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Biodegradable Microcapsules Prepared by Self-Healing of Porous Microspheres

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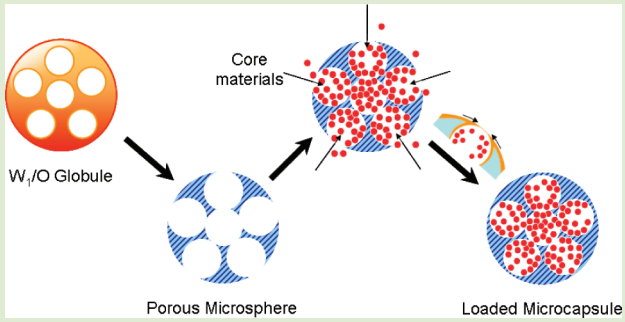
Supporting Information

ABSTRACT: A method is herein proposed to produce biodegradable microcapsules by a self-healing of porous microspheres, which were prepared from water-in-oil-in-water ($W_1/O/W_2$) double-emulsion templates. Methoxypoly(ethylene glycol)-*b*-poly-DL-lactide (PELA) was dissolved in ethyl acetate (EA) as the oil phase (O) of double emulsion, NaCl and poly(vinyl acetate) aqueous solutions serving as internal and external water phases (W_1 and W_2), respectively. Porous PELA microspheres were prepared by a two-step emulsification and solvent extraction method. Core materials, such as proteins or latex particles, could then be loaded by diffusion from the external water phase. Eventually, the pores in the surface could heal up triggered by a solvent swelling or infrared irradiation to form closed microcapsules. Compared with traditional encapsulations which are based on the two-step emulsification, the proposed posthealing approach could overcome some drawbacks, such as the shear destruction, solvent erosion to delicate core materials, or even their unexpected release during the emulsification. Besides PELA, poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) microcapsules were also proved feasible to fulfill such an approach.



During the past several decades, poly(lactic acid) (PLA) has been extensively studied in the field of drug delivery and controlled release, due to their nontoxicity, good bioavailability, and biocompatibility. Furthermore, it has been approved by the Food and Drug Administration for human use.[1−3] Some other degradable molecules with similar functions, such as methoxypoly(ethylene glycol)-*b*-poly-DL-lactide (PELA) and poly(lactic-*co*-glycolic acid) (PLGA), have also attracted intensive attention for their existing or promising applications in the field of pharmaceutical preparation.[4,5]

The water-in-oil-in-water ($W_1/O/W_2$) double emulsion method is one of the most widely applied approaches to prepare biodegradable microcapsules.[6,7] However, there are several common drawbacks of the commonly used method. First, the mechanical shear force, especially during the first step of emulsification, would damage the delicate core materials, for instance, protein drugs.[8,9] Second, organic solvent of the oil and the W_1/O interfaces could also denature the loaded biomolecules.[10−12] Moreover, since the $W_1/O/W_2$ emulsion is a typical unstable system, coalescence between inner droplets (W_1) and teh external aqueous phase (W_2) happens spontaneously, leading to an unexpected release of core materials during the emulsion stage.[2,13]

In 2005, Im et al.[14] first reported a posthealing strategy to fulfill encapsulation based on a type of single hole nanoparticles. The holes in the particles would be closed by thermal annealing or solvent treatment to form nanocapsules. However, such an approach can only be carried out with polystyrene (PS) or poly(methyl methacrylate) (PMMA) as the shell material. If the posthealing strategy could be applied to prepare biodegradable microcapsules, it could be a promising technology to promote research and development in pharmaceutical preparation. Other researchers have tried some pioneering approaches with commonly used biodegradable materials. Yin and Yates[15] prepared porous PLA microspheres by phase separation between polymer and solvent during spray/freeze-drying; then solvent treatment was used to close the pores in the surface. In 2006, Kim et al.[16] synthesized porous PLGA microspheres by the solvent evaporation technique with an unique surfactant Pluronic F127 as porogen, and solvent treatment was also employed to close the pores in the surface.

