Video Article

Fat-Water Phantoms for Magnetic Resonance Imaging Validation: A Flexible and Scalable Protocol

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Abstract

As new techniques are developed to image adipose tissue, methods to validate such protocols are becoming increasingly important. Phantoms, experimental replicas of a tissue or organ of interest, provide a low cost, flexible solution. However, without access to expensive and specialized equipment, constructing stable phantoms with high fat fractions (*e.g.*, >50% fat fraction levels such as those seen in brown adipose tissue) can be difficult due to the hydrophobic nature of lipids. This work presents a detailed, low cost protocol for creating 5x 100 mL phantoms with fat fractions of 0%, 25%, 50%, 75%, and 100% using basic lab supplies (hotplate, beakers, *etc.*) and easily accessible components (distilled water, agar, water-soluble surfactant, sodium benzoate, gadolinium-diethylenetriaminepentacetate (DTPA) contrast agent, peanut oil, and oil-soluble surfactant). The protocol was designed to be flexible; it can be used to create phantoms with different fat fractions and a wide range of volumes. Phantoms created with this technique were evaluated in the feasibility study that compared the fat fraction values from fat-water magnetic resonance imaging to the target values in the constructed phantoms. This study yielded a concordance correlation coefficient of 0.998 (95% confidence interval: 0.972-1.00). In summary, these studies demonstrate the utility of fat phantoms for validating adipose tissue imaging techniques across a range of clinically relevant tissues and organs.

Video Link

The video component of this article can be found at https://www.jove.com/video/57704/

Introduction

Interest in quantifying adipose tissue and triglyceride content using imaging modalities, such as magnetic resonance imaging (MRI), extends across many fields. Research areas include the investigation of white and brown adipose tissue depots and ectopic storage of lipid in organs and tissues such as the liver[1], pancreas[2], and skeletal muscle[3]. As these novel techniques for adipose quantification are developed, methods are needed to confirm that imaging parameters are valid for research and clinical applications.

Phantoms, experimental replicas of a tissue or organ, provide a low-cost, flexible, and controlled tool to develop and validate imaging techniques[4]. Specifically, phantoms can be constructed to consist of fat and water in a volume ratio or fat fraction (FF) comparable to that of the tissue of clinical interest. Clinically, FF values in tissues and organs can vary widely: FF in brown adipose tissue falls between 29.7% and 93.9%[5]; the average liver FF in steatosis patients is 18.1 ± 9.0%[6]; the pancreatic FF in adults at risk for type 2 diabetes ranges between 1.6% and 22.2%[7]; and in some cases of advance disease, patients with Duchenne muscular dystrophy can have FF values of almost 90% in some muscles[8].

Because non-polar molecules such as lipids do not dissolve well in solutions composed of polar molecules such as water, creating stable phantoms with a high target FF remains challenging. For FF up to 50%, many existing methods can be used to create fat water phantoms[9,10,11,12]. Other methods that achieve higher FFs typically require expensive equipment such as a homogenizer or an ultrasonic cell disruptor[13,14]. Although these techniques provide a roadmap for high FF phantoms, equipment constraints and varying amounts of experimental details limit the efforts to create reproducible and robust fat water phantoms.

Building upon these previous techniques, we developed a method to construct cost-effective and stable fat water phantoms across a customizable range of FF values. This protocol details the steps needed to make 5x 100 mL of fat phantoms with FF values of 0%, 25%, 50%, 75%, and 100% using a single hotplate. It can easily be adjusted to create various volumes (10 to 200 mL) and fat percentages (0 to 100%).

The efficacy of the phantom technique was evaluated in the feasibility study comparing fat-water MRI FF values to the target FF values in the constructed phantoms.

Protocol

1. Prepare the Workstation and Materials

1. Adhere to all laboratory safety rules. Wear eye protection and gloves. Read the material safety data sheet for each of the reagents used and take appropriate precautions. Review the materials and equipment list, chemical handling procedures, and glassware precautions.
   Caution: This protocol requires the use of a hotplate at high temperatures. Use caution and wear heat resistant gloves when interacting with hot containers and do not touch the surface of the hotplate.
2. Clear the workspace and clean the surfaces with disinfectant. Wash your hands and put gloves on.
3. Sterilize all instruments and the inside of all glass jars to reduce the potential risk of contamination and increase the longevity of the phantom.
   NOTE: If the phantom will be used for more than a couple of days, periodically clean the surface of the completed phantom with ethanol to prevent bacterial growth.

