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Solvent-Free Strategy Yields Size and Shape-Uniform Capsules

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Supporting Information

ABSTRACT: Capsules with a liquefied core were fabricated via the assembly of polymeric droplets induced by superamphiphobic surfaces. These highly repellent substrates exhibit distinct features such as (i) an easy and precise control over the particle size and shape, (ii) a high encapsulation efficiency, (iii) mild processing conditions, and (iv) the possibility to include any object in either a water or oil-based liquid core, which are not found on the current available strategies. As proof of concept, a photo-cross-linkable derivative of chitosan was used to produce the polymeric shell while a wealth variety of template cores were tested using a reversible cross-linking mechanism, interfacial gelation process or ice. Owing to the widespread application of polymeric capsules, the developed strategy is poised to usher the development of the next generation of materials not only for biomedical purposes but also for cosmetics, agriculture and electronics.

A significant research interest is being devoted toward the use of hollow materials as encapsulation devices for a plethora of different fields, spanning from electronics to cosmetics, including biomedical applications.[1] Core−shell structured particles with a liquid core exhibit (i) a more efficient and homogeneous transfer of solutes, (ii) a higher loading capacity provided by their internal ample space, and (iii) a lighter weight when comparing with their cross-linked-core counterparts.[2] Drawn by these appealing features, distinct strategies to fabricate polymeric capsules have been devised.[3] However, most of them are based on complex and harsh synthesis procedures, eluding the use of coagulating baths, which can ultimately compromise the cargo stability and loading efficiency. Thus, the absence of a simple and solvent-free methodology to prepare liquid-core capsules under mild conditions was the motivation of this work.

Herein, highly repellent substrates were used to design monosized and spherical polymeric capsules with a (i) hydrogel shell made of methacrylamide chitosan (MACHI), a biocompatible and light-sensitive derivative of CHI, and (ii) a liquefied core, wherein different molecules can be dispersed. Recently, surfaces with low wettability were successfully employed to produce compact spherical particles, from a wide range of materials and under mild conditions, by cross-linking pregel spherical droplets formed when in contact with these substrates (SI; S1).[4] However, the use of this solvent-free technology to attain liquefied capsules have not been reported.[5] First, the liquefied core was obtained by dispensing a predefined volume of an alginate (ALG) solution onto a SA surface, which is characterized by contact angles higher than 150° for both water and oil-based liquids (Figure 1I and SI; S1.1). ALG was selected due to its biocompatibility as well as for its ability to reversibly form hydrogels at mild conditions, making it a great candidate for the capsule liquid core (SI, S1.2). As shown in Figure 1a, pregel ALG droplets remained suspended above SA substrates, acquiring an almost spherical shape (SI; S1.1, shape factor of 0.95 ± 0.02). This shape was induced by the extreme wettability of these surfaces, which, in turn, is the result of the

