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Responsive capsules that enable hermetic encapsulation of contents and their thermally triggered burst-release†

John P. Goertz,[a] Kerry C. DeMella,[b] Benjamin R. Thompson,[b] Ian M. White *[a] and Srinivasa R. Raghavan *[b]

Aqueous capsules made from polymers typically allow their encapsulated cargo (*e.g.*, drugs, dyes, proteins) to slowly diffuse out into the solvent through the capsule shell. In many applications, there is a need for a 'hermetic' seal to protect the cargo from the solvent over extended periods of time. Ideally, this hermetic seal should also be capable of being broken on-demand to enable cargo release. We demonstrate a new design for capsules having the above combination of properties. The key is to create the capsule shell from wax materials (alkanes and fatty acids) with a defined melting temperature T_m. Capsules can be loaded with any desired material, including strong acids or bases, reactive or unstable reagents (such as H_2O_2), and biopolymer gels. When sealed capsules are placed in water, no leakage is observed for over six weeks. The capsules can also encapsulate volatile liquids and remain air-tight over at least three weeks. On the other hand, under mild heat (above T_m, *e.g.*, to 45 °C), the shell melts, releasing the core contents into the surrounding solvent. This provides a convenient thermal on–off "switch" for delivering contents from the capsules. The utility of these capsules is shown by implementing a nitrate-detection assay using hazardous chemicals (including H_2SO_4) sealed in the capsules. These "smart" capsules thus constitute a modular, mass-producible platform that could be useful in diverse applications.

Conceptual insights

Controlled encapsulation and release of reagents has the potential to facilitate automation in a variety of integrated systems, yet aqueous-core capsules made with polymers shells typically allow their encapsulated cargo (*e.g.*, drugs, dyes, proteins) to slowly diffuse out into the bulk solution through the capsule shell. In many applications, there is a need for a "hermetic" seal to protect the cargo over long times from the solvent. Ideally, this hermetic seal should also be capable of being broken on-demand. Here, we present a generic encapsulation strategy, adaptable to a range of geometries and cargos, that enables thermal control over reagent release. By creating capsules with shells made from waxy materials, we achieve hermetic encapsulation of liquids, solids, and hydrogels for periods extending to months. Burst-release of the encapsulated materials is triggered only above the melting temperature of the shell. Unlike other methods of encapsulation, such as microfluidics and bulk emulsions, our approach works regardless of the material characteristics of the core. These phase-change capsules provide a simple, modular, mass-producible platform for stable, stimuli-responsive encapsulation.

The term 'capsule' refers to a container structure having a wall or shell that is distinct in composition from the core.[1–7] Aqueous capsules can be made using charged polymers, either by electrostatic complexation or using layer-by-layer (LbL) techniques. The core in aqueous capsules can be liquid-like or gel-like. Solutes such as dyes, drug molecules, or proteins can be encapsulated in the core. Capsules that bear such solutes are used in a variety of industrial formulations, including consumer products, pharmaceuticals, agrochemicals, *etc.*[6,7] The function of capsules in these formulations is generally to hold onto solutes for a desired length of time (*e.g.*, while the formulation is stored on the shelf) and thereafter release the solutes when the material is put to use. Solute release can occur in a sustained and controlled manner over a period of time (such as a day), or in a burst release (over a few minutes) when a stimulus is applied.[2,7]

A crucial issue with aqueous capsules is that solutes in their core can diffuse out of the capsule during storage.[1–7] This is particularly the case when the solutes are hydrophilic small molecules with a molecular weight below ~1000 Da. The sizes of such small solute molecules will be much smaller than the mesh size of the polymer-network constituting the capsule shell—therefore, solute diffusion through the shell is unavoidable. Thus, it is practically impossible to perfectly encapsulate solutions containing salts, acids, bases, dyes, or many drugs within a capsule for an extended period of time. As an example, if conventional capsules with a strong acid in their cores were placed in deionized (DI) water, the acid would inevitably leak out into the water by diffusion, leading to a sharp drop in the pH of the surrounding solution.

[a] *Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA. E-mail: ianwhite@umd.edu*
[b] *Department of Chemical & Biomolecular Engineering, University of Maryland, College Park, Maryland 20742, USA. E-mail: sraghava@umd.edu*

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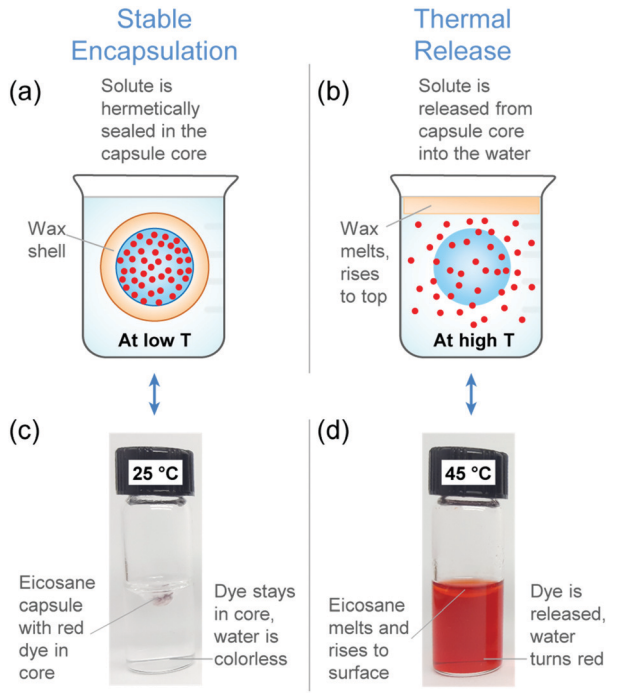

Fig. 1 Concept of responsive hermetic capsules and its practical realization. Capsules are created with aqueous cores and with wax shells having a defined melting temperature T_m. Below T_m, the solute is hermetically sealed in the capsule core and thus does not leak out into the surrounding water even over weeks (a and c). The capsule is also responsive due to the T_m of the wax (around 40 °C). Heating above T_m melts the wax and thus releases the core contents into the water (b and d).

