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Triplet Fusion Upconversion Nanocapsule Synthesis

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Abstract

Triplet fusion upconversion (UC) allows for the generation of one high energy photon from two low energy input photons. This well-studied process has significant implications for producing high energy light beyond a material's surface. However, the deployment of UC materials has been stymied due to poor material solubility, high concentration requirements, and oxygen sensitivity, ultimately resulting in reduced light output. Toward this end, nanoencapsulation has been a popular motif to circumvent these challenges, but durability has remained elusive in organic solvents. Recently, a nanoencapsulation technique was engineered to tackle each of these challenges, whereupon an oleic acid nanodroplet containing upconversion materials was encapsulated with a silica shell. Ultimately, these nanocapsules (NCs) were durable enough to enable triplet fusion upconversion-facilitated volumetric three-dimensional (3D) printing. By encapsulating upconversion materials with silica and dispersing them in a 3D printing resin, photopatterning beyond the surface of the printing vat was made possible. Here, video protocols for the synthesis of upconversion NCs are presented for both small-scale and large-scale batches. The outlined protocols serve as a starting point for adapting this encapsulation scheme to multiple upconversion schemes for use in volumetric 3D printing applications.

Introduction

Moving away from subtractive manufacturing processes (i.e., complex shapes made by carving blocks of raw material) can reduce waste and increase production rates. Accordingly, many industries are moving toward additive manufacturing processes, where objects are built layer-by-layer[1] by means of three-dimensional (3D) printing. Many are working to develop additive manufacturing processes for numerous classes of materials (e.g., glass[2], ceramics[3,4], metals[5], and plastics[6,7]).

This layer-by-layer curing limits resin selection and impacts the print's mechanical properties[6,7]. Considering light-based 3D printing for making plastics, two-photon absorption (2PA)-based printing moves away from the layer-by-layer processes by printing volumetrically[8]. The 2PA process

requires simultaneous absorption of two photons to initiate polymerization. This not only increases the requisite power inputs, but also increases the complexity and cost of the printing system, limiting the print sizes to the mm^3 scale or smaller[9].

Recently, a new 3D printing methodology using triplet fusion upconversion (UC) has made volumetric 3D printing with UC possible on the cm^3 scale[10]. Excitingly, this process requires relatively low power density irradiation[10] as compared to 2PA-based printing[9,11,12]. The upconversion process converts two low energy photons into one high energy photon[13], and the upconverted light is absorbed by the photoinitiator to initiate polymerization. Deploying triplet fusion UC materials has traditionally been challenging due to high material concentration requirements, poor solubility, and oxygen sensitivity[13,14,15]. Encapsulating UC materials using a variety of nanoparticle schemes has been well studied[16] but falls short of the durability required in organic solvents. The silica-coated oleic acid upconversion nanocapsule (UCNC) synthetic protocol described here overcomes this durability challenge for dispersion of UC materials in a wide variety of organic solvents, including 3D printing resins[10]. The upconverted light generated from materials inside of the nanocapsules is patterned in multiple dimensions to generate support structure-free solid objects, which allows for printing high resolution structures with a resolution as small as 50 μm[10]. By removing support structures and printing in an oxygen-free environment, new resin chemistries are accessible to achieve both improved and novel material properties inaccessible with traditional stereolithography.

Here, the UCNC synthetic protocol is outlined for encapsulating the sensitizer (palladium (II) *meso*-tetraphenyl tetrabenzoporphine, PdTPTBP) and the annihilator (9,10-bis((triisopropylsilyl)ethynyl)anthracene, TIPS-an) at two different scales. Synthesis on a large scale provides material to provide ~10 g of upconversion nanocapsule paste for use in 3D printing resins. Synthesis on a small scale for ~1 g of upconversion nanocapsule paste allows for the optimization of new nanocapsule contents. This protocol will support the successful integration of triplet fusion UCNCs into a variety of 3D printing workflows and other applications.