2. Prepare the Water Solution

1. Prepare the workspace for the water solution. Position the following materials and equipment on the bench: graduated cylinder, 400 mL beaker, stir bar, scale, 2x weigh boats, spatula, 2x 1.0 mL syringes with needle, distilled water, gadolinium-diethylenetriaminepentacetate (DTPA) contrast agent, water-soluble surfactant, agar, and sodium benzoate.
   NOTE: Syringes can be used with or without needles. However, using needles will improve the accuracy of the measurement and help prevent splatter when the contents are being added to the water or oil solutions.
2. Place a stir bar into a 400 mL beaker. Use a 100 or 200 mL graduated cylinder to measure 300 mL of distilled water and pour the water into the beaker. Place the beaker on the hotplate and set at 90 °C with a stir rate of 100 rpm.
   NOTE: High temperatures are used in this protocol to achieve quick results. Because the solutions are not left on the hotplate for long periods of time, the set-point temperature for the hotplate does not reflect the temperature of the solution.
3. Use a calibrated scale to measure 0.30 g of sodium benzoate into a weigh boat. Add sodium benzoate to the water solution.
4. Use a syringe to measure 0.6 mL of the water-soluble surfactant. Make sure that there are no air bubbles. Hold the needle a few millimeters over the center of the solution, and slowly release the water-soluble surfactant to avoid splatter on the walls of the beaker.
5. Using a clean syringe, measure 0.24 mL of the gadolinium-DTPA contrast agent. Add it to the beaker, using the same technique as in step 2.4.
   NOTE: Gadolinium-DTPA is used to adjust the phantom's MRI relaxation properties to match those of the tissue of interest. The reader can adjust the volume of added gadolinium-DTPA to better match the relaxation properties of the tissue of interest.
6. Measure 9.0 g of agar into a weigh boat. Slowly spoon the agar with a spatula into the beaker with water.
7. Once everything has been added to the water solution, increase the hotplate temperature to 350 °C and stir bar speed to 1100 rpm for 5-10 min to melt the agar.
   1. To check if the agar is melted, briefly remove the water solution from the hotplate, stop stirring, and check the color of the solution. Melted agar should be clear (no streamers or clumps) and yellow or amber in color.
8. Once the agar is fully melted, use a syringe or pour about 3.5 mL of the water solution into a small vial. If the test solution does not set or separates after 5-10 min, the agar is not melted. Increase the hotplate temperature back to 350 °C and continue heating the solution.
9. Repeat step 2.8 until water solution in the test vial sets properly.
10. Leave the water solution on the hotplate at 50 °C and 100 rpm. Clean the work space and prepare for the oil solution.
    1. Remove the following materials from the bench: scale, 2x weigh boats, spatula, 2x 1.0 mL syringes with needle (used), distilled water, gadolinium-DTPA contrast agent, water-soluble surfactant, agar, and sodium benzoate.
    2. Position the following materials and equipment on the bench: 400 mL beaker (clean), stir bar (clean), 2.0 mL syringe with needle, peanut oil, and oil-soluble surfactant.

3. Oil Solution

1. Place a new stir bar into a clean 400 mL beaker. Use a graduated cylinder to measure 300 mL of peanut oil and pour into the beaker. Remove the beaker containing the water solution and place the oil solution beaker on the hotplate. Set to 90 °C with a stir rate of 100 rpm for 1 min.
   NOTE: Peanut oil is used because it has a similar nuclear magnetic resonance spectrum compared to triglycerides in human adipose tissue[15].
   1. Do not leave the oil on the hotplate unattended. If the oil gets too hot and begins to smoke, remove it from the hotplate and reduce the temperature before returning the oil to the hotplate.
2. Measure 3.0 mL of the oil-soluble surfactant with a clean syringe. Using the same technique described in step 2.4, add the oil-soluble surfactant to the beaker. Set the hotplate to 150 °C and 1100 rpm for 5 min to fully mix the oil solution.
3. Take the oil solution off the hotplate and clean the workspace in preparation for creating the phantom.
   1. Remove the following materials from the bench: 2.0 mL syringe with needle (used), peanut oil, and oil-soluble surfactant.
   2. Position the following materials and equipment on the bench: 250 mL Erlenmeyer flask, stir bar (clean), volumetric pipettes, volumetric pipette holder, and 5x 120 mL glass jars.