The challenge then is to completely prevent leakage of dissolved solute from the aqueous core of a capsule. Put differently, the goal is to design a capsule shell that provides a hermetic seal, *i.e.*, a perfect seal that ensures separation of the core contents from the surrounding solvent over long periods of time (Fig. 1a). For a seal to be hermetic, one approach would be to create it from a hard, non-porous material such as a metal, or an inorganic solid (*e.g.*, silica), or a thermoset polymer (*e.g.*, an epoxy). However, the contents in the capsule core must be eventually put to use, and hence a hermetic, but unbreakable seal is not useful. In an ideal scenario, the hermetic seal should also be breakable when a trigger is applied, allowing the core contents to leak out into the solvent. Most hard materials, however, do not readily break apart under mild conditions. This is why the above problem presents a challenge, and to our knowledge, there are no examples in the literature of capsules that exhibit a hermetic, yet responsive seal. As relevant comparisons, there have been reports on capsules that have been creatively configured to exhibit delayed[8,9] or slow (but not zero)[10–14] release. In all these cases, the pertinent capsules were not switchable by an external trigger to a fast or burst-release mode.

Here, we present a design for a "smart" capsule that exhibits the following combination of properties: no detectable release of its contents in the initial state (*i.e.*, at room temperature) and a burst-release of the contents when subjected to mild heat (*e.g.*, 45 °C). This unique set of properties is enabled by constructing capsule shells out of wax materials (alkanes and fatty acids) having defined melting temperatures T_m. Below T_m, the wax shell provides a hermetic seal (Fig. 1a), whereas above T_m, the wax melts into a liquid, allowing release of core contents (Fig. 1b). Waxes are widely prevalent materials, and the concept of using wax shells for capsules is inspired by our recent work demonstrating that wax layers can separate liquid reagents in tubes.[15,16] This is illustrated further in Fig. S1 in the ESI.†

Independently, there have been a few studies that have explored encapsulation with waxy shells. Specifically, Buchwald *et al.* recently created centimeter-scale cylindrical vessels with a shell of paraffin wax.[17] These were filled with reagents and used as air-tight containers to carry out oxygen-sensitive reactions without the need for a glove box. The authors, however, did not investigate the leakage-characteristics of their vessels. Also, Weitz *et al.* synthesized wax microcapsules using a microfluidic double-emulsion method.[18] Their study also did not focus on the leakage of small molecules out of capsule cores. Moreover, to implement the double emulsion method with molten waxes, microfluidic devices must be carefully operated at high temperatures, which is quite difficult. This method is also largely incompatible with gel-like or biological payloads for which prolonged contact with hot oil is undesirable.

Here, we will describe two simple and scalable methods to synthesize wax-shelled capsules (Fig. 2). Method I (Fig. 2A) involves directly adding droplets of an aqueous solution into molten wax. The solution is first cooled to 5 °C and the temperature of the wax is maintained above its T_m, but not much higher. For example, in the case of paraffin wax ($T_m \sim 57$ °C), it is held at 65 °C. The temperature difference between the hot liquid and the cold capsule is key to forming the wax shell. The shell forms within 5 to 20 s, and after 20 s, the wax-shelled capsules must be removed from the solution (with tweezers or a mesh filter). Cross-sections of such capsules (cut in half) in Fig. 2A show that the wax shell is about 0.7 mm in thickness. The concurrent importance of temperature and incubation time during capsule synthesis is further elaborated in Fig. S2 (ESI†). If the molten wax is held at a temperature T much greater than T_m or if the incubation time in the hot liquid is more than 30 s, then the wax shell that was formed initially (Fig. S2a and b, ESI†) melts and disintegrates (Fig. S2c and d, ESI†).

Method II for capsule synthesis is analogous to injection molding (Fig. 2B). Briefly, we create arrays of capsule molds, either by direct 3D-printing in a flexible material or by casting in PDMS from a master 3D-printed in a rigid material. Molten wax is poured into the cup-shaped molds, then a 3D-printed stamp consisting of an array of posts is inserted into the molten material to form cup-shaped hollow cavities. Once cooled, the wax forms a wall of thickness 0.5 or 1 mm depending on the mold size used. After these cups ($\sim$10 μL volume) are removed from the stamp, they are loaded with core material. Next, the cup is capped with molten wax, thereby forming closed capsules. On the whole, Method II has the advantage of being more amenable to mass-production of capsules whereas Method I is simpler and suitable for lab-scale studies. Both methods allow for a variety of core materials to be encapsulated

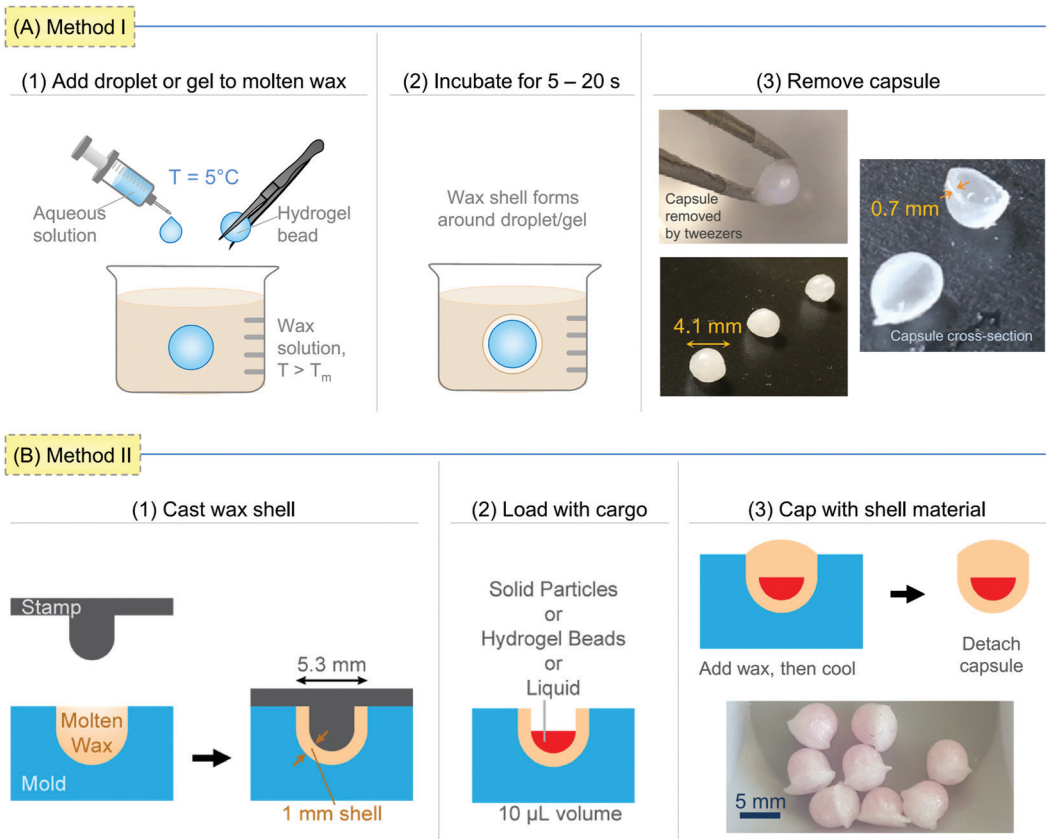

Fig. 2 Methods for synthesizing wax-shelled capsules. (A) In Method I, aqueous liquids or gels are cooled to 5 °C and then dropped into molten wax for 5–20 s. A wax shell rapidly forms around the structure, and intact capsules are then removed. An image with several capsules and the cross-section of a cut capsule are shown. (B) In Method II, molten wax is poured into molds and a stamp is inserted to create hollow cavities. On cooling, a wax shell of 1 or 0.5 mm is formed. Then, contents (liquid, solid, or gel) are loaded into the cavity. Finally, additional wax is used to cap the cavity, and upon cooling, the capsules are detached. An image with several capsules is shown.