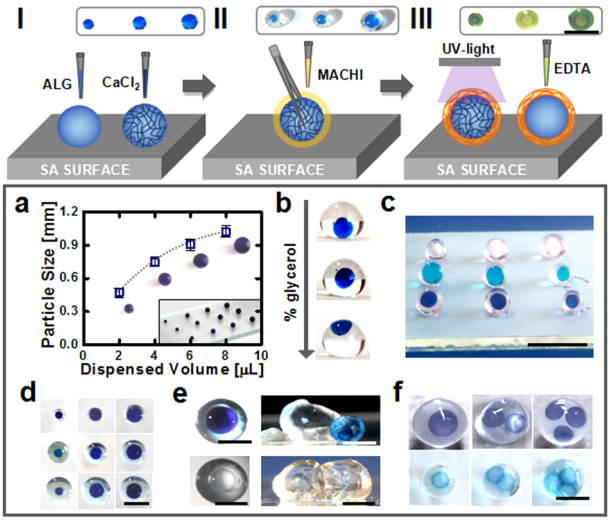

Figure 1. (I) Spherical ALG droplet induced by a SA surface and their subsequent Ca^{2+}-mediated cross-linking. (II) Entrapment of a ALG core within a MACHI droplet. (III) UV-mediated cross-linking of the MACHI shell followed by the core dissolution via EDTA action. Scale bar stands for 1.2 mm. (a) Effect of the dispensed volume of ALG solution on the size of the obtained ALG particles after 15 min of Ca^{2+}-mediated gelling. Scale bar corresponds to 6 mm. (b) Effect of glycerol on the position of the ALG core inside a MACHI pregel droplet (0, 16 and 20% (v/v) of glycerol/water). (c) Scale-up of the developed strategy to attain simultaneously polymeric capsules containing cores with different sizes and entrapping different compounds. Scale bar stands for 2 mm. (d) Multicompartmental hydrogel particles with distinct shell thickness. Scale bar is 1.2 mm. (e) MACHI capsule before (upper panel) and after (lower panel) the EDTA treatment. Scale bar stands for 400 μm. (f) Hydrogel particles with a multicore structure before (upper panel) and after (lower panel) EDTA treatment. Scale bar corresponds to 700 μm.

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presence of a hierarchical topography with micro- and nanofeatures and a low surface energy (SI; S1.3).

Afterward, calcium chloride ($CaCl_2$) was added above the preformed ALG droplets to prompt their gelation (Figure 1I). Figure 1a shows the possibility of controlling the ALG particle size with high precision by simply tuning the dispensed volume above the SA surface. By changing the droplet volume from 2 to 8 μL, the particle size increased from 0.5 ± 0.05 mm to 1.0 ± 0.05 after 15 min of Ca^{2+}-mediated cross-linking, which corroborates with previous studies ($R^2 \approx 0.99$) (SI; S1.4).[6]

The preformed ALG hydrogel particles were then entrapped within a larger volume of MACHI solution, previously dispensed above a SA surface, to form a shell around it (Figure 1II). As shown in Figure 1b (upper panel), the ALG core sank almost instantaneously onto the SA due to a density mismatch between the MACHI solution (liquid) and the ALG particle (hydrogel). Consequently, the external MACHI droplet may not surround the core in the contact area with SA surface, forming a hole from which the immobilized cargo may be released in an uncontrolled way (SI; S1.4). To overcome this main issue, glycerol was used to increase the density of the external MACHI solution and compensate for the higher density of the ALG particle. As can be observed in Figure 1b, the ALG particle position inside the MACHI droplet can be tuned by adjusting the amount of glycerol added. Moreover, the inclusion of this compound ensures the scale-up of this process to attain simultaneously polymeric capsules with different core sizes (Figure 1c).

Afterward, the MACHI hydrogel shell was cross-linked upon exposing this photosensitive polymer to UV-light for 1 min (Figure 1III and SI; S1.5). By varying the dispensed volume of MACHI polymer, the capsule thickness ranged from around 100 to 400 μm (Figure 1d).

Finally, the ALG core was dissolved upon dropping an ethylenediaminetetraacetic acid (EDTA) solution above the previous particle, yielding a MACHI capsule with a liquid core (Figure 1III). EDTA, a divalent ion chelating agent, can disrupt the ALG/Ca^{2+} matrix as demonstrated by the conversion of the ALG solid core into a liquid (Figure 1e and SI; S1.2). Further control over the internal structure was demonstrated by synthesizing capsules exhibiting multiple-cores (Figure 1f). To produce these particles, different number of the preformed ALG templates were assembled simultaneously within a droplet of a MACHI precursor solution, which was subsequently gelled by UV-light exposure (Figure 1f; upper panel) and its cores liquefied upon EDTA action (Figure 1f; lower panel). Capsules with a hierarchical architecture of more than two core assemblies could be useful for individual reagent loading in each of the created subcompartments, being attractive as artificial organelles, bioreactors for confined synthesis or as drug carries.[7]