4. Create Phantom Emulsion

1. Prepare volumetric pipettes for the water and oil solutions. Pipettes should only be used with their respective solution to prevent cross-contamination.
    1. Match the size of the pipette to the volume being used in the protocol. For example, use 2x 50 mL volumetric pipettes (50 mL water solution + 50 mL oil solution) to create a 100 mL phantom with a target FF of 50% fat.
2. Place the water solution on the hotplate and set the hotplate to 300 °C and 1100 rpm. After 4-5 min, turn the stirrer off.
3. Using a volumetric pipette, check if the water solution is ready for extraction by partially filling the pipette with a small amount (5-10 mL) of the solution and releasing back into the beaker. If the water solution can be easily removed and released without excessive remnants in the pipette, move on to next step, otherwise, leave it on the hotplate and check again in 2-3 min.
   NOTE: The components of the water solution are more susceptible to setting and separating, so it is best to keep the water solution stirring and/or warm as often as possible. If the water solution is not warmed and stirred before transferring, it will be very difficult to measure accurate volumes due to the tendency of agar to congeal when cooled.
4. Carefully add a clean stir bar to a 250 mL Erlenmeyer flask. Take the water solution off the hotplate, measure the proper volume (**Table 2**), and transfer it to the Erlenmeyer flask.
5. Place the oil solution on the hotplate and set at 90 °C and 1100 rpm to ensure the solution is homogeneous. After 1-2 min, remove the oil solution from the hotplate and replace it with the Erlenmeyer flask.
6. Measure proper amount of the oil solution (**Table 2**) and slowly add to the water solution in the Erlenmeyer flask.
7. Once all oil solution has been added, increase the temperature to 300 °C and maintain the stirring at 1100 rpm. Stir the combined solutions for 4-5 min (there should be vortex from the stir bar). The emulsion should be white, with a creamy texture.
8. Use a magnetic stir bar retriever to remove the stir bar.
   NOTE: The stir bar retriever should be used to remove the stir bars from all future emulsions. Clean it thoroughly between each use.
9. Use heat resistant gloves to carefully pour the mixture in the Erlenmeyer flask into a clean 120 mL glass jar. Slowly pour the mixture down the side of the glass jar to prevent bubbles in the mixture as it cools.
10. Clean the Erlenmeyer flask and stir bar, then repeat steps 4.2-4.8, adjusting the amounts of water and oil solutions, until all phantoms are created.
    NOTE: Make sure the glass is cool before cleaning.

Representative Results

If the water solution has been prepared correctly, a small amount of the solution should congeal quickly in a test vial (**Figure 1**, **left**). If the solution separates (**Figure 1**, **right**), the solution should be prepared again (as instructed in step 3.8 of the protocol). If the emulsion separates (examples in **Figure 2**, **left** and right), the phantom is not viable and should be discarded. When this occurs, it's usually because the emulsion did not reach a high enough temperature.

Successful phantoms will congeal to form a homogenous mixture, which can be imaged and measured via MRI. (**Figure 3**). A high concordance correlation coefficient (0.998; 95% confidence interval: 0.972-1.00) and the inclusion of the line of identity within the 95% confidence band of the regression line suggests the mean MRI-observed fat signal fraction (FSF) values measured in a region of interest in the images did not differ significantly from the known FF values in the fat-water phantoms (**Figure 4**).

Figure 1. Illustration of congealed (left) and separated (right) water solution test vials. A small test vial should be sampled to assess the viability of the water solution. If the water solution congeals (left), proceed with the next step in the phantom construction protocol. If the water solution separates (indicated by the two arrows on the right vial), the water solution needs to be re-prepared before it can be used for the formation of the phantom emulsion.

Figure 2. Example of unsuccessful phantom emulsions. Visually inspect the phantom approximately 10 min after pouring to determine if the emulsion will properly set. If the phantom begins to separate (**left**) or appears inhomogeneous (**right**), the phantoms need to be remade.