in the capsules: neat liquids, liquids containing suspended particles, or gelled beads (such as those formed by crosslinking the anionic biopolymer sodium alginate with Ca^{2+} cations). Method II also permits solid powders or granules to be placed in the cores. Further details about each method are provided in the Materials and Methods section (ESI†).

Using these procedures, we have successfully fabricated capsules from paraffin wax (which is a mixture of hydrocarbons of different chain lengths); purified *n*-alkanes such as eicosane (C_{20} chains, with a T_m of 38 °C), docosane (C_{22}, 42 °C), and tetracosane (C_{24}, 52 °C); and fatty acids such as lauric (C_{12}, 43 °C), and palmitic acid (C_{16}, 63 °C). The shell formed by palmitic acid is uniform and very similar to that formed by paraffin wax. However, with the lower-melting waxes like eicosane, the shell is thinner and less uniform. To obtain a leak-proof shell, we subsequently immersed these capsules in molten octadecane (C_{18} alkane with a T_m of 28 °C) at a temperature of 34 °C for around 5 s. This second 'sealing' step is necessary to ensure that the capsules are stable and leak-proof at room temperature.

An initial experiment to test the encapsulation ability of wax-shelled capsules is shown in Fig. 1c and d. Here, a sealed eicosane capsule loaded with a red food dye is placed in DI water at room temperature. The dye remains sealed within the capsule and does not leak out—therefore the water appears colorless (Fig. 1c). Next, we demonstrate the responsive properties of the above capsule. For this, the sample is heated to 45 °C, which is above the T_m of eicosane. The higher temperature causes the wax shell to melt. Within a minute of heating, the dye is released completely into the water, which accordingly appears red (Fig. 1d). Note that the molten eicosane moves to the top of the vial due to its lower density. The results thereby show that temperature can be used as an "on–off switch" for solute release from the capsules. Fig. S3 (ESI†) shows similar temperature-responsive behavior of capsules made with other waxes. In all cases, the solutes are small, hydrophilic molecules (various food dyes) and they are perfectly encapsulated with zero leakage at room temperature. Conversely, the solute is completely released when heated above the T_m of the wax shell.

In addition to dyes, hazardous and caustic reagents can also be sealed in wax-shelled capsules and subsequently released. This includes strong bases and acids, as shown in Fig. S4 (ESI†). The paraffin-wax-shelled capsule in Fig. S4A (ESI†) contains a very strong base, *i.e.*, 2 M NaOH, in its core. It is placed in a solution of 10 mM phenolphthalein in 70–30 water–ethanol. Phenolphthalein is an acid/base indicator that turns bright pink at basic pH. Panel 1 shows that there is no leakage of base

from the capsule at room temperature. However, when heated above T_m, the wax shell melts and the base diffuses into solution, resulting in a bright pink color (Panels 2 and 3). Fig. S4B (ESI†) shows a similar capsule containing glacial acetic acid in its core and placed in a solution of 10 mM methyl red in ethanol. Again, there is no release at room temperature (Panel 1), but release does occur when heated above T_m (Panels 2 and 3).

The results thus far show that all kinds of hydrophilic solutes, regardless of their molecular size, can be encapsulated with no detectable leakage in wax-shelled capsules. The impermeability of wax shells to solutes is likely due to the wax being a dense, non-porous, and hydrophobic material. But is the wax shell indeed a hermetic seal, *i.e.*, can it ensure zero leakage over long timescales? To test this, we encapsulated a 1 mM fluorescein solution in several paraffin-wax-shelled capsules and placed each capsule in separate vials containing 10 mL of DI water. The absorbance in the external solution was measured over a period of six weeks. As shown by Fig. 3, the absorbance (at 490 nm) remained at zero over the entire time in all the test vials. After six weeks, the wax shells were melted by heat, whereupon the absorbance jumped to 0.5. Based on the sensitivity of the spectrometer and the configuration of the test, we would have been able to detect if even 1% of solute had leaked from the capsule into the solution. We have also continued such experiments with capsules over several months, and the capsules have continued to remain leak-proof over this period.

We have performed several variations of the above test. For example, a more sensitive test relying on fluorescence rather than absorbance, also showed that the leakage of solute into water was indistinguishable from zero. The impermeability also works in the opposite direction, *i.e.*, solutes in the external solution do not enter into a wax-shelled capsule; see Fig. S4C in the ESI.† Also, the results are the same regardless of the wax used for the shell and regardless of the method used for capsule preparation (Method I or II) (note that, for shells such as eicosane, the second sealing step with octadecane is necessary to ensure zero-leakage; this is shown by Fig. S5 in the ESI†). We also assessed if solutes could leak out by evaporation. For this, capsules with a paraffin-wax shell were loaded with acetone, a volatile compound, and the capsules were then left exposed to air. No detectable change in capsule weight was observed for a period of three weeks, consistent with earlier work.[17] On the whole, our capsules permit hermetic encapsulation of solutes (stability to leakage in water and evaporation in air) at room temperature and also a burst release of the solutes under mild heat.

