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Preparation of aqueous gel beads coated by lipid bilayers

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Novel giant liposome microcapsules have been fabricated based on aqueous gel cores encapsulated with a lipid bilayer. The method involves templating of lipid-stabilised water-in-oil emulsions after gelling the aqueous phase with a suitable hydrocolloid.

Liposomes consist of an aqueous core encapsulated within one or more phospholipid bilayer membranes and have great potential for applications in cosmetics, pharmaceutical formulations and food products as delivery vehicles for drugs and other supplements.[1,2] They can be produced by a variety of techniques including lipid swelling,[3] electroformation[4] and membrane extrusion.[5] Pautot *et al.*[6] have developed an emulsion-transfer method for the production of asymmetric liposomes in which the phospholipid compositions of the inner and outer monolayers of the bilayer membrane can be independently controlled. However, the stability of the lipid-stabilised emulsion template limits the maximal size of the liposomes produced by this method to a few micrometers. In addition to liposome variations based on lipids, a range of vesicular structures can be synthesised using assembly of polymeric amphiphiles.[6–11] Recently, giant vesicles have proved to be extremely useful tools for studying lipid membrane mechanics and various inter-membrane interactions.[12] Taylor *et al.*[13] reported a novel preparation method for monodisperse giant vesicles based on a combination of microcontact printing plus electroformation. Microcontact printing is used to deposit a micro-pattern of lipid "patches" onto an indium tin oxide (ITO) electrode surface, which forms the base of an electroformation cell. Application of an AC field across the cell causes liposomes to grow simultaneously on each patch of lipid and the final result is an array of monodisperse giant liposomes, the size of which is controlled by the size of the pattern features.

Here we report the fabrication of novel hybrid giant liposomes with cores of an aqueous gel based on an extension of the Pautot technique.[6] The idea of the method and the structure of the produced capsules are illustrated in Fig. 1. It involves the following three steps: (i) a lipid-stabilised water-in-oil emulsion is prepared in the presence of a gelling hydrocolloid in the aqueous phase (Fig. 1A). (ii) The water drops, coated with a lipid monolayer are gelled at low temperature to produce gel beads (Fig. 1B). (iii) The gelled beads are transferred from the oil phase through the planar oil–water interface where they pick up a second lipid monolayer and convert into giant liposomes of gelled aqueous cores (Figs. 1C and 1D). The novelty here is that we maintain a saturated lipid monolayer at the planar oil–water interface by injecting lipid solution in a spreading solvent. Also, in addition to ref. 6, the presence of a gelled aqueous cores leads to an increased stability of the template upon transfer through the oil–water interface and allows us to prepare gel beads coated by a lipid bilayer of sizes ranging from 1–2 μm to 30–40 μm. Details of the method are given below.

The oil phase was squalane (Sigma) mixed with 0.05 g mL^{-1} of egg lecitin (Sigma) and 0.5 mg L^{-1} lissamine rhodamine B (Avanti Polar Lipids, Inc.). The gelling agent was κ-carrageenan (GENUGEL® type X-902-02, CPKelco). In part of the experiments, the water-soluble fluorescent dye Texas Red (Fluka) was used to stain the aqueous phase. In a typical recipe, 200 μL of hot aqueous solution of 1 wt% κ-carrageenan was emulsified in 4 mL of the oil phase with IKA at 11 000 rpm for 60 s at 70 °C. The emulsification was carried out in a polypropylene tube to avoid deposition of the water drops on the tube walls. The water-in-oil emulsion produced was rapidly cooled to 25 °C to set the gel by 5-fold dilution in squalane and left for 3 h to allow the formation of a complete lipid monolayer on the interface of the gel beads. 3 mL of squalane were put in a beaker (diameter 34 mm) containing 3 mL of milliQ water. The beaker was kept at 35 °C using a hot plate. The planar squalane–water interface was kept saturated with a lipid monolayer by injecting 60 μL of 53 μg mL^{-1} egg lecitin solution in isopropanol (Sigma) doped with 1 mol% Fluorescein-lipid (Avanti Polar Lipids, Inc.) at the interface. Then, 100 μL of the gelled water-in-oil emulsion were carefully added to the oil phase in the beaker. During this process the oil phase becomes clear while the water phase becomes turbid, which indicates that the gel beads are completely transferred in the water phase. This procedure was repeated several times.