Capsules with a core−shell structure were subsequently loaded with cells to assess the suitability of the purposed strategy to encapsulate highly sensitive compounds. To this end, the viability of human fibroblasts entrapped in five distinct cell carrier formulations was assessed. First, cells were homogeneously distributed within an ALG/Ca^{2+} matrix, exhibiting good viability rates due to (i) the mild processing conditions used, (ii) the efficient exchange of essential molecules with the surroundings provided by the particle small size, and (iii) the ALG biocompatible character (Figure 2A). Then, these cell carriers were entrapped within a second polymeric layer made of MACHI (SI; S2). As shown on Figure 2B, some nonviable cells appeared on the core center after the incorporation of this barrier between the core and the culture medium. This may be ascribed to the increase of the overall diameter of the particle, which hampered the diffusion of nutrients/O_2/cell residuals and, hence, compromised the cell viability. Therefore, these particles were subjected to a EDTA step to create liquefied cell-laden capsules. The results suggest the formation of a cell-friendly liquid environment wherein cells are metabolically active, highlighting the potential of these capsules as cell encapsulation devices (Figure 2C). Indeed, previous works have shown higher cell viability rates for higher core dissolution degrees, which can justify the attained high viability levels.[8] Other alternative to enhance the diffusion rates was tested by entrapping fibroblasts within the thin (≈200 μm) MACHI shell (Figure 2D). When comparing with compact cell-laden MACHI particles, which revealed nonviable cells at the inner areas (Figure 2E), most of the cells enclosed on the MACHI shell were viable, which further strengths the potential of the developed liquefied capsules.

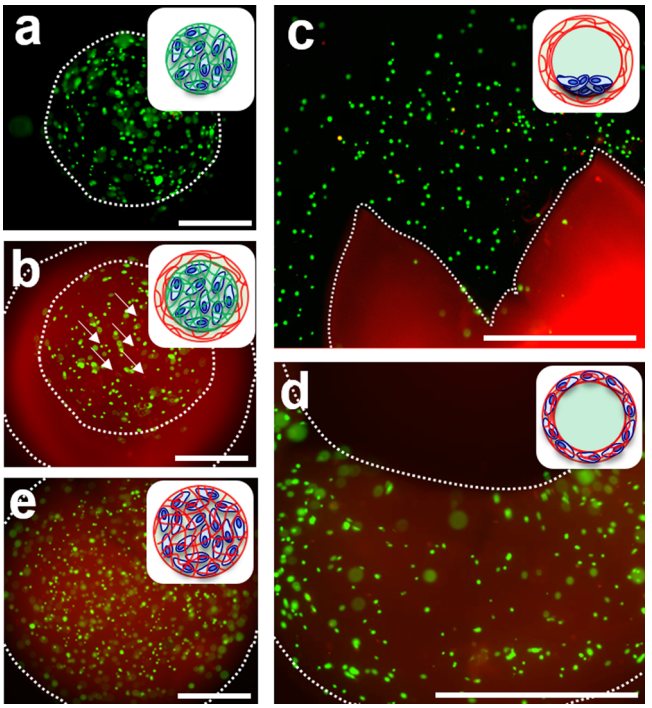

Figure 2. LIVE/DEAD images of cell-laden ALG microparticles (A), MACHI capsule with a cross-linked cell-laden ALG core (arrow indicates some nonviable cells) (B), a ruptured MACHI capsule releasing the encapsulated cells (C), cell-laden MACHI shell (D), and cell-laden compact MACHI particle (E) using calcein (green; living cells) and Ethd-1 (red; dead cells) dyes. Scale bar corresponds to 200 μm.