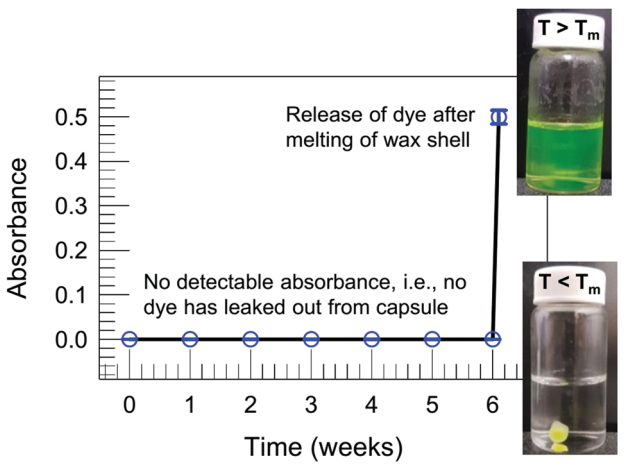

Fig. 3 Wax-shelled capsules enable hermetic encapsulation of small, hydrophilic solutes. Capsules (n = 6) with a paraffin-wax shell are loaded with fluorescein and placed in water. The absorbance of the external solution is measured to be zero over six weeks, showing that the shell is leak-proof over this period. The capsules are then melted by heating above T_m, whereupon the absorbance in the solution reaches 0.5. Photos of a vial before and after melting the capsule are shown. Error bars indicate the standard deviation from the mean.

We proceed to demonstrate two applications for our capsules. First, we show the ability to encapsulate hydrogen peroxide (H_2O_2) and subsequently release it as needed. H_2O_2 is employed to drive signal generation in numerous *in vitro* diagnostic assays.[19–22] Typically, it is manually added after all other reaction steps have completed, due to its potential to interfere with biochemical reagents. Here, our capsule design is flexible enough to accommodate H_2O_2 in capsule cores in either solid, liquid, or gel form. In the solid case, we used sodium percarbonate granules, which dissolve to release H_2O_2 in water. In the gel case, we first made gel beads of $\sim$1 mm diameter by dropping a 1.5 wt% sodium alginate solution through a syringe into 1 M calcium chloride ($CaCl_2$). The gel beads were then incubated in a 30% H_2O_2 solution for an hour. Granules and gel beads were placed in capsule cores and covered with a shell of eicosane. The capsules were then immersed in a solution of dichlorofluorescein diacetate (DCF-DA), an oxidation-sensitive probe that reacts with H_2O_2 to yield intense fluorescence. As shown by Fig. 4a, the capsules successfully isolated their cargo at 25 °C. When heated to 45 °C, the eicosane shell melts, thereby liberating H_2O_2 into the bulk solution. This presents a convenient approach for storage and on-demand-release of H_2O_2.

Next, we demonstrate how these capsules can facilitate assays with hazardous components. In this regard, we explore a capsule-based version of the blue-violet test for nitrates (NO_3^-).[23] Nitrates are pervasive contaminants in groundwater that can have severe effects on health.[24,25] The classic blue-violet assay requires sequential addition of a test reagent (diphenylamine, DPA) in concentrated sulfuric acid (H_2SO_4) followed by pure H_2SO_4. Naturally, the handling of such a potent acid is extremely dangerous, and it would be beneficial if its exposure to the user and the environment was minimized. Our capsules provide a way to shield the user from such hazardous reagents. Fig. 4b(i) shows a tube with a liquid sample at the bottom (a 30 mM solution of $NaNO_3$) along with one eicosane capsule containing DPA and three eicosane capsules with concentrated H_2SO_4. The reaction was initiated by immersing the capped tube in near-boiling water, causing first the DPA capsule to burst (since it was lowest in the tube and therefore closest to the tube walls), followed by the H_2SO_4 capsules (Fig. 4b(ii)). A blue color developed only when nitrate

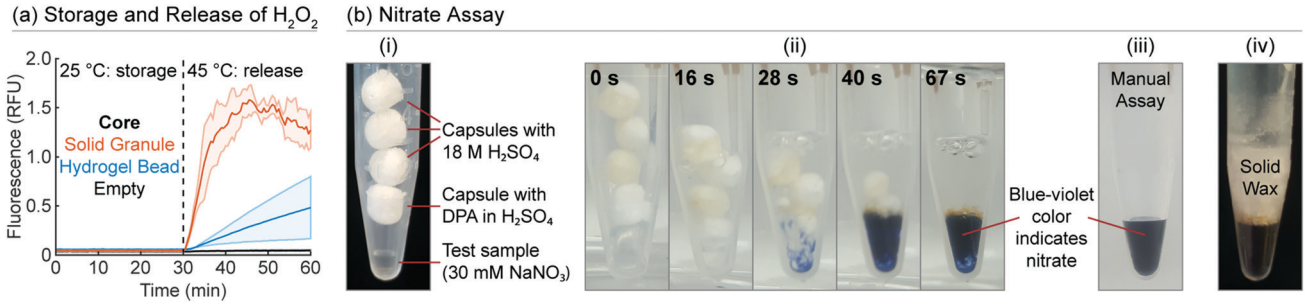

Fig. 4 Applications for responsive hermetic capsules. (a) Eicosane capsules successfully sequester both solid percarbonate granules (precursors for H_2O_2) and alginate hydrogel beads soaked in H_2O_2. No release of H_2O_2 from the capsule core is seen at 25 °C, but when the shells are melted by heating to 45 °C, released H_2O_2 activates the fluorescence of DCF-DA. Shaded region represents standard deviation. (b) Eicosane capsules are used to conduct an assay for nitrate that requires hazardous reagents. (i) The test sample (a 30 mM $NaNO_3$ solution) is placed at the bottom of a tube with one capsule of diphenylamine (DPA) in 12 M H_2SO_4 and three capsules of 18 M H_2SO_4. (ii) The tube is placed in near-boiling water and the photos are shown for successive times. The DPA capsule melts first, mixing its cargo with the test solution. Then, when the H_2SO_4 capsules melt, a blue-violet color appears, indicative of nitrate. (iii) A manual, solution-based assay with the same reagents yields a similar color. (iv) After the capsule-based assay cools, the shell material re-solidifies in a layer on top of the solution, isolating the hazardous chemicals.

was present in the liquid sample (Fig. 4b(ii); negative control in Fig. S6, ESI†); note that the color is comparable in intensity to that from a manual, solution-based assay (Fig. 4b(iii)). Afterwards, the tube was allowed to cool, whereupon the molten eicosane re-solidified as a thick waxy layer above the test solution (Fig. 4b(iv)), thus continuing to shield the user from exposure to the caustic components.