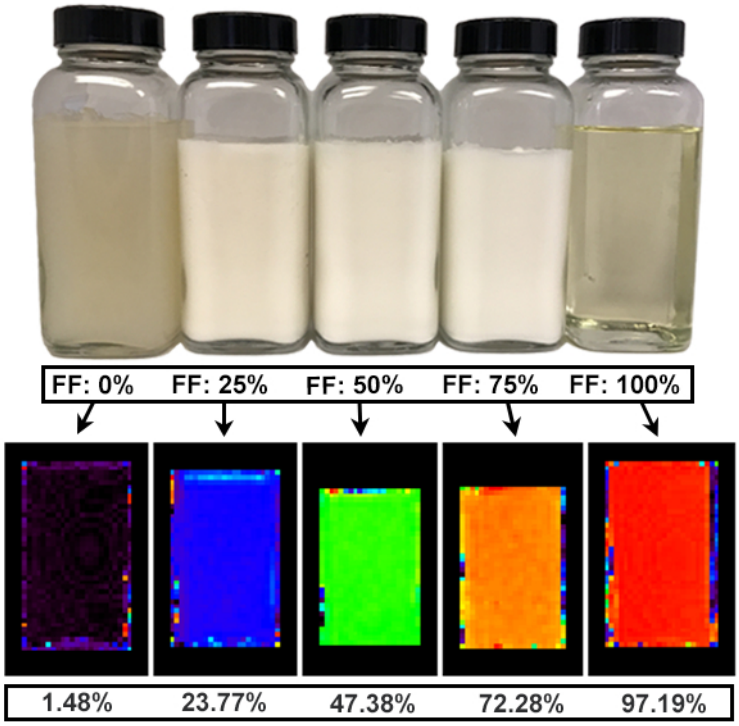

Figure 3. Schematic representation of a range of phantoms and their respective magnetic resonance imaging (MRI) results. Pictures show slight color differences in the constructed phantoms (0%, 25%, 50%, 75%, and 100%; top). Proton-density fat-signal-fraction (FSF) maps reveal a homogenous FSF measurement similar to the target fat content (middle). Distinct edge effects due to the imaging properties of the glass containers are apparent on the borders of each FSF map.

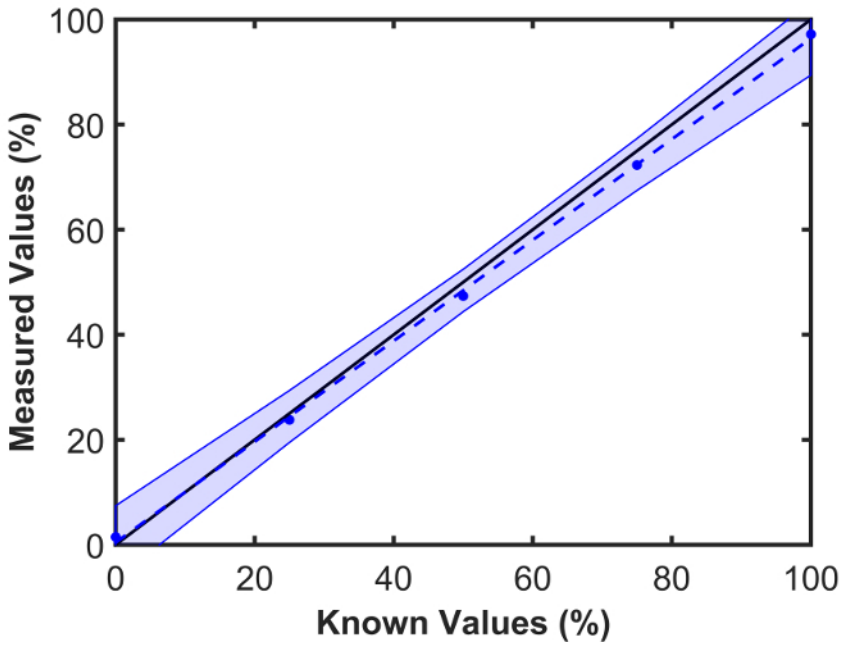

Figure 4. Scatterplot showing measured FSF values as a function of known FF values (blue points). The black solid line indicates identity. The blue dashed line indicates the line of best fit. The shaded area indicates the 95% confidence interval of the estimates. Please click here to view a larger version of this figure.