Figure 3 summarizes different strategies to produce polymeric capsules using different sacrificial cores along with distinct removal methods. Gelatin, the denaturated form of collagen protein, was used as a core due to its temperature-responsive behavior. At low temperatures, its chains undergo a conformational change from a random coil to a triple helix, resulting on the formation of a 3D cross-linked network (Figure 3a). Interestingly, this aggregation process can be reversibly disrupted above 30 °C to yield liquefied capsules (Figure 3b).[9] The use of gelatin as template constitutes a simplification over the process described on Figure 1 because it avoids the addition of any compound to either cross-link or liquefy the core.

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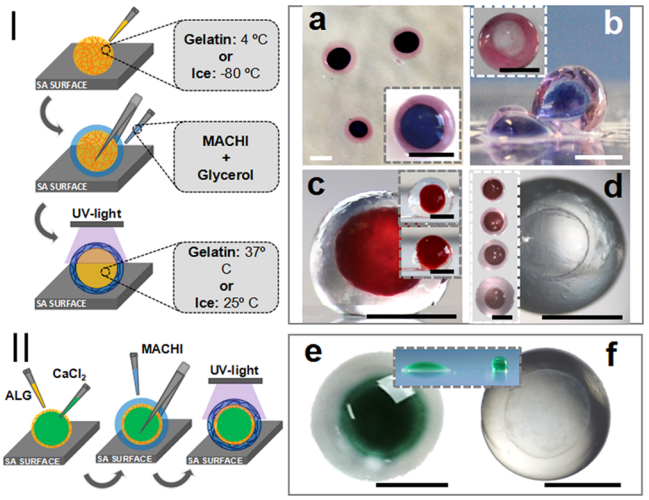

Figure 3. Fabrication of polymeric capsules: (I) Thermoresponsive sacrificial cores: examples of MACHI capsules with a gelatin (a,b) or ice core (c,d) before (a,c) and after (b,d) the core removal, respectively. Regarding the ice-core capsules, ethanol was added to decrease the density of the surrounding MACHI solution (c, upper inset), the temperature was controlled to avoid the core melting (c, lower inset) and the shell thickness tuned by controlling the volume of MACHI solution dispensed (e, inset). (II) Interfacial gelation process: example of a MACHI capsule with a $CaCl_2$ liquid-core before (e) and after (f) the dye release.

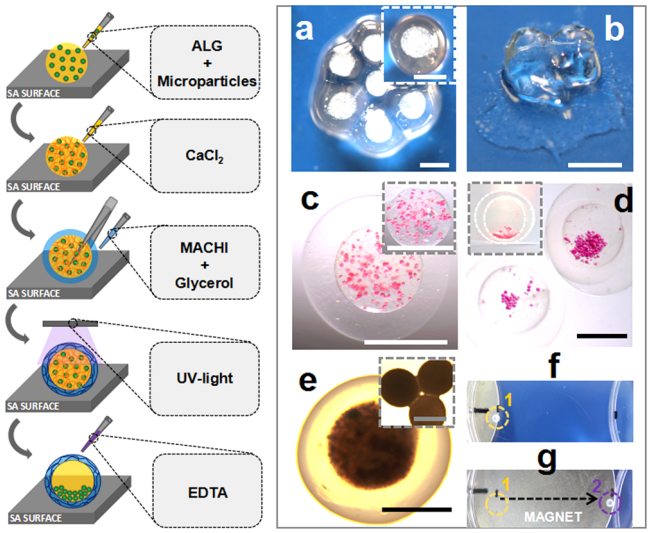

Figure 4. Fabrication of hierarchical capsules containing either $CaCO_3$ (a,b) or PLLA microparticles (c,d), or Fe_3O_4 nanoparticles before (a,c,e) and after (b,d,f,g) EDTA treatment. Motion control of the produced capsules using a permanent magnet (surface magnetic intensity of 0.075 T; f,g). Scale corresponds to 400 μm.

