

Golf ball-shaped PLGA microparticles with internal pores fabricated by simple O/W emulsion†

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Simple oil-in-water emulsion led to structural complexity at both the surface and interior of the PLGA microsphere. A golf ball-like dimpled surface comes from the heteroaggregation of volatile nonsolvent colloid originating from the inside of the organic droplet as supported by *in situ* optical microscopy. The internal porous structure and encapsulation of hydrophobic agent inside the microparticle implies its potential application as a drug carrier.

Biodegradable polymer microparticles have been widely used as sustained release depot systems for drugs, personal care ingredients, and medical imaging agents.¹ Physical parameters such as nano-/micron-scale internal structures, surface morphology, and size distributions are critical, in that they can play an important role in the control of loading efficiency, release kinetics and biological activities of the encapsulated substances. Thus, considerable efforts are being made to precisely control these features. In particular, considering the microparticle as a carrier for active agent, the main focus is on **the control of internal pore structures of microparticles** such as providing microparticles with a controlled hollow interior and porous wall.² Recently the ability to implement structural features on the surface morphology in the biomedical applications has been a topic of intense interest for the polymer film surface.³ With the concept being extended to microparticles which exhibit **a dimpled surface combined with complex internal porous morphology**, advantages of increased surface area for drug release, providing additional loading sites on the particle surface which can be used as enhanced biomolecular imaging systems⁴ and multiple drug dosing systems, and potential for modified interaction with biological systems can be expected. As a golf ball with dimple structures on the surface can prolong the distance of flight by reducing the drag force by air, mimicking its structure in microparticles is also expected to reduce the drag force exerted on the microparticles when it is necessary to move in the fluid.⁵

Fabrication of such golf ball-shaped microspheres has been previously reported by two principal methods; (i) seeded dispersion or emulsion polymerization; (ii) stabilizing emulsions using small colloidal particles (*i.e.*, Pickering emulsions or

armoured emulsions).^{6,7} However, the former techniques cannot be applied to biodegradable aliphatic polyester and the latter cannot control the internal structures of microparticles. Polymer microspheres having a dimpled surface structure as well as controlled porous internal morphology can be hardly found in the literature.

Herein we report a facile and reliable method to fabricate poly(D,L-lactic-*co*-glycolic acid) (PLGA) microspheres with **controlled surface and internal pore morphologies**. A single oil-in-water (O/W) emulsification technique was used to prepare polymer microspheres,⁸ and **an organic phase change material (PCM) was incorporated into the organic phase to generate the structural complexity**. PCM based on low volatile hydrocarbon is a material that can induce a phase separation in the organic phase, eventually producing diverse nano-/micro-scale structures within and on the surface of polymer microspheres. Our system is illustrated using PLGA because of its well-known biodegradability and biocompatibility for biomedical and pharmaceutical applications.⁹ Moreover, a hydrophobic chemical agent (Rhodamine 6G) and a hydrophilic silica colloid were encapsulated within micron-sized PLGA golf balls to investigate their potential application as a depot system for drug delivery and a bio-imaging system, respectively.

A 10 wt% mixture of PLGA (lactic acid : glycolic acid = 65 : 35) and **2-methylpentane was dissolved in dichloromethane (DCM)**. **2-Methylpentane was selected as a PCM because it is in a liquid state (b.p. = 62 °C) during the fabrication of microparticles and can be easily removed through evaporation after microsphere preparation**. Considering PLGA, the Hansen's solubility scale of DCM is 1 (a clear solution),¹⁰ and **PLGA is not soluble in 2-methylpentane** as examined by the result of no change of PLGA which was placed in the PCM for 24 h. 2-Methylpentane acts as a nonsolvent for PLGA. Because the amount of 2-methylpentane used was small (less than 4 wt%) and 2-methylpentane was fully miscible with DCM, a clear organic solution was obtained. O/W emulsions were prepared by dropping the organic phase into 1 wt% polyvinyl alcohol (PVA) aqueous solution using a syringe (21G needle) with magnetic stirring at 300 rpm. **DCM was removed via evaporation at ambient temperature for 3 h, resulting in hardened microparticles**. A PLGA microsphere mimicking a golf-ball surface is depicted in Fig. 1. **The incorporation of an organic PCM into the organic phase for the fabrication of porous microparticles is comparable with a supercritical anti-solvent (SAS) technique using supercritical CO₂ as a anti-solvent, where CO₂ is completely removed as a gas.**¹¹ However, our approach is advantageous in that a smaller amount of PCM is used, the spherical shape of the microparticles can be preserved during the fabrication