In summary, we have put forward modular strategies for mass-producing wax-shelled capsules that hermetically seal their core contents from the external environment. We have leveraged these capsules for sequestration of labile chemicals (such as H_2O_2) and hazardous reagents (such as concentrated NaOH and H_2SO_4). The ability to release such contents on-demand by mild heat may facilitate a range of portable, thermally-automated assays for medical diagnostics, environmental screening, and many other applications. These capsules could also constitute a generic controlled-release platform (on–off switch) for the delivery of pharmaceuticals, agrochemicals, and cosmetic agents. Using the methods presented here, wax-shelled capsules/containers can be created in arbitrary shapes (not just spheres) and with a range of core volumes. An example of such containers in different shapes (star, cube, doughnut) is shown in Fig. S7 in the ESI.† Future investigations should attempt to fabricate smaller capsules that adequately retain barrier functionality while minimizing the ratio of shell to core volume. We also envision the extension of our technique to multilayered or multicompartment constructs and complex 3D fluidic networks that can all be actuated by heat.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Supplementary Information for

Responsive Capsules that Enable Hermetic Encapsulation of Contents and their Thermally Triggered Burst-Release

John P. Goertz,[1] Kerry C. DeMella,[2] Benjamin R. Thompson,[2] Ian M. White[1]* and Srinivasa R. Raghavan[2]*
[1]Fischell Department of Bioengineering and
[2]Department of Chemical and Biomolecular Engineering
University of Maryland, College Park, MD 20742

Materials and Methods

Materials

Alkanes and fatty acids were purchased from Alfa Aesar; Sylgard 184 polydimethylsiloxane (PDMS) monomer and crosslinker from Ellsworth Adhesives; all other chemicals from Sigma; nylon mesh sieves from Component Supply Co.; and well plates from Greiner. A Formlabs Form2 printer was used for stereolithographic 3D printing with ≤ 0.05 mm layer height.

Fatty Acid Characterization

Fatty acids were characterized for their ability to act as phase-change partitions in a similar manner to the purified alkanes reported previously.[15,16] To determine the optimal temperature for rapid layer melting, 50 µL of the respective fatty acid was layered above 50 µL of 3 µM tetramethylrhodamine (TAMRA) (Figure S1a and b). The pH sensitivity of fluorescein amidite (FAM) was leveraged: at high pH, fluorescein displays strong fluorescence but is almost completely quenched at low pH (Figure S1c and d). 50 µL of fatty acid was layered between 50 µL of a pH 8 fluorescein solution and 10 µL of a 1 M HCl solution. Increasing the temperature melted the fatty acid barrier, allowing the fluorescein solution to sink through and mix with the HCl layer, resulting in the expected fluorescence quenching. To demonstrate independent actuation of different barriers, tubes were filled with 50 µL octadecanoic (stearic) acid, then 10 µL brilliant blue dye, then 50 µL of the indicated fatty acid, then 50 µL thioflavin T dye (Figure S1e). All tubes were incubated for 5 min at the indicated temperatures, then allowed to cool to room temperature before being photographed.

Capsule Synthesis (Method I)

Wax-shelled capsule synthesis according to Method I is illustrated in Figure 2A. In the case of liquid cores, deionized (DI) water or an aqueous solution of 2% sodium alginate was used. In the case of gel cores, these were prepared by extruding a 2% sodium alginate solution into 0.5 M $CaCl_2$ through a blunt 22G needle and incubating for 20 min. The Ca^{2+} ions crosslink the alginate chains, thus converting droplets into gelled beads (diameter 4-5 mm), which were then washed and used. Solutions or gel beads from the above step were cooled to 5°C and subsequently dropped into a reservoir of molten wax held at a temperature above its T_m (but within about 10°C). For example, in the case of paraffin wax (T_m ~ 57°C), the molten wax was heated to 65°C. A thin wax layer forms around the cold droplet/bead within seconds. After 5 – 20 s, the wax-shelled capsule was removed with tweezers. If the incubation time is longer or if the wax temperature was higher than T_m + 10, a stable shell does not form, as shown by Figure S2. For capsules with a shell of eicosane or other low-melting waxes, a second sealing step was needed to ensure

a leak-proof shell. For this, the capsules were immersed for 10 – 30 s into octadecane at 34°C. To prepare wax-coated gels in specific shapes (Figure S7), a 2% solution of agar containing 5 mM of fluorescein was heated to 90°C, then cooled to room temperature to form a gel. The stiff agar gel was cut into specific shapes using a stencil and a blade. The gels were then cooled and dropped into hot paraffin wax to form the wax layer around them.

Capsule Synthesis (Method II)

Capsule synthesis by Method II is illustrated in Figure 2B. Capsules were designed to have a 10 µL hemispherical core with a 15 µL cylindrical headspace. Molds were made by 3D printing, either directly or via a master template. The master was printed with Formlabs High Temp Resin and coated in mold-release (Ease Release 200, Mann Release Technologies). An elastomer mold was made by filling the master with PDMS (prepared at a 10:1 ratio), curing in an 80°C oven for 30 min, removing the partially-cured mold, and replacing it in the oven for an additional 30 min. Molds were also directly 3D-printed in Formlabs Flexible Resin. A stamp, used to produce the hollow core of the phase-change cups during casting, was 3D-printed in High Temp Resin. Molds and stamps were coated with mold-release prior to each use. PDMS molds were cheaper and easier to produce in quantity than directly-printed molds, but slight (~1%) deformation during curing led to mis-alignment of the post array and produced many failed capsules with discontinuous walls. The directly-printed mold gave a higher yield, but imprecisions in the printing process still led to some failures.

Shell material was melted and held at 200°C, then poured into the cup-shaped molds. This high of a temperature was found to be necessary to delay solidification of the poured material to allow for positioning of mold components. The 3D-printed stamp consisting of an array of posts was then inserted into the molten material to form cup-shaped hollow cavities. The stamp was secured with heavy-duty binder clips. Air was allowed to circulate underneath the casting assembly during cooling. Once cooled, the mold was peeled away. The wax forms a shell of thickness 0.5 or 1 mm depending on the mold size used. Often, the resulting cups remained lightly trapped to stamp posts and were removed with a razor blade. After filling with the desired core material, the cups were capped with additional 200°C shell material. First, 15 µL was deposited and allowed to solidify, followed by an additional 5 µL. This was necessary because the high thermal gradients experienced by the small amount of wax often led to formation of a visible pore through the center of the solidified layer; the second shell addition adequately sealed this pore. The cap is ultimately much thicker than the molded cup walls, so the nominal shell thickness should be interpreted as the maximum thickness of the thinnest portion of the resulting capsule. For capsules with a shell of eicosane or other low-melting waxes, a second sealing step (immersion into octadecane at 34°C for 10 – 30 s) was implemented to achieve the final capsule.