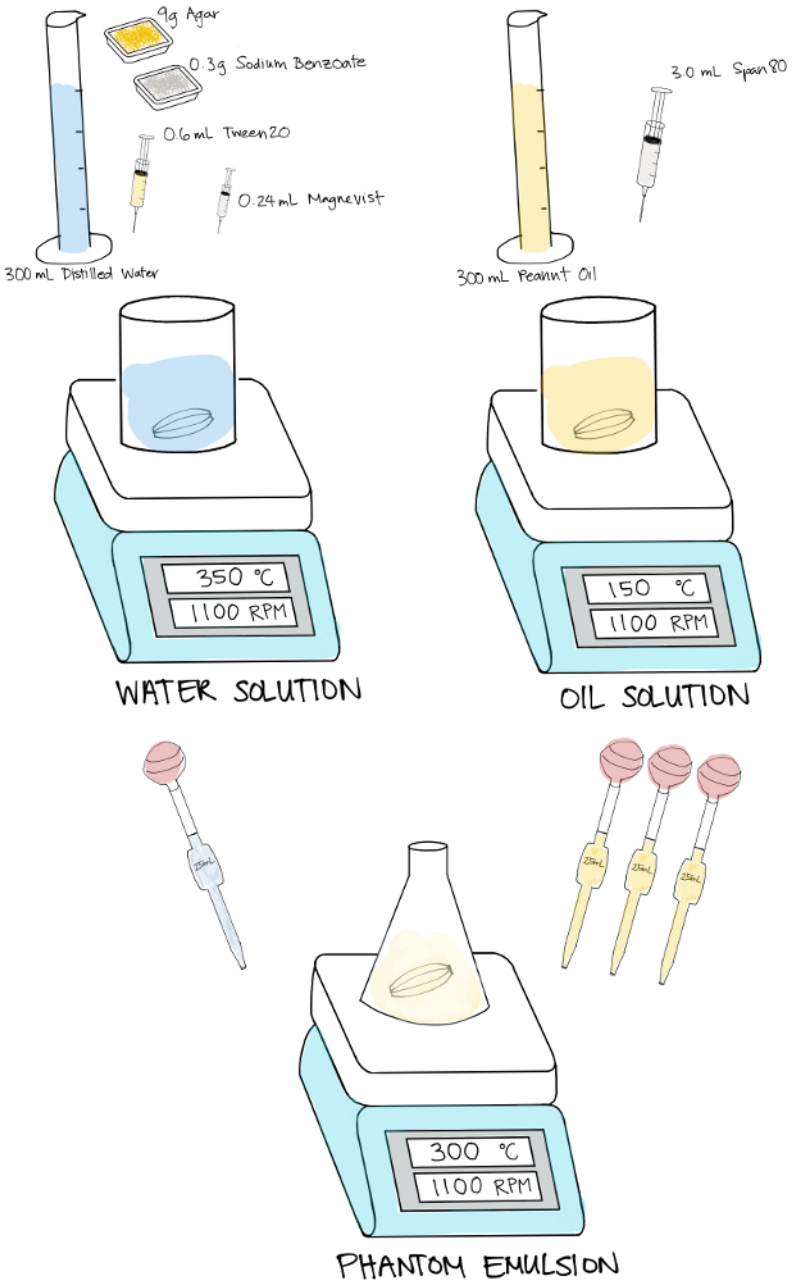

Figure 5. Sketch illustrating high level overview of protocol. The upper left of the diagram shows the ingredients, materials, and hotplate settings for preparing the water solution, and the upper right of the diagram shows the ingredients, materials, and hotplate settings for preparing the oil solution. The bottom shows the hotplate settings for combining the oil and water solutions to form the emulsion. Please click here to view a larger version of this figure.

Quantity	Equipment/Material
300 mL	Distilled Water
9.0 g	Agar
0.6 mL	Water-Soluble Surfactant
0.24 mL	Gadolinium-DTPA Contrast Agent
0.3 g	Sodium Benzoate
300 mL	Peanut Oil
2.0 mL	Oil-Soluble Surfacant
1*	Hotplate w/ Stirrer
3	Stir bars
2	400 mL Beaker
1	250 mL Erlenmeyer Flask
2	25 mL Volumetric Pipette
1	3.0 mL Syringe
2	1.0 mL Syringe
3	Syringe Needles
1	Spatula
1	Scale
2	Weigh Boats
5	120 mL Glass Jars
1	Heat Resistant Gloves (pair)
1	1-3 dram vial
2	50 mL volumetric pipette
2	75 mL volumetric pipette

Table 1. Quantity of materials and equipment required for 5x 100 mL phantoms (0%, 25%, 50%, 75%, and 100%).