Protocol

1. Large-scale upconversion nanocapsule synthesis

1. In a glovebox (see **Table of Materials**) with an inert atmosphere under red lighting, prepare saturated solutions of the sensitizer (PdTPTBP) and annihilator (TIPS-anthracene) (see **Table of Materials**) in 99% oleic acid at room temperature (~22 °C).
    1. Add 2 mL of oleic acid to 20 mg of PdTPTBP in a vial with a stir bar. Then, cover the vial with foil to protect from ambient light. Add 2 mL of oleic acid to 25 mg of TIPS-anthracene in a vial with a stir bar.
    2. Stir the mixtures at 600 rpm for at least 4 h before filtering with a 0.45 μm PTFE syringe filter. Each solution should visibly have undissolved solid to be removed by filtration, signifying that each solution is saturated.
    3. Using a syringe, prepare 1.75 mL of the upconversion material stock solution by mixing 0.7 mL of the filtered TIPS-anthracene solution, 0.35 mL of the filtered PdTPTBP solution, and 0.7 mL of oleic acid.

NOTE: The upconversion solution used for the nanocapsules has a ratio of 2:1:2 of TIPS-anthracene to PdTPTBP to oleic acid by volume.

2. Measure 4 g of 10K MPEG-silane in a clean 20 mL vial so that it is ready for use during synthesis. This can be conducted inside or outside of the glovebox. If this material is measured outside of the glovebox, secure the vial lid with sealing film or electrical tape before bringing it into the glovebox.

3. In a 250 mL Erlenmeyer flask sealed with a septum, chill 200 mL of ultrapure deionized water in an ice bath for at least 1 h to reach ~5 °C . Typically, this takes a few hours.

4. Secure the septum to the flask using at least six pieces of sealing film. This is to ensure that the septum remains affixed when the flask is under vacuum in the glovebox antechamber.

5. Bring the chilled water into the glovebox immediately before preparing the nanocapsules. Only pull a light vacuum on the antechamber when bringing in the water by pulling 20% vacuum based on the measurement on the antechamber pressure gauge.

6. After bringing the water into the glovebox, immediately turn on the glovebox purge feature to bypass the column. This removes the oxygen introduced when bringing in the water under light vacuum and prolongs the life of the column. Keep the purge on until the synthesis is complete, and all waste has been removed from the glovebox.

7. Ensure all chemicals and consumables are ready to use, including syringes and needles for dispensing (3-aminopropyl)triethoxysilane (APTES) and tetraethyl orthosilicate. Make sure the 10K MPEG-silane is within reach. For cleaning, nylon cloths are also useful to have available.

8. Plug in the blender (see **Table of Materials**). Cover the electrical sockets with a plastic bin or nylon cloth. This barrier allows for protection in the case of an unexpected blender leak. Make sure the blender is powered off.

9. Carefully pour the water into the blender. Add 1.45 mL of the upconversion material stock solution (prepared in step 1.1.3) in one portion with a syringe into the center of the water in the blender.

10. Affix the lid and cover it with a nylon wipe in the case of an unexpected leak. Blend at the maximum speed (22,600 rpm) for exactly 60 s while holding the blender lid to prevent small leaks.

11. Turn off the blender and move it out of the way to ensure adequate working space.

12. Transfer the emulsion to a 500 mL round bottom flask. Secure the flask to a stir plate with a clamp. Mix the emulsion vigorously at 1200 rpm with an egg-shaped stir bar (see **Table of Materials**).

13. Using a syringe, add 0.75 mL of APTES to the emulsion to generate a clear solution of micelles.

14. Add 4 g of 10K MPEG-silane to prevent capsule aggregation. Shake the flask if needed to ensure it is dispersed. Stir at 1200 rpm for approximately 10 min.

15. During this time, dry the blender and lid with a nylon cloth. Use tongs to keep hands far away from the sharp blender blades.

16. After 10 min have passed, add 15 mL of tetraethyl orthosilicate in one portion using a 20 mL syringe. Add another 15 mL of tetraethyl orthosilicate in one portion

using a 20 mL syringe for a total of 30 mL. Affix a septum to the flask and stir at 1200 rpm for 30 min.

17. Remove the flask and waste from the glovebox and turn off the glovebox purge.

18. Affix the flask to a stir plate with a heating element, such as an oil bath or aluminum heating block. Connect the flask to a Schlenk line so the reaction is held at a constant pressure under an inert gas such as nitrogen or argon.