Another strategy also based on the use of thermosensitive templates consists in using ice as template. Contrarily to cross-linked cores, an ice core floats when placed above a MACHI droplet (Figure 3c). Thus, ethanol was added to lower the density of the surrounding droplet (Figure 3d). Such capsules may be extremely attractive for the cryopreservation of living cells, an issue that has received increasing attention. Contrarily to some living organisms, most mammalian cells are unable to survive when exposed to subzero temperatures unless they are placed in solutions with specific additives and following defined freezing protocols.[10] Recently, the entrapment of the desired structures inside hydrogels emerged as an alternative to the established protocols since they allow the cell protection from mechanical damage upon ice crystallization and preserve the cell−cell interactions.[11] With this in mind, polymeric capsules containing both cells and cryopreservatives could be fabricated following this methodology, envisioning cell preservation for future outcomes. Polymeric capsules were also templated on a liquid core by depositing a $CaCl_2$ droplet above another of ALG, resulting on a thin, elastic interfacial membrane (Figure 3II). Following this methodology, bicompartmental hydrogel particles were formed by assembling this core inside a MA-CHI shell (Figure 3e,f). Using this strategy, the addition of any compound to adjust the density or to remove the core is avoided.

Hierarchical systems were fabricated by incorporating different objects inside the core during the synthesis process, proving once more the versatility of this strategy. Herein, calcium carbonate ($CaCO_3$) particles were evenly distributed within the ALG core as visualized by an arrangement of white dots, characteristic of these particles (Figure 4a). These subcompartments can also be disrupted through the action of EDTA, which turn the core into a liquid (Figure 4b). Such compartmentalized systems may find biomedical utility, which is imparted by their proven biocompatibility.[12] Similarly, PLLA microparticles were also enclosed within MACHI capsules and may have an increased importance, for example, as supporting points of anchorage-dependent cells as they are enable to grow in suspension (Figure 4c,d). Actually, it was previously reported higher cell viability levels, around 50%, when cells were encapsulated within particles containing anchorage points, highlighting the importance of these particulate devices for application in Tissue Engineering rather than be merely used as cell carriers.[13] Furthermore, the stability of the obtained capsules was assessed through a rotational test using capsules with two layer thicknesses, i.e., 250 and 350 μm. Interestingly, after 1 h at 200 rpm, both capsules maintained their integrity avoiding the release of their contents, thus proving the production of stable capsules. Furthermore, Fe_4O_3 particles were successfully encapsulated within MACHI capsules (Figure 4e), empowering these capsules with magnetic-responsiveness that can be used to guide them over a surface (Figure 4f and 4g).

In summary, SA surfaces were successfully employed to fabricate ready-to-use and stable multiscaled liquefied capsules enclosing different objects. This strategy benefit from its (i) solvent-free character enabling a loading efficiency of almost 100%, (ii) reproducibility as demonstrated by the great control over the particle size and shape, (iii) versatility as shown by the fabrication of a wide variety of core−shell capsules, (iv) mild processing conditions as proved by the safe encapsulation of metabolically active cells, and (v) its cost-effective character inasmuch as it is based on a simple setup. Based on all these features, this simple, yet efficient strategy is envisioned to constitute an innovative approach to produce liquid-core polymeric systems to entrap a variety of sensitive molecules including not only cells but also proteins, genes, enzymes, and drugs, with minimal adverse effects on their functionality. Moreover, due to the simultaneously superhydrophobic and superoleophobic character of the used substrates, capsules may contain virtually any type of liquid make it possible to broad the application spectrum to diverse technological purposes such as agriculture, biotechnology, cosmetics, and electronics, where

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solvents different than water are often required. Owing to the widespread application of polymeric capsules like the produced ones, modifications to the conventional fabrication techniques are likely to have a strong impact and open new prospects for the development of the next generation of engineered polymeric assemblies for both science and technology.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b11925.

Preparation and characterization of the polymeric capsules, the superamphiphobic surfaces, the methacrylamide chitosan, cell carriers, and hierarchical polymeric capsules (PDF)

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Notes

The authors declare no competing financial interest.

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