Herein, we first proposed a method to prepare biodegradable microcapsules by combining the $W_1/O/W_2$ emulsion method and self-healing of porous microspheres, as illustrated in Figure 1. Porous microspheres were first fabricated from $W_1/O/W_2$ emulsion globules; then core materials were loaded through diffusion, from the external water phase, and the pores in the

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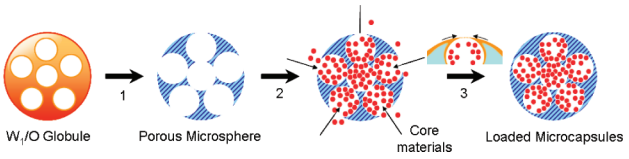

Figure 1. Flowchart for the preparation of biodegradable microcapsules. 1: W_1 escaping and oil phase solidification, 2: loading of core materials, 3: self-healing of porous microspheres.

surface of microspheres were eventually closed by solvent treatment or infrared irradiation to form closed microcapsules. Compared to other methods with the posthealing process, double emulsion globules were first employed to prepare the semiproducts of the final microcapsules, which can be fabricated by the most commonly used materials including PLA, PLGA, and PELA. Such a $W_1/O/W_2$ emulsion template could greatly facilitate the morphology control and would offer a biofriendly internal compartment for core materials. Such a strategy is obviously different from traditional encapsulations by the double emulsion method, because the core material was loaded after the two-step emulsification; thus the common disadvantages including shear destruction, solvent erosion to core materials, and their unexpected release would never exist.

During the preparation of double emulsion, a certain amount of the internal aqueous phase (W_1) was first emulsified into an oil phase (O) by a homogenizer, which was conducted at 24 000 rpm for 25 s. In this process W_1 was broken into fine droplets, usually less than one micrometer in diameter. The W_1/O simple emulsion was further dispersed into an external water phase (W_2) by the homogenizer, which was conducted at 3500 rpm for 60 s. W_1/O globules in W_2 were prepared at tens of micrometers in diameter and were first endowed with multicore morphology.[17] It is widely accepted that double emulsion is an unstable system. There are several possible evolution trends for the collapse of a $W_1/O/W_2$ system. Most of the time, the evolution will jeopardize the loading capacity, such as the coalescence of W_1 and W_2. During our previous study,[17] we succeeded in restricting the coalescence of W_1 droplets within every single oil globule; thus multicell W_1/O globules would transform to single-cell ones spontaneously. We call such an evolution behavior emulsion ripening, by which the desired internal morphology can be obtained in a given time. There are two key factors to affect the evolution behavior: the stability of interfaces and osmotic gradient between W_1 and W_2. It was found that, when the external interfaces were better stabilized than the internal ones, coalescence between W_1 droplets would dominate the emulsion evolution. The higher NaCl concentration in W_1 than W_2 would lead to the faster swelling up of W_1 droplets, to the higher frequency of coalescence of W_1 droplets, and to the faster ripening at last.[17] In our recent experiments, it is proved that the solidification process is another key factor to determine the final morphology, especially on the surface porosity. When solidification happened at the same time of W_1 escaping, namely, the coalescence between W_1 and W_2, pores would be formed in the surface and be interconnected inside.

During our experiments, PELA was first employed as shell materials to test the posthealing strategy. Thereafter, both PLA and PLGA were also proved feasible. Ethyl acetate (EA) was chosen as the organic solvent to dissolve the shell materials. The solidification of the oil globules was realized by solvent extraction, the speed of which was precisely adjusted to cooperate with the ripening of the double emulsion.