19. Stir and heat the reaction at 65 °C at a speed of 1200 rpm for 40 h.

20. After 40 h, disconnect the reaction from the Schlenk line to add 4 g of 10K MPEG-silane. Reconnect the reaction to the Schlenk line. Stir and heat the reaction at 65 °C at 1200 rpm for 8 h.

21. After 8 h, turn off the heat and allow the reaction to cool to room temperature while stirring at 1200 rpm.

22. When the reaction is cool, transfer the reaction into centrifuge tubes.

    1. For a centrifuge (see **Table of Materials**) that holds 50 mL centrifuge tubes, split the reaction equally among 10 centrifuge tubes.

    2. For a centrifuge that holds 0.5 L centrifuge tubes, split the reaction equally among two centrifuge tubes.

23. Centrifuge the suspension at 8670 x *g* for 1 h at a temperature of 20-22 °C. Discard the pellet and retain the supernatant containing the nanocapsules.

24. Centrifuge the supernatant at 8670 x *g* for 14-16 h at 20-22 °C.

25. Discard the supernatant and collect the pellet containing upconversion nanocapsules.

    1. Using a pipette, carefully rinse the top surface of the nanocapsule pellet with ultrapure deionized water (2 x 10 mL). This should be conducted at a low flow so the pellet is not dislodged from the centrifuge tube.

    2. Transfer the nanocapsule paste into two or three separate 20 mL scintillation vials with a spatula and immediately bring the vials into the glovebox. Approximately 7-10 g of nanocapsule paste should be recovered.

       NOTE: For further use, it is recommended that the nanocapsules are dispersed into a solvent such as a monomer for 3D printing or deoxygenated ultrapure deionized water within 48 h of synthesis. Water will evaporate from the nanocapsule paste and leave the nanocapsules unusable after 48 h.

26. Perform scanning electron microscopy (SEM), dynamic light scattering (DLS), and upconversion photoluminescence to characterize the nanocapsule preparation.

2. Small-scale upconversion nanocapsule synthesis

1. Prepare the stock solutions of the sensitizer and the annihilator as described in step 1.1. Scale down the volume of the solution used to make upconversion nanocapsules to 250 µL instead of the 1.75 mL described in step 1.1. Mix 100 µL of the filtered TIPS-an solution with 50 µL of the filtered PdTPTBP solution and 100 µL of oleic acid.

2. Vigorously sparge 20 mL of ultrapure deionized water in a 40 mL scintillation vial (see **Table of Materials**) with an inert gas, such as nitrogen or argon, using a Schlenk line for at least 10 min. Affix the lid with electrical tape or sealing film prior to bringing the vial into the glovebox.

NOTE: If making multiple small-scale samples at once, larger volumes of water can be sufficiently degassed by blending 200 mL of chilled water as outlined in section 1 using a clean, unused blender pitcher. Sparging water with an inert gas on a Schlenk line is not effective at volumes greater than 20 mL.

3. Measure 400 mg of 10K MPEG-silane so that it is ready for use during synthesis into a clean 10 mL vial. This can be conducted inside or outside of the glovebox. If this is measured outside of the glovebox, secure the vial's lid with sealing film or electrical tape before bringing it into the glovebox.

4. Bring the sparged water into the glovebox, and immediately turn on the glovebox purge feature to bypass the column. This whisks away oxygen introduced when bringing in the water under light vacuum and prolongs the life of the column. The purge should remain on until the synthesis is complete, and all waste has been removed from the glovebox.

5. Ensure all chemicals and consumables (5 mL syringes and a micropipette with tips) are ready to use.
    1. Using a syringe, remove 1 mL of (3-aminopropyl)triethoxysilane from the bottle and dispense it into a clean, labeled 20 mL vial for later use.
    2. Using a syringe, remove 5 mL of tetraethyl orthosilicate and dispense it into a clean, labeled 20 mL vial for later use.
    3. Make sure the 10K MPEG-silane is within reach in the glovebox.
    4. For cleaning, extra nylon cloths are also useful to have available.