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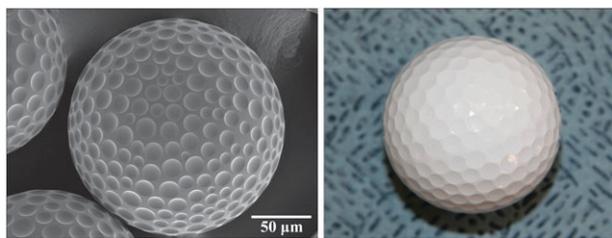


Fig. 1 SEM image of PLGA (LA : GA = 65 : 35) microspheres with golf-ball mimicking dimple structure on the surface prepared by a single O/W emulsification/solvent evaporation method (left). Photo image of real golf ball (right). Ratio of PLGA:PCM is 7:3. The weight ratio of DCM to the mixture of polymer and PCM was fixed at 9:1. 1 wt% PVA in distilled water is used as aqueous phase.

method, and the method is simple and does not involve using a high pressure apparatus. In addition, it is anticipated that a dense surface without pores at the dimple structure of the PLGA microparticle would prevent the abrupt release of drugs encapsulated within the microparticles.

Dimpled surface structures of the microspheres are generated when the weight ratio of PLGA : PCM becomes less than 4. The size of the dimples on the surface of the microspheres increased with increasing the amount of PCM, while the spherical shape of the microspheres was unaltered. Cross-sectional images of PLGA microspheres showed that **PCM induced the formation of small pores distributed throughout the internal matrix of the microspheres.** Above the 8 : 2 weight ratio of PLGA to PCM, hollow core structures with a porous shell are also produced, though the former structures are dominantly formed. At the ratio of 6 : 4, a macroscopic hollow core with a microporous shell structure is observed in most of the prepared microspheres. The internal porous structures can be easily controlled by varying the polymer : PCM ratio while maintaining the golf ball-like dimples on the surface. From the duplication of the preparation varying the ratio of PLGA and PCM, a reproducible image was obtained, indicating that our approach is highly controllable for generating complex structures within polymer microspheres (Fig. S2 in ESI†).

As suggested by the formation of dimple structures *via* Pickering emulsion,⁷ **small colloids in the dispersed media adsorb onto the surface of droplets (water or oil or emulsion) to reduce the total surface energy.**¹² Despite no small colloid being dispersed in the initial state of the aqueous phase for the new system, monitoring of the temporal changes of the organic phase at the early stage of O/W emulsions revealed similar structure to Pickering emulsion (Fig. 2). This indicates that it is important to understand the role of PCM in the formation of structural complexity both at the surface and at the interior of the microparticle.

Generally spreading coefficient, λ_j ($\lambda_j = \gamma_{jk} - \gamma_{ij} - \gamma_{ik}$) is used to predict the final equilibrium morphology of the **droplets of immiscible liquids** (phases 1 and 3) when brought into contact in a third mutually immiscible liquid (phase 2).¹³ Initial interfacial tension values were used to interpret the final morphology. Our calculation result of the new system was $\lambda_1 < 0$, $\lambda_2 < 0$, and $\lambda_3 > 0$, which predict the formation of a **core-shell structure.** (See ESI for the detail of steps for spreading coefficient calculation†). Although the result of

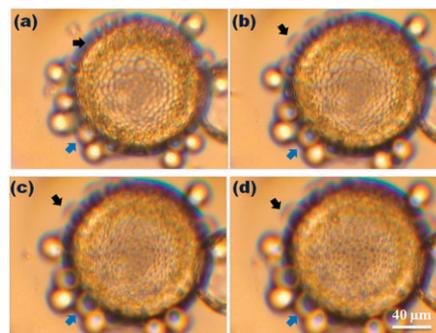


Fig. 2 Optical microscope images of oil droplets dispersed in the aqueous phase taken every two seconds starting from (a). Black and blue arrows indicate the bubble enlargement on the surface with the time (See a movie in Supplemental Information†).

core-shell prediction from the spreading coefficient calculation explains the formation of the internal porous structure by the PCM droplet, it cannot explain the observed fact that the PCM colloid resides on the surface of the oil phase as shown in Fig. 2. This clearly indicates that there is a limitation on explaining the mechanism of the formation of a dimple structured microparticle with internal pores using a conventional microparticle formation mechanism. We propose a new explanation of the formation of golf ball-like dimple structures by the O/W emulsification method. It was reported that when **the PLGA/DCM solutions are emulsified in the aqueous phase for the fabrication of microspheres, DCM diffuses to the aqueous phase with a diffusion coefficient of $2 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$.** Thus the solvent extraction is a very fast process, which does not take longer than a few minutes for particles as large as 100 μm .¹⁴ However, it takes time for the evaporation of the extracted DCM in the aqueous phase below to 0.2 wt% when non-aggregated particles can be obtained.¹⁴ In our system, the critical feature is the positioning of the PCM liquid bubbles at the surface of the oil droplet as much like particle-stabilized emulsions owing to the fast DCM extraction from the surface region of the organic phase at an early stage. Since the fabrications are conducted at ambient temperature (which is far below the b.p. of the 2-methylpentane), liquid bubbles evolving from the organic phase formed heteroaggregation on the surface instead of undergoing abrupt removal from the surface. Meanwhile, **during the evaporation of DCM in the aqueous phase, solidification will proceed from the surface to the core of PLGA microparticles.** However, **owing to the extracted DCM in the aqueous phase, microspheres are likely to show a soft surface till the residual DCM is lowered to 0.2 wt%,** which might take over several tens of minutes. With the depletion of DCM in the organic phase, **the phase separation between PLGA and PCM begins inside the oil phase,** and PCM seems to be dispersed in the matrix by the cross-sectional SEM image of the resulting particle. When the PCM in the organic phase exceeds a critical amount, PCM might be dispersed at the shell and the remainder will move to the core region of the embryonic microspheres.