Capsule Encapsulation Tests

For the tests shown in Figure 3, the following procedure was employed. 1 mM fluorescein in 90-10 glycerol-water was encapsulated in paraffin-wax-shelled capsules (diameter ~ 4 mm) using Method I. Each capsule was then placed into a separate vial containing 10 mL of deionized water. Over six weeks, the absorbances of the surrounding solutions in the vials were periodically measured by a UV-Vis spectrometer (Cary 50). The absorbance peak of fluorescein was at 490 nm. After 6 weeks, the capsules were melted at 65°C to release their contents into solution and the absorbances of the solutions were again measured.

For the tests shown in Figure S5, the following procedure was employed. Eicosane-shelled capsules (0.5 mm shell) were prepared using Method II, and these were filled with 10 µL 100 µM resorufin in 50 mM Tris-HCl, 320 mM NH_4OAc, pH 8.7. The capsules were then immersed in individual wells of a 96-well plate containing 200 µL de-ionized water (DI). At the indicated time points, 50 µL was removed from each well and placed in a black-walled half-area plate for fluorescent analysis in a Spectramax m5 plate reader. A reading ten-fold above the noise floor of the instrument (corresponding to ~0.1% leakage) was considered a failure. The solutions were then replaced with the capsules in their original wells. The test was continued for 5 days.

For the experiment in Figure S4A, the following procedure was employed. An alginate gel bead, prepared as described above, was immersed in 2 M NaOH for 30 min. This gel bead was cooled and encapsulated in a capsule with a paraffin-wax shell using Method I. The capsule was placed in a solution of 10 mM phenolphthalein in 70-30 water-ethanol at room temperature. After confirming that there was no leakage of base under these conditions, the capsule was heated to 65°C to melt the wax shell, and this process was recorded as a movie. The images shown in the figure are stills from this movie.

For the experiment in Figure S4B, the following procedure was employed. An alginate gel bead, prepared as described above, was immersed in glacial acetic acid for 30 min. This gel bead was cooled and encapsulated in a capsule with a paraffin-wax shell using Method I. The capsule was placed in a solution of 10 mM methyl red in ethanol at room temperature. After confirming that there was no leakage of acid under these conditions, the capsule was heated to 65°C to melt the wax shell, and this process was recorded as a movie. The images shown in the figure are stills from this movie.

For the experiment in Figure S4C, the following procedure was employed. An alginate gel bead, prepared as described above, was cooled and encapsulated in a capsule with a paraffin-wax shell using Method I. The capsule was placed in a solution of 10 mM methylene blue in water at room temperature for 3 days. Thereafter, the capsule was extracted from the vial using tweezers, rinsed with water, and then examined.

Controlled Release of H_2O_2

Sodium percarbonate was sieved to obtain granules approximately 1-2 mm in diameter, placed in eicosane-shelled capsules (0.5 mm shell, two per capsule), and capped. 1-2 mm alginate beads were prepared by extrusion of a 1.5% sodium alginate solution into 1 M $CaCl_2$ through a blunt 25G needle. Beads were allowed to crosslink for 1 h, vacuum-drained, immersed in 30% H_2O_2 for 1 h, vacuum-drained again, placed in eicosane-shelled capsules (0.5 mm shell, two per capsule), and capped. Capsules with no core material were also prepared. Capsules were placed in 200 µL PCR tubes with 100 µL 10 µM DCF-DA, then analyzed with a BioRad MiniOpticon thermocycler for 30 min at 25°C followed by 30 min at 45°C.

Blue-Violet Test for Nitrates

Test reagent was prepared by mixing 6 mg diphenylamine into 1.2 mL 3 M H_2SO_4, then adding 300 µL 18 M H_2SO_4 (this two-step process is necessary: the heat evolved during the addition of pure sulfuric acid allows the diphenylamine to fully dissolve). The traditional test is performed by mixing sample solution, test reagent, and pure sulfuric acid sequentially at a 3:3:10 ratio, producing a deep blue color in the presence of nitrate (improper order slows color development). Eicosane-shelled capsules with 0.5 mm shells containing 10 µL test reagent or 18 M H_2SO_4. 10 µL sample solution was placed in a 0.6 mL centrifuge tube with one test reagent capsule and three pure sulfuric acid capsules then immersed in near-boiling water.