6. Plug in the vortex mixer (see **Table of Materials**) and set the speed to the highest setting (3200 rpm).

7. Using a micropipette, add 145 µL of sensitizer/annihilator stock solution to a vial of water (20 mL). Affix the lid with electrical tape or sealing film.

8. Vortex the solution at the highest speed of the vortex mixer (3200 rpm) for 7 min to ensure nanodroplet formation similar to the large-scale synthesis. Hold the vial close to the base and never hold on to the vial lid during vortexing, as the lid can become loose and detach from the vial.

9. Affix the vial to a stir plate. Stir the emulsion at 1200 rpm with an octagon-shaped stir bar (see **Table of Materials**).

10. Using a micropipette, add 75 µL of APTES to generate a clear solution of micelles.

11. After generating the clear solution, immediately add 400 mg of 10K MPEG-silane. Affix the lid and shake the vial to efficiently mix the reaction. Return the vial to the stir plate.

12. Using a syringe, add 3 mL of tetraethyl orthosilicate in sequence while the reaction is being stirred at 1200 rpm. Affix the lid and shake the vial to efficiently mix the reaction. Stir the reaction at 1200 rpm until it is removed from the glovebox.

13. Seal the vial with electrical tape or sealing film and remove the vial from the glovebox.

14. Heat the solution at 65 °C using an oil bath or aluminum heating block. Stir the reaction at 1200 rpm for 40 h.

15. After 40 h, add 400 mg of 10K MPEG-silane. Reseal the vial with electrical tape or sealing film. Stir the reaction at 1200 rpm for 8 h.

16. Allow the reaction to cool to room temperature while stirring at 1200 rpm. When the reaction is cool, combine the reaction mixtures into one 50 mL centrifuge tube.
17. Centrifuge the suspension at 8670 x *g* for 1 h at a temperature of 20-22 °C. Discard the pellet and retain the supernatant containing the nanocapsules.
18. Centrifuge the supernatant at 8670 x *g* for 14-16 h at 20-22 °C.
19. Discard the supernatant and retain the pellet containing upconversion nanocapsules. Using a pipette, carefully rinse the top surface of the nanocapsule pellet with 2 x 1 mL of ultrapure deionized water. This should be conducted at a low flow so the pellet is not dislodged from the centrifuge tube.
20. Transfer the nanocapsule paste into a 20 mL scintillation vial with a spatula and bring the vials into the glovebox immediately. Approximately 700-1000 mg of nanocapsule paste should be recovered.
    NOTE: For further use, it is recommended that the nanocapsules are dispersed into a solvent, such as a monomer for 3D printing or deoxygenated ultrapure deionized water, within 48 h. Water will evaporate from the nanocapsule paste and will leave the nanocapsules unusable after 48 h.

Representative Results

Figure 1 shows a cartoon depiction of the upconversion nanocapsule synthesis protocol. The parallels among the small scale and large-scale UCNC preparation are emphasized, such as the oil in water emulsion generation and the addition of chemicals to synthesize the silica shell. From the small-scale synthesis, 700-1000 mg of UCNC paste is typically collected, while 7-10 g of the UCNC is typically collected from the large-scale synthesis.

The nanocapsules were characterized using a combination of spectroscopic and microscopy techniques[10]. To prepare samples for SEM, a film was drop-cast from a solution of 100 mg mL^{-1} nanocapsule paste dispersed in water onto an appropriate conductive SEM substrate and allowed to dry. The conductivity of the nanocapsules is inherently low, but still sufficient for characterization without the addition of another conductive material. A representative SEM image (**Figure 2A**) shows the relatively monodisperse nanocapsules with diameters of ~50 nm obtained with this protocol. One limitation of using SEM to characterize the morphology of the UCNCs is that they are unstable under ultrahigh vacuum for long periods of time. Under ultrahigh vacuum necessary for SEM measurements, the UCNCs can be successfully imaged if working efficiently, typically within 30 min. UCNCs fuse under high vacuum after approximately 30 min under ultrahigh vacuum (**Figure 2B**). This fusion is not observed under ambient conditions following the procedure outlined in this protocol (*vide infra*). Even in light of the stability considerations under vacuum, electron microscopy is still a beneficial method to assess the typical morphology of the UCNCs.