At the beginning stage of a $W_1/O/W_2$ system, there were quite a number of small W_1 droplets within every single oil globule. Water molecules were diffusing across the oil layer, from W_2 to W_1, driven by an osmotic gradient, which could be adjusted by the NaCl concentration of the water phases. When solidification happened in the early stage of ripening, W_1 droplets expanding and bursting out with the oil transforming to solid, honeycomb-like morphology would be obtained, as shown in Figure 2b,e. After a certain period of ripening, there

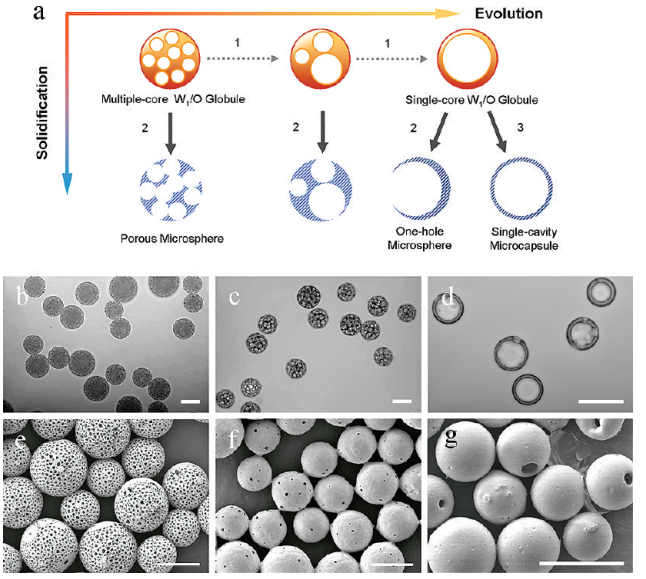

Figure 2. (a) Morphology evolutions of $W_1/O/W_2$ globules and solid microcapsules/microspheres illustrated by a cartoon chart. (b) Optical and (e) SEM images of the $W_1/O/W_2$ emulsion templated microspheres after ripening for 360 min. (c) Optical and (f) SEM images of the $W_1/O/W_2$ emulsion templated microspheres after ripening for 10 h. (d) Optical and (g) SEM images of the $W_1/O/W_2$ emulsion templated microspheres after ripening for 52 h; the scale bars represent 50 μm in both figures. 1: W1 coalescence, 2: W_1 escaping and oil phase solidification, 3: oil phase solidification.

were fewer and larger W_1 droplets left in the oil globule. When the solidification was initiated, fewer pores would be formed in the surface, as shown in Figure 2c,f. With the ripening going on, single-core W_1/O globules appeared, and it would cost 52 h to get the maximal proportion of the single-core morphology. When the solidification happened in the final stage of double emulsion, single-pore microspheres and single-cell microcapsules would be obtained, as shown in Figure 2d,g. Thereafter, the $W_1/O/W_2$ emulsion would transform to O/W_2 simple emulsion gradually.

By adjusting the time of ripening and the speed of solidification, as illustrated in Figure 2, microspheres with desired morphologies can be obtained. The pore size distribution and the compartmental structure could be adjusted by the concentration of PELA, solidification manner, and osmotic pressure between W_1 and W_2. More information on these effects is listed in the Supporting Information.

As illustrated in Figure 1, the pores in the surfaces of these porous microspheres can be closed after the loading of core materials. Based on the porous PELA microspheres, an addition of a small amount of ethyl acetate (EA) (10% v/v) to the

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aqueous suspension of the microspheres was proved effective to initiate the healing of the pores, which could be completely closed within 5 h at room temperature, as shown in Figure 3b,c.

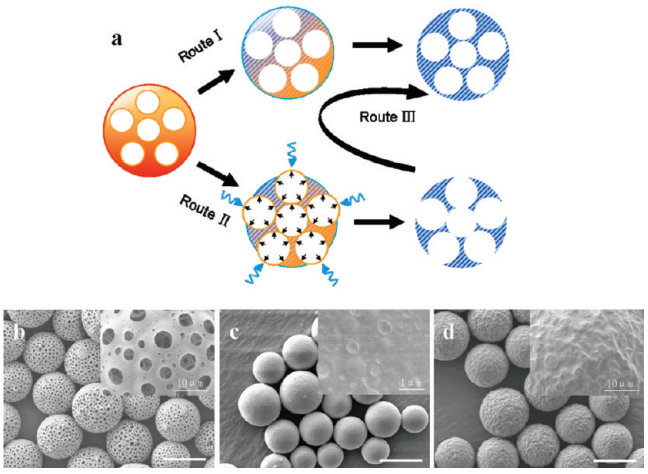

Figure 3. (a) Schematic illustration for formation of multicell microcapsules and porous microspheres. The SEM images of porous microspheres (b) before self-healing, (c) after solvent swelling, and (d) after infrared irradiation. The scale bars represent 50 μm in both figures.