Supplementary Figures

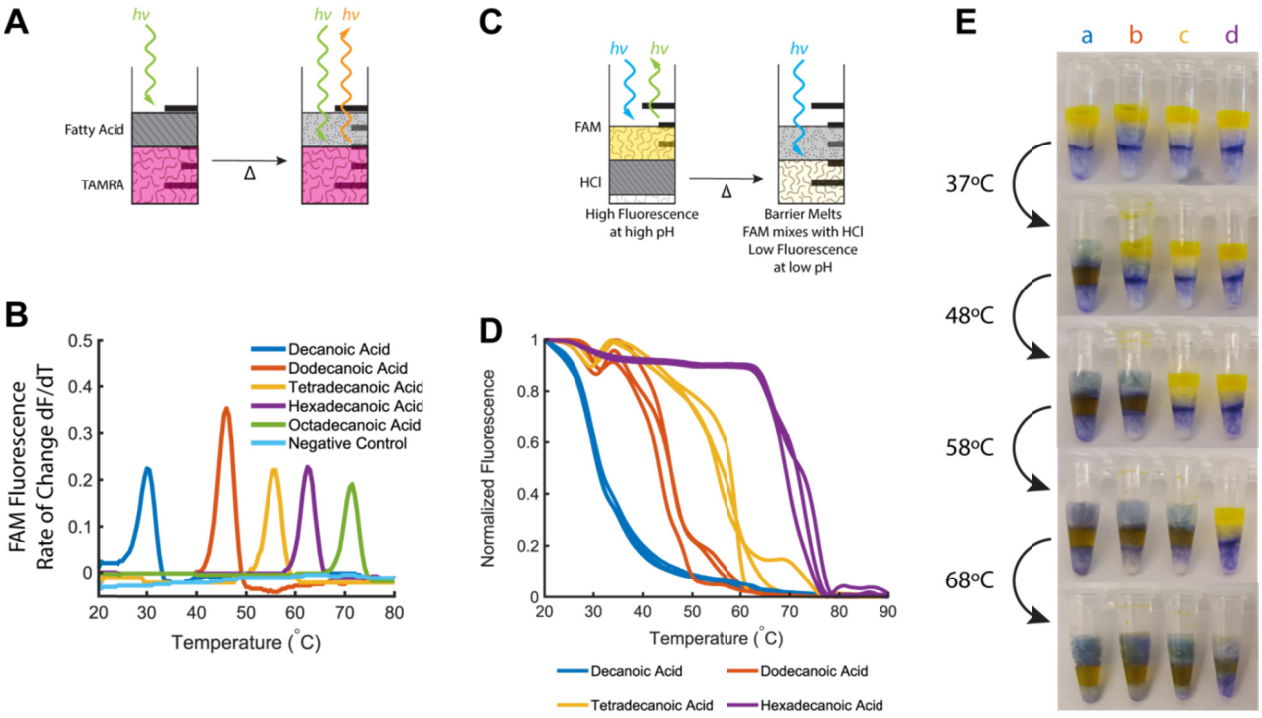

Figure S1. Characterization of fatty acids as phase-change partitions. A) Melting behaviour was characterized by placing a layer of the respective fatty acid on top of a fluorescent solution. B) The rate of change in observed fluorescence is indicative of the rate of melting at the given temperature. Fatty acids displayed well-defined melting points separated by several degrees Celsius. C) The pH sensitivity of fluorescein amidite (FAM) allowed quantification of the "breakthrough" point for each fatty acid. D) The fatty acids displayed well-defined breakthrough curves as the FAM solution mixed with the HCl solution, quenching the fluorescence. E) Stepwise increases in temperature melted (a) decanoic ("capric") acid, (b) dodecanoic ("lauric") acid, (c) tetradecanoic ("myristic") acid, and (d) hexadecanoic ("palmitic") acid sequentially, allowing solutions of yellow and blue dye to mix in a discrete, predictable manner. All tubes pictured were brought to the indicated temperature then allowed to cool to room temperature before being photographed.

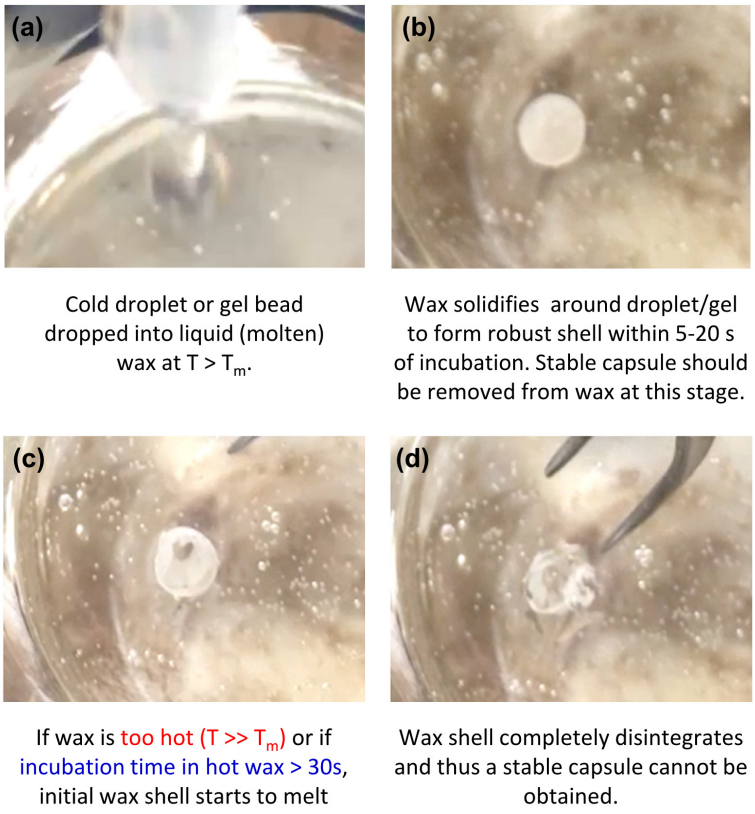

Figure S2. Considerations during synthesis of wax-shelled capsules by Method I. The importance of wax temperature and time of incubation in the molten wax are shown by the images. The molten (liquid) wax must be above its T_m, but not much higher. If the liquid is too hot (T >> T_m) or if the droplet/gel is incubated in the liquid for too long (> 30 s), the wax shell formed initially will melt and disintegrate.

Figure S3. Capsules with different wax shells showing responsive hermetic behavior. Capsules are created with aqueous cores having solutions of different food dyes and with different wax shells (each with a defined melting temperature T_m), as indicated. In all cases, the dye is hermetically sealed in the capsule core at room temperature and there is no leakage into the surrounding water (top images). Heating above T_m melts the shell and releases the dyes into water (bottom images). The image for the eicosane-shelled capsule is the same as in Figure 1.