As shown in Fig. 2, the size of **a liquid PCM bubble** increases with time which can be possible by the continuous supply of PCM to the surface located liquid bubbles. It is well known that **volume contraction occurs in O/W emulsions and**

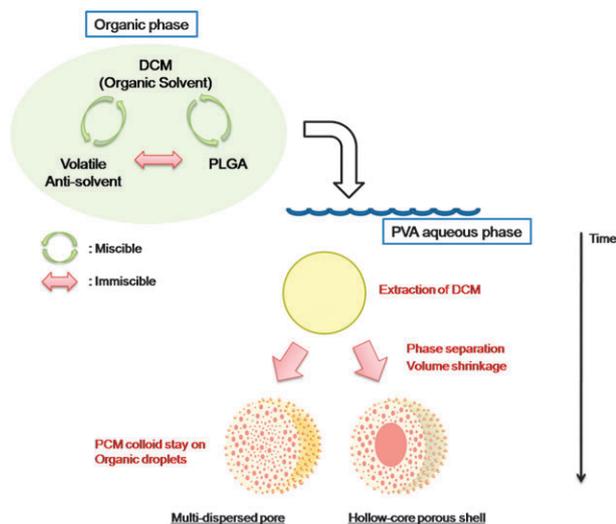


Fig. 3 Schematic diagram of the O/W system and the proposed mechanism of the formation of golf ball like PLGA microspheres. Steps (red) proceed with the time.

final microspheres are smaller than original emulsions.¹⁵ Thus, PCM droplets dispersed near the surface of the organic phase act as a source of PCM bubble expansion and also form a PCM bubble replacing the PCM droplet leaving from the surface of the organic phase with the volume contraction of the organic droplet until the complete solidification of the micro-particle. Continuous stirring of the aqueous medium facilitates the removal of the expanded PCM bubble from the surface of the organic phase, and consequently the dimples on the surface could be formed by the traces of PCM colloid. The concept and proposed mechanism are summarized in Fig. 3.

The approach of emulsions combined with a solvent evaporation method has been widely used to encapsulate drugs in polymers for preparing drug loaded microspheres. In order to evaluate the encapsulation capability of the golf ball microspheres, rhodamine 6G and monodisperse silica particles were selected for the encapsulants, which represent

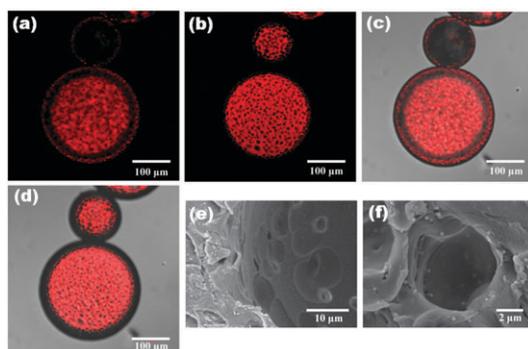


Fig. 4 Confocal fluorescence microscopy images of PLGA microspheres fabricated by an O/W emulsification technique. Rhodamine 6G (0.1 wt%) in DCM was incorporated in the organic phase to illustrate the distribution of the internal pores within the microspheres. Focus on the interior ((a) and (c)) and focus on the surface ((b) and (d)) are made for observation. SEM images of monodisperse sized hydrophilic silica encapsulated in the microspheres ((e) and (f)) (Enlarged image can be found in ESI†).

hydrophobic and hydrophilic agents, respectively. The 7:3 ratios of the polymer and PCM in the DCM were used for the encapsulation. Rhodamine 6G within the microsphere was observed using confocal fluorescence microscopy, and variation of focus enabled the observation of the distribution of the dye at core and shell separately.

Fig. 4 clearly shows that two different internal structures exist, which are a hollow core and a porous structure with small pores. Hydrophilic monodisperse silica particles were also successfully encapsulated into the pores of the microspheres. These results imply that golf ball like PLGA microspheres with internal pores can be used for a potential carrier system.

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