In a separate series of experiments we also stained the aqueous phase with Texas Red (10^{-5} M) to visualise the emulsion drops, compared to the case when only membrane dye is present on their surface. Fig. 2A shows the fluorescence images of the water drops in the squalane phase where the aqueous phase is doped with Texas Red, while Figs. 2B and 2C show similar emulsions where the position of the lipid monolayer is visualised by doping with lissamine rhodamine. We found that the quality of the produced template of gelled emulsion is crucial for the successful fabrication of the giant liposomes by using the aforementioned technique. We studied the effects of the lipid concentration in the oil phase and the

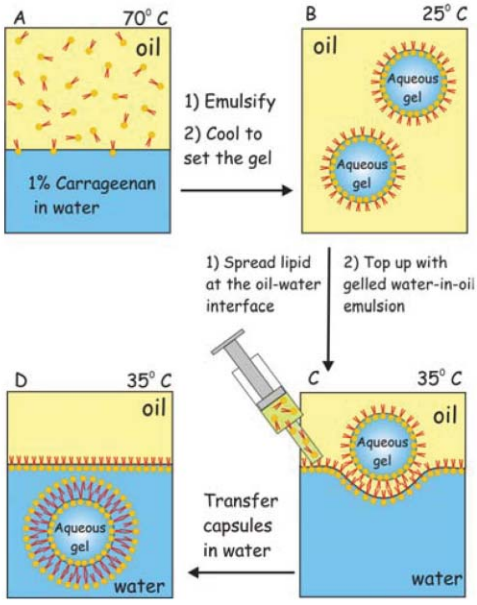

Fig. 1 Schematics of the preparation of the giant liposomes of gelled cores by templating lipid-stabilised water-in-oil emulsions.

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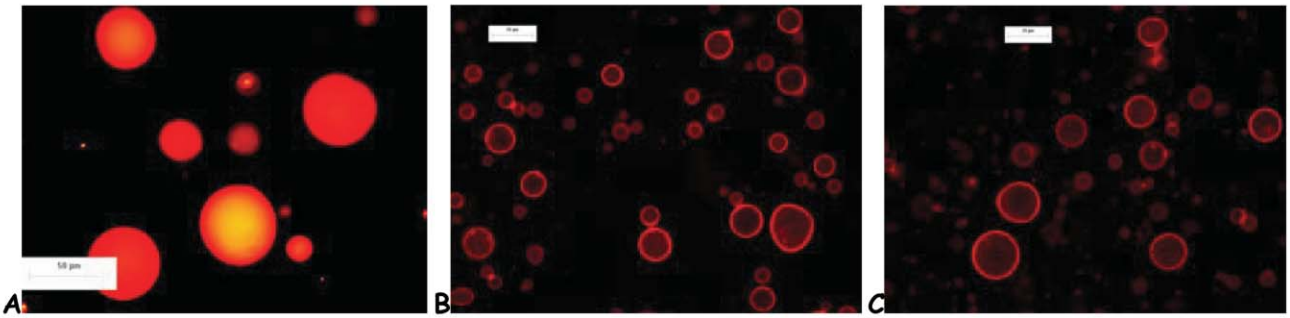

Fig. 2 (A) Fluorescence microscope image of gelled water-in-squalane emulsion stained with the hydrosolube dye Texas Red. (B and C) Images of gelled water-in-squalene emulsion stained with the membrane probe lissamine rhodamine. All images are taken with fluorescence filter for TRITC.