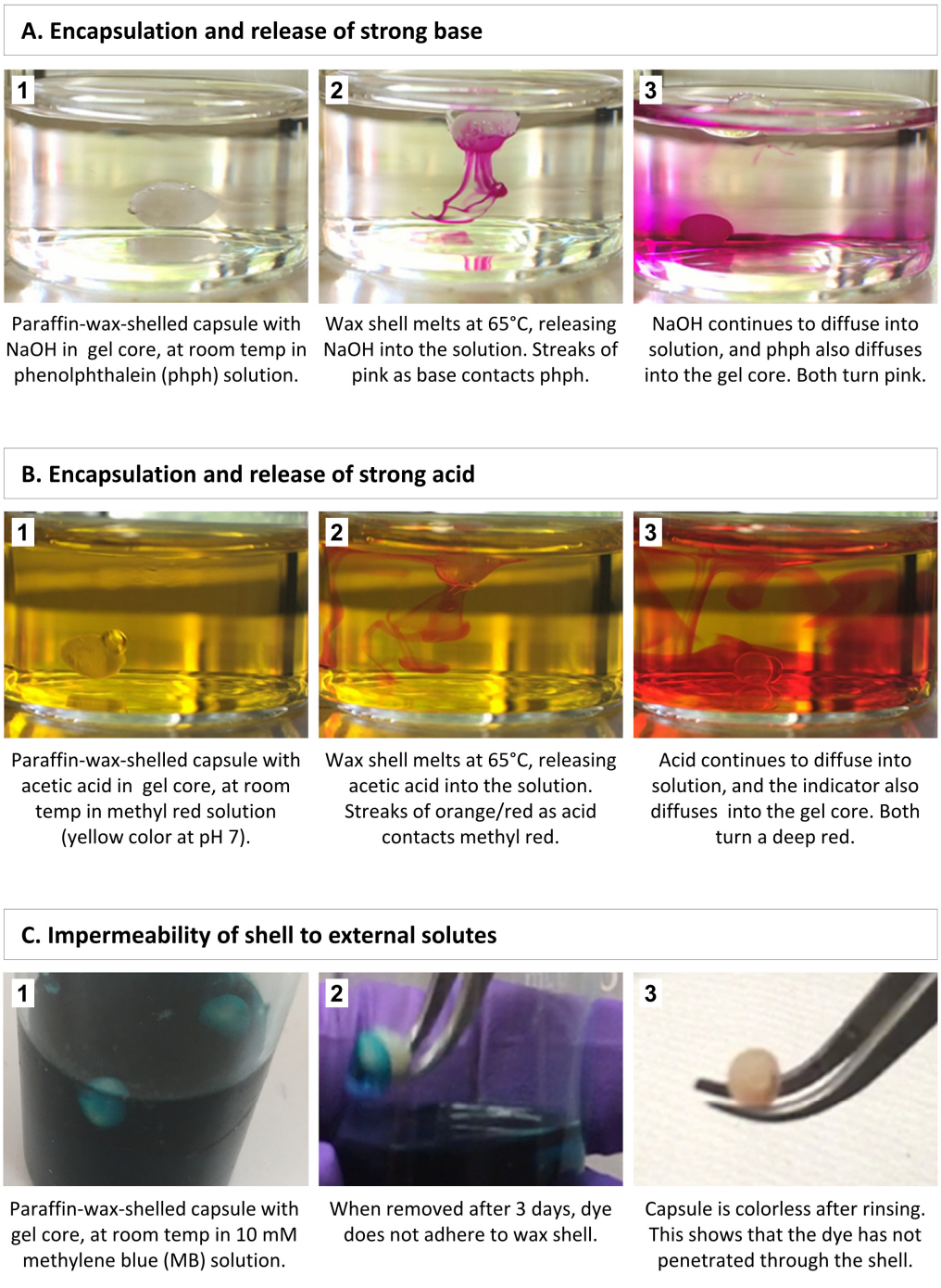

Figure S4. Wax-shelled capsules showing hermetic encapsulation and burst solute release. In all cases, the shell is made of paraffin wax. (A) Hermetic encapsulation of strong base (NaOH) at room temperature and its burst release at 65°C. (B) Hermetic encapsulation of strong acid (glacial acetic acid) at room temperature and its burst release at 65°C. (C) External solute (methylene blue dye) is unable to penetrate through the wax shell at room temperature. After 3 days of incubation, the capsule when removed from solution still looks clear.

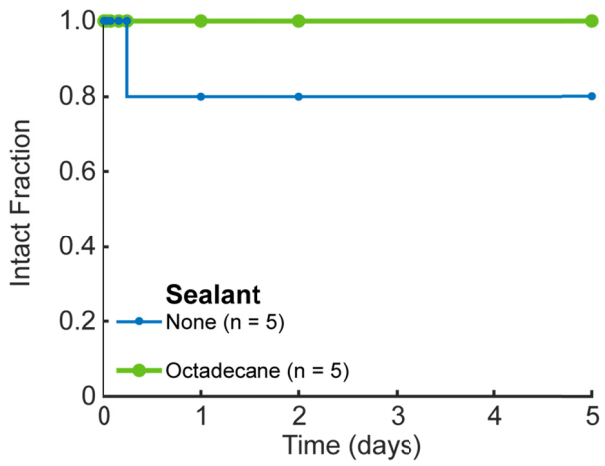

Figure S5. Eicosane-shelled capsules need to be 'sealed' to protect their cargo against leakage. Eicosane capsules are loaded with the fluorescent solute, resorufin and then sealed by briefly dipping into octadecane. Sealed capsules exhibit no leakage over five days, whereas one unsealed capsule failed in this time.

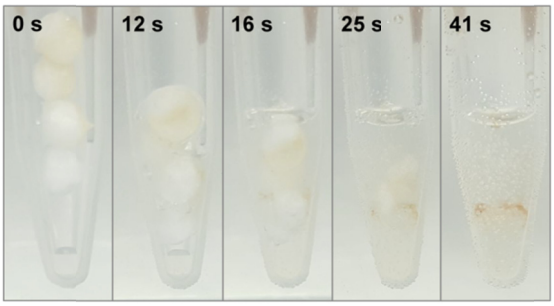

Figure S6. Negative results in nitrate assay for control sample. This is a control experiment to accompany Figure 4b in the main paper. The capsule-based assay is shown to yield no color change in the absence of nitrates in the test solution (bottom of the tube).

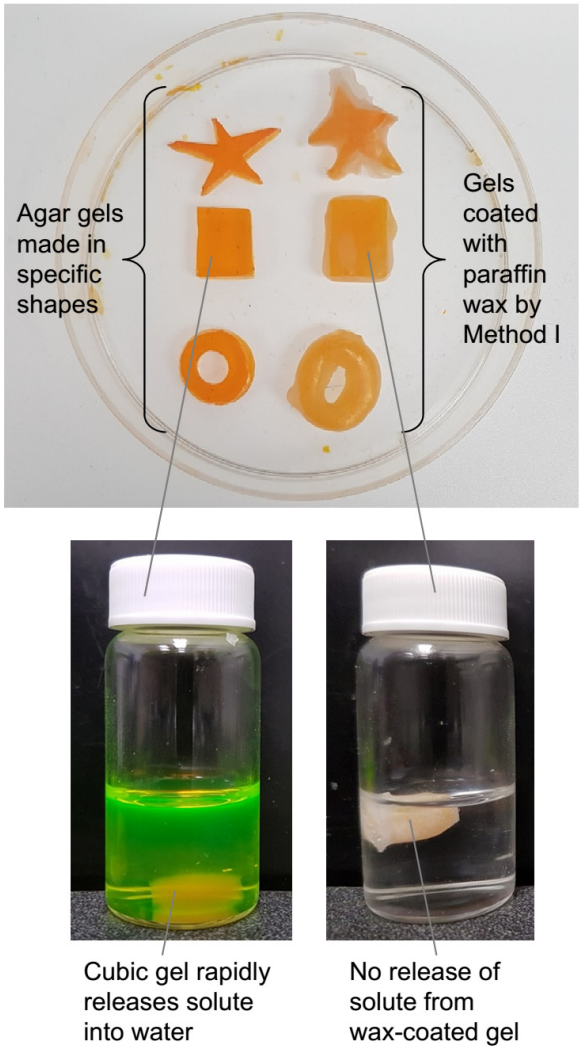

Figure S7. Wax-coated gels in specific shapes. In the top panel, agar gels in specific shapes (star, cube, doughnut) are shown on the left, and their counterparts with a layer of paraffin wax (formed by Method I) are shown on the right. As expected, the uncoated gels release their internal solute (fluorescein) rapidly (within an hour) when placed in water (bottom panel, vial on the left). However, no solute is released from the wax-coated gels placed in water (vial on the right).