Dynamic light scattering (DLS) is another useful technique to characterize the average nanocapsule hydrodynamic diameter in solution. The samples for DLS can be easily prepared with a sample of diluted UCNCs. Here, a sample of the supernatant recovered after the first centrifuge (step 1.23 or 2.17) was characterized by DLS. The supernatant was diluted by a factor of 10x with ultrapure deionized water and filtered with a 0.2 µm PVDF filter to remove large particulates and dust. Alternatively, one can characterize the UCNC paste

at a concentration of 100 mg mL^{-1} in ultrapure deionized water diluted 10x and filtered with a 0.2 µm PVDF filter. The hydrodynamic diameter was measured using DLS to be <100 nm from batch to batch, typically in the range of 65-90 nm[10]. Nanoparticle aggregation is not observed under these characterization conditions, removing the need for an additional electrolyte[10]. Similar UCNC diameters can be generated from large scale or small-scale protocols; representative traces from one scan are presented in **Figure 2C**. Due to Brownian motion and the mathematical fitting process to the Stokes-Einstein equation, many scans are averaged together to determine the average hydrodynamic diameters[17]. The average hydrodynamic diameters for the samples shown in **Figure 2C** are ~75 nm for the large batch (polydispersity, PDI: 0.21) and ~66 nm (PDI: 0.15) for the small batch presented. This variation in hydrodynamic diameter is typical from batch to batch, irrespective of the reaction scale.

Finally, optical characterization is vital to assess the integrity of the silica shell encapsulation (**Figure 2D**). Here, a sample of the supernatant recovered after the first centrifuge was diluted by 10x in deoxygenated acetone in the glovebox. The sample was diluted in acetone to test the structural integrity of the UCNCs. In **Figure 2D**, the anthracene upconversion emission is clearly present upon irradiation with a 635 nm laser, signifying the average silica shell remains intact. If the silica shells are too thin, bright upconversion is extremely low upon irradiation with a 635 nm laser. This is due to the upconversion contents being dissolved and diluted in acetone to a concentration too low to generate bright upconverted emission[10].