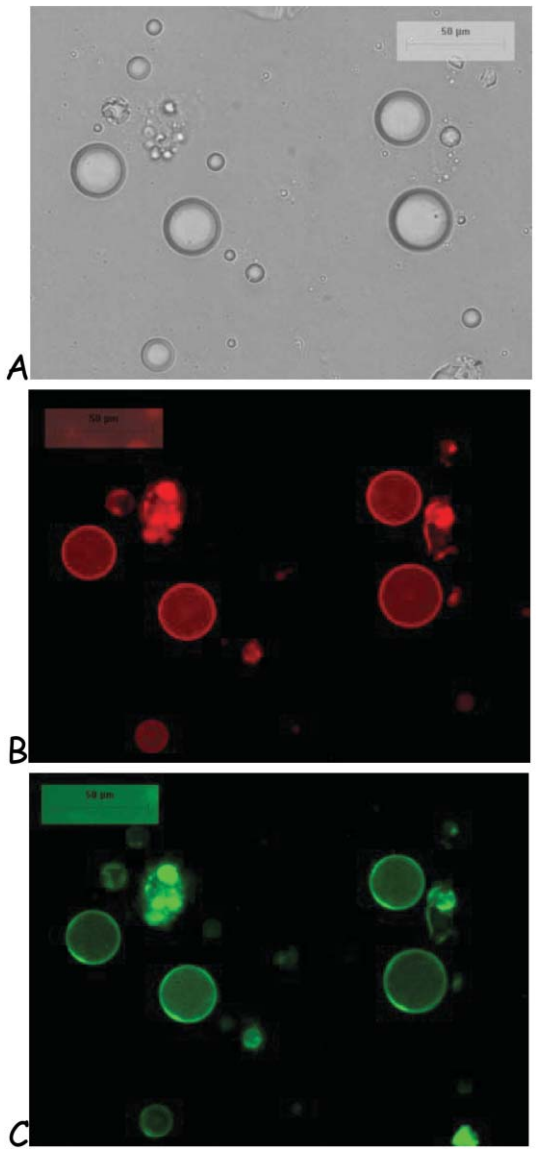

Fig. 3 Optical microscopy images of giant liposomes with gelled aqueous cores obtained by transferring the lipid stabilised gel beads from the squalene phase into water through a planar squalene–water interface with a saturated lipid monolayer doped with Fluorescein-lipid. The lipid monolayer on the gel beads has been doped with lissamine rhodamine. (A) A sample of the liposomes imaged in transmitted light. The same sample imaged by fluorescence microscopy with filter sets for TRITC (B) and FITC (C).

amount of spread lipid at the planar oil–water interface on the quality of the produced giant liposomes with gelled aqueous cores. It was also found that these types of liposome microcapsules are much more stable and easier to handle than conventional giant liposomes while presenting the same way of encapsulation. Fig. 3 shows asymmetric giant liposomes produced by using different fluorescent membrane probes to stain the inner and the outer part of the lipid bilayer. The inner lipid monolayer has been doped with lissamine rhodamine, while the outer lipid monolayer has been stained with Fluorescein-lipid (spread at the oil–water interface), as confirmed by fluorescence microscopy (Figs. 3B and 3C). To demonstrate the integrity of the lipid bilayer around the gelled liposomes we studied the retention of hydrosoluble fluorescent dyes encapsulated within the liposomes. We found a significant decrease of the "release" rate of the dye from the lipid bilayer encapsulated beads compared with "naked" gel beads. These results will be reported in a follow-up paper.

In summary, we report the first preparation of giant liposomes with gelled aqueous cores by templating of lipid-encapsulated aqueous gel beads in oil. These novel microcapsules have much higher stability and mechanical strength than conventional liposomes and may find applications as drug delivery vehicles and for controlled release of proteins, vaccines, cosmetic and food supplements.

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Notes and references

1 A. J. Khopade, F. Caruso, P. Tripathi, S. Nagaich and N. K. Jain, *Int. J. Pharm.*, 2002, **232**, 157.
2 X. Shi and F. Caruso, *Langmuir*, 2001, **17**, 2036.
3 E. Evans and D. Needham, *J. Phys. Chem.*, 1987, **91**, 4219.
4 M. I. Angelova, S. Soléau, Ph. Méléard, J. F. Faucon and P. Bothorel, *Prog. Colloid Polym. Sci.*, 1992, **89**, 121.
5 G. Zhang, F. Farooqui, O. Kinstler and R. L. Letsinger, *Tetrahedron Lett.*, 1996, **37**, 6243.
6 S. Pautot, B. Frisken and D. A. Weitz, *Biophys. J.*, 2002, **82**, 156; S. Pautot, B. J. Frisken and D. A. Weitz, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 10718.
7 D. E. Discher and A. Eisenberg, *Science*, 2002, **297**, 967.
8 J. C. M. Lee, H. Bermudez, B. M. Discher, M. A. Sheehan, Y. Y. Won, F. S. Bates and D. E. Discher, *Biotechnol. Bioeng.*, 2001, **73**, 135.
9 D. A. Hammer and D. E. Discher, *Annu. Rev. Mater. Res.*, 2001, **31**, 387.
10 K. Yu, A. Hurd, A. Eisenberg and C. J. Brinker, *Langmuir*, 2001, **17**, 7961.
11 L. Luo and A. Eisenberg, *Angew. Chem., Int. Ed.*, 2002, **41**, 1001.
12 *Giant Vescicles*, ed. P. L. Luisi and P. Walde, John Wiley & Sons, Chichester, 2000.
13 P. Taylor, C. Xu, P. D. I. Fletcher and V. N. Paunov, *Chem. Commun.*, 2003, 1732.