (Fig. 2). Interestingly, increasing the phosphate level to 300 mM dramatically reduced the mechanical resistance to fracture, such that nearly all of the microcapsules were broken within 500 minutes. The results, which are consistent with the change in surface textures shown by SEM (Figs. 1b–d), indicate that the outer membrane becomes brittle for relatively high levels of ACP deposited in the alginate/chitosan membrane.

The storage and release properties of the mineralized and non-mineralized alginate/chitosan microcapsules were assessed by encapsulation of a large water-soluble protein (haemoglobin, $M_w \approx 65000$, size $\approx$ 6 nm) or water-soluble drug molecule (ibuprofen, $M_w \approx 228$) within the capsules.‡ In both cases,

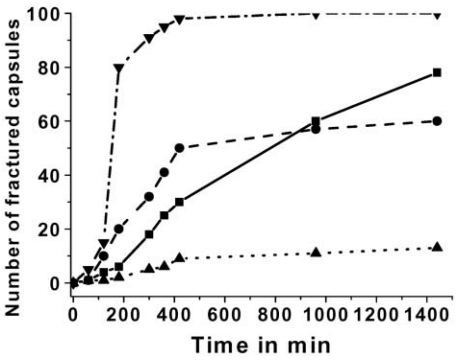

Fig. 2 Plot of mechanical resistance to fracture for microcapsules prepared with different levels of calcium phosphate. ■, 0 mM, ●, 50 mM, ▲, 100 mM, ▼, 300 mM phosphate.

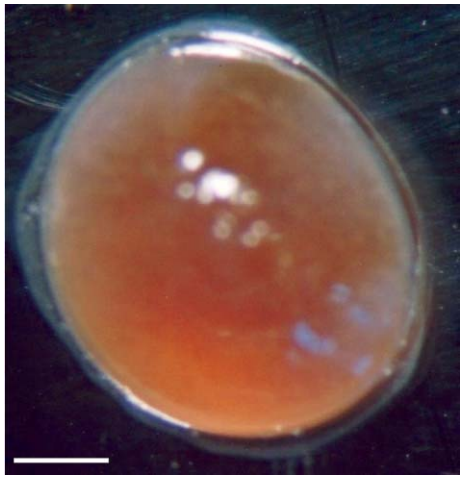

Fig. 3 Optical micrograph of a haemoglobin-encapsulated polysaccharide microcapsule prepared in the presence of 300 mM phosphate. The calcium phosphate mineralized alginate/chitosan membrane (white outline) can be clearly observed around the protein-rich alginate red interior. Scale bar, 100 μm.

stable microcapsules were prepared with no immediate leaching of the entrapped molecules from the alginate gel interior (Fig. 3). However, extended time studies indicated that over 70% of the entrapped heamoglobin or ibuprofen molecules were released from the unmineralized microspheres to the external solution within a period of a few hours (Fig. 4), indicating that the molecular mass cut-off for diffusion through the alginate/chitosan outer membrane was greater than 65 kDa. In contrast, the amount of haemoglobin or ibuprofen released, as well as the respective rates of release, were reduced progressively by increasing the level of calcium phosphate associated with the alginate/chitosan membrane. In particular, microcapsules prepared from 300 mM phosphate retained approximately 70% of the trapped protein or drug molecules even after 10 days, indicating that the *in situ* mineralization

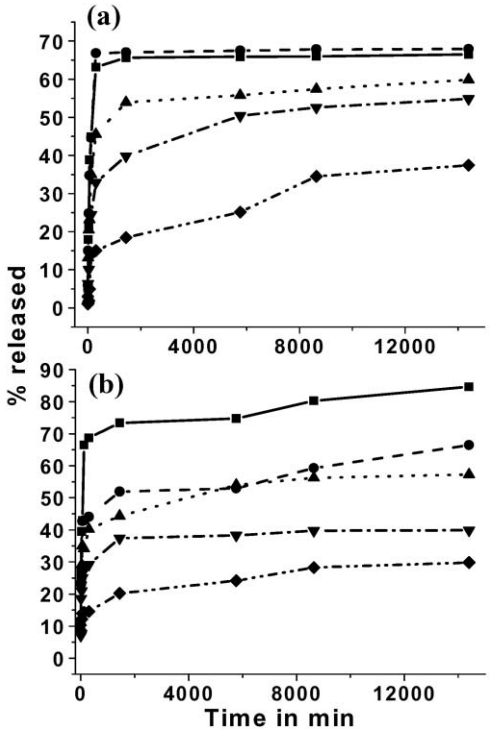

Fig. 4 Plots of percentage release of (a) haemoglobin and (b) ibuprofen molecules from microcapsules prepared with different levels of calcium phosphate; ■, 0 mM, ●, 30 mM, ▲, 50 mM, ▼, 100 mM, ♦, 300 mM phosphate.

process was successful in significantly reducing the membrane permeability.

In conclusion, semi-permeable calcium phosphate–polysaccharide microcapsules can be prepared by a one-step method involving the counter-diffusion of co-precipitating ions through an incipient alginate/chitosan membrane produced specifically at the surface of millimetre-sized aqueous droplets. The approach, which is related conceptually to natural processes of biomineralization, is facile and environmentally benign, and produces biocompatible materials capable of high encapsulation efficiency, variable mechanical strength and controlled release properties. Such bioinorganic hybrid materials should have significant use in a range of technological and biomedical applications.

Acknowledgements

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Notes and references

†Ca-alginate/chitosan microspheres were prepared by addition of droplets (*ca.* 0.1 ml in volume) of a slightly viscous solution of sodium alginate (2 wt%, Aldrich) in aqueous NaCl (0.15 M) to an aqueous solution of chitosan (1 wt%, Aldrich) containing $CaCl_2$ (50 mM) and 1 wt% acetic acid at pH 6.2–6.5. The droplets were added *via* a 0.4 mm diameter needle syringe and left for 1 h in the chitosan solution. Typically, a hundred microcapsules or so were prepared in a single experiment. Mineralized alginate/chitosan microcapsules were similarly prepared except that Na_2HPO_4 (30 to 300 mM) was added to the alginate solution prior to droplet formation. Samples were rinsed with distilled water and dried in an oven overnight at 40 °C prior to optical microscopy, SEM, XRD and FTIR spectroscopy studies.

‡Sodium alginate solutions containing 0 to 300 mM Na_2HPO_4 and 1 wt% haemoglobin (Sigma) (or 1 wt% ibuprofen (Sigma)) were prepared and added dropwise to Ca-containing chitosan solutions to produce microcapsules as described above. Approximately 0.1 g of the washed microcapsules was then added to 1 mL of deionised water and the time-dependent concentration of haemoglobin or ibuprofen released into solution was monitored by changes in the UV-visible absorption bands at 406 or 260 nm, respectively, using predetermined calibration curves.

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