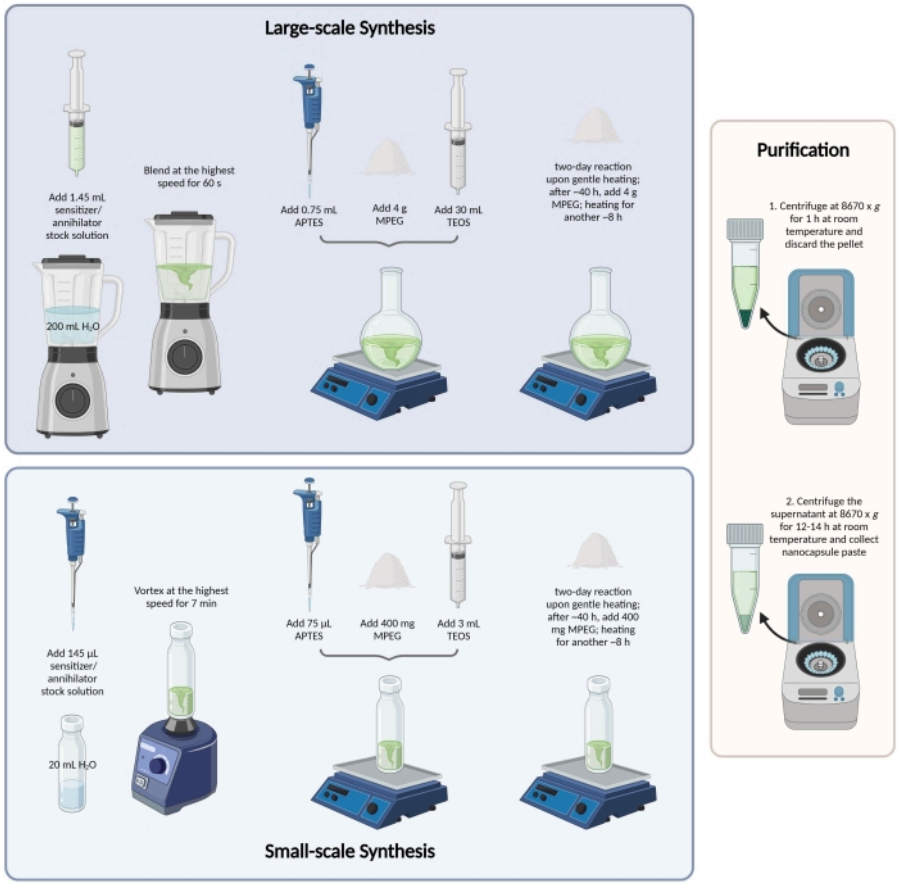

Figure 1: A cartoon depiction of the upconversion nanocapsule synthetic process on the small and large scale. This figure was created with Biorender.com. Please click here to view a larger version of this figure.

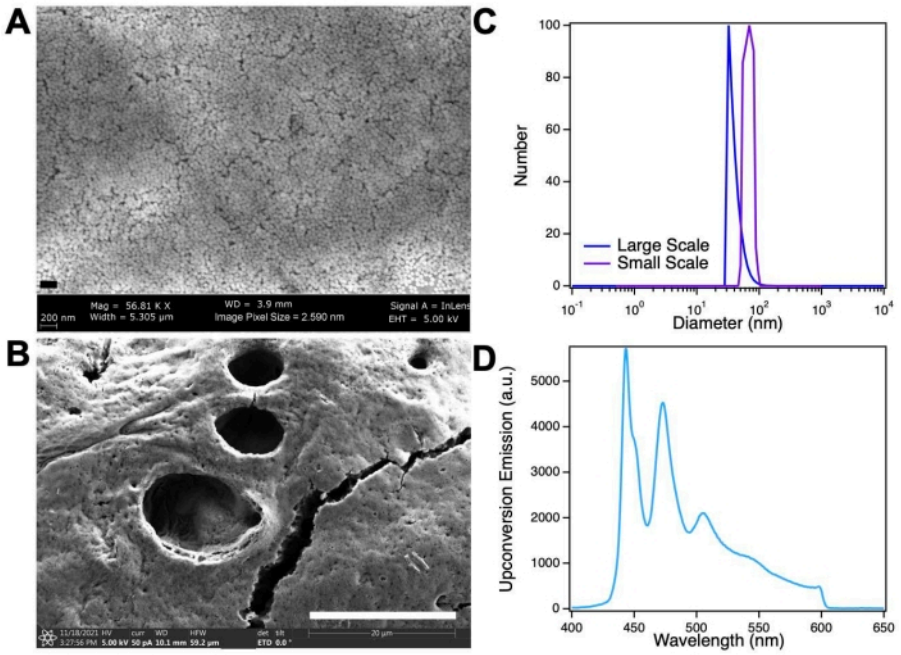

Figure 2: Representative nanocapsule characterization using microscopy and spectroscopy. (**A**) SEM of the UCNCs shows the scale and uniformity of the upconversion nanocapsule synthesis. Scale bar = 200 nm. (**B**) SEM of the UCNCs that have fused under ultra-high vacuum over the course of ~30 min. SEM samples were prepared by drop-casting solutions of UCNCs in deionized ultrapure water. Scale bar = 20 µm. (**C**) Representative DLS traces of upconversion nanocapsules prepared on a small scale and large scale. UCNCs were diluted in deionized ultrapure water. (**D**) The upconversion emission of TIPS-an in UCNCs diluted in acetone was generated upon irradiation with a 635 nm laser at ~65 W cm^{-2}. This bright upconversion signifies the silica shells are thick enough to prevent the nanocapsule contents from spilling out. Please click here to view a larger version of this figure.