In addition to the self-healing triggered by organic solvent, we also found an even milder way to induce the self-healing, by infrared irradiation. It is believed that the effect of infrared irradiation is the same as thermal annealing, though the infrared irradiation affect directly on the solid microspheres rather than on whole system. The T_g of the PELA being used is 42 °C, while the self-healing can be realized at as low as 38 °C. Figure 3b,d shows the surface morphologies of microspheres before and after infrared irradiation. It took 2.5 h to close all of the pores in the surfaces; more images are given in Supporting Information.

Figure 3a illustrates three different routes to explain the forming and transforming mechanisms of the microspheres/microcapsules. Under relative equilibrium conditions, where no burst escaping happened during the process of solidification, hollow microcapsules with a smooth surface could be obtained, as shown in Figure 3a, route I. On the other hand, when the burst escaping of W_1 droplets and the solidification of the oil occurred at the same time, porous morphology was obtained, as shown in Figure 3a, route II. A nano-CT reconstructed slice image of the porous microsphere in Figure 4a shows a multicell internal structure and proved that the pores did not only exist in the surface but also interconnected the inner compartments. After the forming of the porous microspheres, we induced a suitable solvent or infrared irradiation on the porous polymeric microspheres, which would become soft and return to melted state as in the earlier stage of double emulsion. According to the principle of thermodynamics, interfacial free-energy minimization,[18] pores in the surface would heal up under a relative equilibrium condition without internal swelling or burst escaping, as shown in Figure 3a, route III.

Self-healed microcapsules from different routes exhibited very different surface appearances. After solvent treatment the surface of microcapsules was relatively smooth, while after infrared irradiation a wrinkled surface would appear, as shown in Figure 3c,d. We supposed that the solvent could affect the

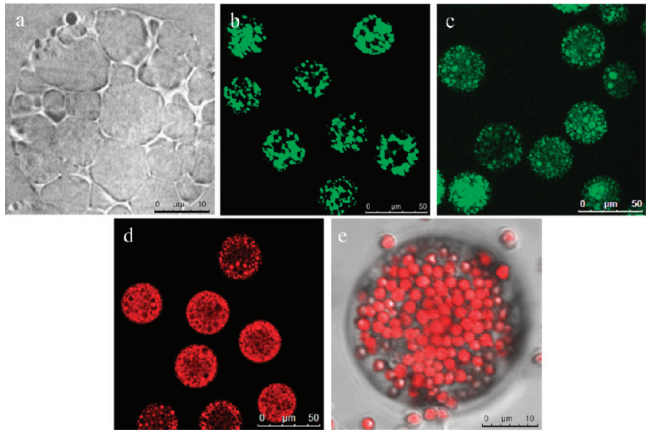

Figure 4. (a) Nano-CT reconstructed slice images of porous microspheres. LCSM images of microcapsule loaded with various core materials: (b) AF488; (c) AF488-BSA; (d) 500 nm PS fluorescent nanoparticles; (e) 2.0 μm PS fluorescent microparticles.

whole skeleton of porous microspheres leading to reconstruction of the whole structure and finally to a more spherical morphology; however, the infrared irradiations might only affect the surface layer, and thus the surface cannot stretch evenly to form a smooth surface.

Such a self-healing strategy offers a convenient loading for core materials. To test the loading versatility, core materials with different sizes were employed to fill into porous microspheres. Core materials were added in the external aqueous phase with porous microspheres and further concentrated by centrifugation to fill these microspheres under a relatively higher concentration. After the treatment by the solvent swelling or infrared irradiation, extra core materials in the aqueous phase were removed by filtration.