Phantom Water/Oil Measurements		
Fat Percentage	**Water Solution**	**Oil Solution**
0%	100 mL	0 mL
25%	75 mL	25 mL
50%	50 mL	50 mL
75%	25 mL	75 mL
100%	0 mL	100 mL

Table 2. Measurements of oil and water solutions to create 5x 100 mL phantoms (0%, 25%, 50%, 75%, and 100%).

Discussion

We describe a robust method to create fat water phantoms suitable for validating the medical imaging techniques used to quantify adipose tissue and triglyceride content *in vivo*. By creating two reservoirs (one for the oil solution and one for the water solution), stable phantoms with a variety of FF values – including values exceeding 50% – were constructed without the need for expensive equipment. High FF phantoms (>50%) provide the utility to ensure imaging techniques for adipose quantification are valid for tissues or organs with high FF values, such as brown adipose tissue[5]. The MRI estimates of FSF were well correlated with the known FF values.

When only a single hotplate is available (as described in this protocol), the logistics of maintaining heat in each solution is a primary concern. Without heating or stirring, the water solution may cool and begin to congeal. To avoid this, place the water solution on the hotplate (<100 °C, ~100 rpm) whenever possible and always between mixing phantoms. Importantly, both the oil and water solutions should be well mixed when each solution is extracted to create the phantom. Always place the respective solution on the hotplate for at least 30 s (< 100 °C, ~ 100 rpm) prior to extracting the solution. In an ideal case, separate hotplates should be used for the water solution, the oil solution, and the phantom emulsion. Follow the same steps as described above to create each solution. Once fully mixed, set both hotplates to 50 °C and 100 rpm to prevent congealing and settling. Before extracting the solution from the beaker, turn the stirrer off and wait for the stir bar to completely stop moving.

While the precision and accuracy of the oil to water ratio in the emulsion is critical, the measurements of each component in the oil and water solutions allow for more flexibility. At its foundation, the MRI-observed FSF is a measure of "fat" versus "non-fat" signals in the total volume; therefore, "non-fat" can be any compound that contributes to the image signal intensity (water, agar, surfactant, *etc.*). We still advise measuring the water and oil solution components as accurately as possible, as those proportions were found to create the most stable and repeatable phantoms. Small deviations of the amount of the agar in the water solution (*e.g.*, 8.9 instead of 9.0 g), however, should not affect the overall FF of the emulsion if the oil to water solution ratio is maintained. The measurement of the volumes of the water and oil solutions above room temperature may also result in a small error due to the effects of thermal expansion on the volume of each component. Taking into account the volumetric temperature expansion coefficients of water and oil, as reflected in their densities[16,17], and the relatively small change in temperature, we estimate the error of the overall FF due to thermal expansion to be less than 0.5%. We also note the possibility that the relaxivity of gadolinium-DTPA for water and lipids may differ. If so, and depending on pulse sequence parameters, the quantitative accuracy of the MRI FSF measurements could be diminished. The MRI-observed FSF may also vary with the spectral model used to analyze the data.

Although the method described here has only been used to make phantoms between 10 mL and 200 mL, the technique can be used to produce smaller or larger volume phantoms. Notably, it is difficult to extract volumes of <10 mL from the reservoirs due to the viscosity of the solutions. Small volume phantoms, therefore, require excess emulsion from which to draw the desired volume to maintain the FF accuracy of the final phantom. For example, a 10 mL phantom with a 10% target FF necessitates a 10 mL extraction from a 100 mL emulsion. When creating large phantoms (>100 mL), the size of both the stir bar and glassware must be scaled up together (and the ratio of solution to glassware capacity) to create a vortex in the solution when the stirrer is set to > 500 rpm. The emulsion likely will not achieve homogeneity without a vortex.

Given the complexity of creating high FF phantoms, small deviations from the protocol may have a profound effect on the stability and quality of the final phantom. Environmental conditions, such as room temperature, altitude, and humidity, may alter the phantom preparing process in an inconsistent manner and adversely affect the final product. Intermediate checks of the water solution provide opportunities to detect and mitigate these possible effects. However, it is possible that even with rigorous attention to the protocol details, the final phantom may separate, and the process will need to be repeated.

Disclosures

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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