Discussion

There are several considerations when preparing bright upconverting nanocapsules. First, the synthesis is completed in a glovebox because the upconversion materials must be protected from oxygen-it is well established that upconverted light output is reduced in the presence of oxygen[13,14,15,16]. Additionally, the sensitizer and annihilator stock solutions should be prepared fresh for every batch. PdTPTBP and other metalated porphyrins have been shown to demetalate in ambient lighting in the presence of acid[18], and anthracenes are known to aggregate over time[19]. These effects can be minimized by preparing fresh solutions under red lighting for each synthesis. The authors note that rigorous red lighting is no longer required once the metalated porphyrin and anthracene are mixed, and ambient lighting is acceptable to use after this step. Finally, for the large-scale synthesis, it is recommended that at least 1.75 mL of the upconverting stock solution is prepared, since adding less than 1.45 mL of this solution to make UCNCs will alter the proportions of all other required reagents as well as the

concentration-dependent nanodroplet formation. Similarly, for the small-scale synthesis, it is recommended that 250 µL of the upconverting stock solution is prepared in the same proportions. Finally, when using a micropipette for dispensing the oleic acid stock solutions, slowly release the plunger and wait for it to fully rise to dispense the desired volume. The oleic acid will slowly fill the pipette tip due to its high viscosity and it is easy to inadvertently dispense less solution than expected.

It is important to understand that the oleic acid nanodroplet generation is sensitive to blending time, speed, and significant temperature changes. For instance, the blender selection is significant and can impact the formation of oleic acid nanodroplets. Multiple blender brands were tested in the initial development stages. The blender recommended in the **Table of Materials** led to the generation of relatively superior and reproducible nanocapsules described in this protocol. Notably, powerful blending increases the temperature of the emulsion and reduces the oleic acid nanodroplet formation efficiency. The blender blades must be completely submerged in water to best control the temperature, which was one consideration for determining the required water volume presented here[10]. Additionally, chilling the water in advance reduces droplet aggregation in the emulsion, which ultimately improves the nanocapsule yield for the large-scale synthesis. On the other hand, for the small-scale synthesis, chilling the water does not significantly alter the oleic nanodroplet formation, probably because holding the 40 mL vial does not increase the temperature of the water as much as the blender blades.

The APTES addition is a significant synthetic step, as APTES stabilizes the oleic acid nanodroplets generated by blending or vortexing. The initial nanodroplet emulsion is a cloudy, turbid dispersion. Upon addition of APTES, the solution becomes clear and transparent as the nanodroplets are stabilized. On average, the APTES volumes required are very close to what is presented in the protocol, but sometimes slightly less or slightly more APTES is required for the solution to become clear. Thus, the APTES addition should be treated in an analogous manner to conducting other titrations[20]. Adding too much APTES (i.e., beyond a "just clear" solution) will disrupt nanocapsule shell formation and decrease yield. To that end, if significantly different volumes of APTES are required to produce a clear suspension, or a clear suspension is never reached, this indicates troubleshooting is required to optimize the oleic acid nanodroplet formation. For instance, if the nanodroplet generation is inefficient, the droplet volume and thus surface area of the nanodroplet will be larger than expected and may require more APTES. This has been observed in the small-scale synthesis, and can be remedied in a variety of ways, such as the force used to hold a vial against the vortex mixer or by increasing the vortexing time.

Additionally, the 10K MPEG-silane must be added immediately after APTES to prevent aggregation and cannot be omitted[10]. Without the addition of 10K MPEG-silane, irreversible aggregation is observed within ~30 min in the form of precipitate generation. Although 5K MPEG-silane can be substituted for 10K MPEG-silane, lower molecular weight MPEG-silanes do not sufficiently prevent aggregation at a constant concentration.

The silica shell formation is key to impart UCNC durability when dispersed in various solutions. While silica shell growth is generally well studied[21,22,23], the often-used[21] acid or base catalysis to promote silica growth is not used here, as the heating is sufficient for generating a durable, cross-linked silica shell. To monitor the silica shell formation over time, bright upconversion should be observed after 100x dilution

of a nanocapsule reaction aliquot in an organic solvent, such as acetone, with minimal sensitizer phosphorescence for the PdTPTBP/TIPS-an system (**Figure 2D** and reference[10]). Typically, bright upconversion is observable after about 24 h, but 48 h will increase the relative emission, signifying that a larger population of the UCNCs possess a durable shell. Note that the UC emission is dependent upon the irradiation power and sufficient power densities should be employed. For instance, in the system described here, power densities on the order of ~65 W cm^{-2} are required to see bright upconverted PL.