As shown in Figure 4b–e, core materials of different sizes ranging from small molecules of 643 M_w to microspheres of 2 μm can be loaded successfully. All of these core materials can penetrate deeply and be accommodated well in these PELA microcapsules. Compared with traditional encapsulation by the double emulsion method, this posthealing strategy was even milder. Furthermore, redundant core materials not loaded can be easily recovered for another cycle of loading.

Compared to the reported methods with the posthealing process, these porous microspheres herein presented were prepared from a more biofriendly double emulsion method, leading to an easy control on the final morphology. Besides the commonly used solvent treatment, infrared irradiation was first proved effective to initiate the self-healing, and it could be more promising for the encapsulation of active biomaterials.

To evaluate the loading capacity of the PELA microcapsule, BSA as a model drug was employed to fill into porous microspheres which were further healed to form BSA-loaded microcapsules by infrared irradiation. The healed microcapsules showed a relatively high loading amount (12.67%), while the encapsulation efficiency after self-healing was 45.22%. Green fluorescent protein (GFP) was employed as a tracer to test the loading stability of PELA microcapsules. As shown in Figure 5, no obvious escaping of GFP was observed for a 20 day storage, and the fluorescent signal remained at the same level as just being fabricated.

While the PELA microcapsules were successfully prepared by the posthealing strategy, PLA and PLGA were also tested by a very similar strategy. During the preparation of porous

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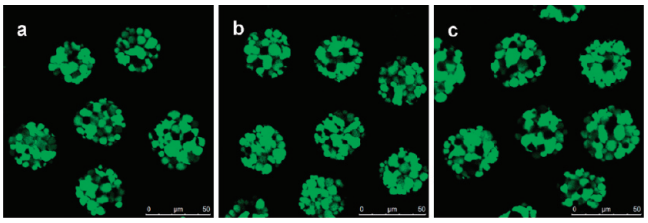

Figure 5. LCSM images of microcapsule loaded with GFP suspended in water. (a) 0 d; (b) 15 d; (c) 20 d.

microspheres, PLA or PLGA took a major proportion of the shell material, while PELA was also employed as an essential amphiphilic stabilizer, which took about 10% in the polymer component to form a porous structure. After infrared irradiation for 4 h, these pores in the surface would heal up completely, as shown in Figure 6. Although the final microcapsules showed different surface morphologies, the posthealing strategy proved effective with PLA or PLGA as the shell material.

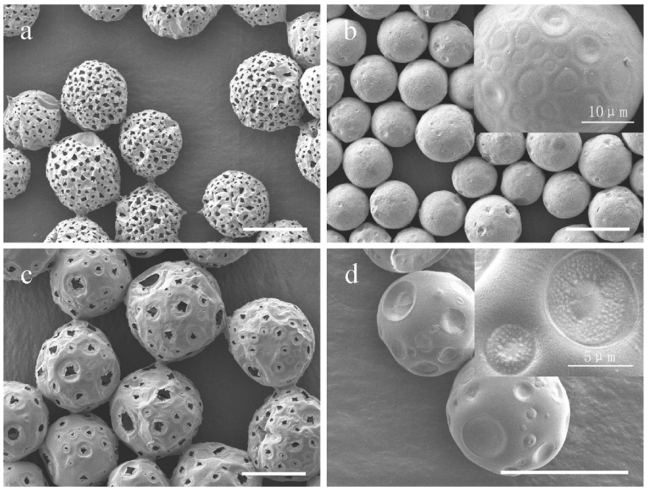

Figure 6. SEM images of PLA porous microspheres (a) before self-healing and (b) after infrared irradiation. SEM images of PLGA porous microspheres (c) before self-healing and (d) after infrared irradiation. The scale bars represent 50 μm in both figures.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, detailed characterization data, and additional results and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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