The second addition of 10K MPEG-silane after 40 h of silica growth improves nanocapsule dispersibility in organic solvents. While the UCNCs will still be dispersible in multiple solvents without this second 10K MPEG-silane addition, the second addition is highly recommended to increase the UCNC loadings by mass in solution. For instance, for use in a 3D printing resin, 0.67 g mL^{-1} of nanocapsule paste was dispersed in acrylic acid[10].

Exposing the UCNCs to oxygen during the entire multi-day fabrication process results in the ingress of oxygen in concentrations that significantly reduce upconversion photoluminescence. To ensure an inert atmosphere is maintained during the 48 h of stirring in an ambient atmosphere, different protocols are invoked depending upon the reaction scale. At large scales, the ethanol generated during silica growth can produce significant pressures which can lead to the removal of an affixed septum or the loss in structural integrity of the reaction vessel[24]. Thus, the 500 mL flask should be connected to a Schlenk line to allow for a pressure release in an inert atmosphere. At small scales, sealing a 40 mL glass vial with sealing film or electrical tape maintains the seal's structural integrity. Without sealing the vial's lid, the increase in pressure will slowly unthread the lid and allow for the ingress of oxygen.

The reaction purification by centrifugation separates the UCNCs from other undesired side products. Multiple centrifuge brands and rotors are compatible with this purification if the *g* force provided in the protocol is accessible. The *g* force can be converted to rotations per minute based upon the centrifuge rotor dimensions[25]. Exposing the UCNCs to an ambient atmosphere briefly during centrifugation is acceptable as long as they are stored in an inert atmosphere after purification. One limitation of this synthesis is that atom yield is difficult to quantify in relation to the input chemicals. After centrifugation, this large-scale nanocapsule synthesis should yield roughly 10 g of paste and the small-scale synthesis should yield roughly 1.0 g of capsule paste. It is unclear how much of the TEOS is incorporated into making the UCNC shell. The pellet discarded after the first centrifugation is comprised of large molecular weight silica that is not incorporated into the UCNCs. After the second centrifugation, the supernatant can be centrifuged again to increase the mass collected. It is not recommended to increase the centrifugation time beyond 16 h, as the soft capsule paste will solidify into a compact film that cannot be dispersed in other solvents. Even so, the capsule paste masses collected from batch to batch are consistent and are sufficient for subsequent use and characterization.

The UCNC durability can vary from solvent to solvent as well as with storage conditions. While the UCNC paste collected by centrifugation is unusable after 48 h as water evaporates, the nanocapsules are durable in a variety of solvents. In water, the UCNC durability is in the order of several months. In acrylic acid, the durability is reduced to days mostly because the acrylic acid solvent is unstable and can undergo

polymerization when stored in oxygen-free conditions[10,26]. Further solvent-dependent investigations of UCNC durability are ongoing.

The small-scale synthesis is especially useful for relative comparisons of upconversion photoluminescence among different formulations. The NC paste collected after the second centrifugation should be dispersed in water at concentration of 100-200 mg mL^{-1} and diluted in acetone (or another solvent as desired). A minimum of 25% of the solution volume must contain water (e.g., 25/75 water/acetone v/v) to keep the NCs suspended and prevent precipitates from forming. Comparing the relative upconversion emission among batches was required to determine the concentrations of sensitizer and annihilator in this protocol. Perhaps counterintuitively, the ratio of sensitizer to annihilator required to maximize the light output in UC nanocapsules for 3D printing may not be equivalent to the ratio that maximizes the UC quantum yield[27] in oleic acid stock solutions.

In conclusion, a detailed protocol and best practices for synthesizing upconversion nanocapsules is expanded upon in a step-by-step fashion[10]. Since other methods to encapsulate upconversion materials for use in real-life applications are only compatible with aqueous environments[16], this synthesis is significant because it allows for upconversion materials to be deployed into diverse chemical environments, such as organic solvents. These methods will serve to increase approaches to access volumetric 3D printing for precision additive manufacturing and in any application requiring high-energy light beyond the surface.

Disclosures

Harvard University has filed several patents based on this work. SNS, RCS, and DNC are co-founders of Quadratic3D, Inc.

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