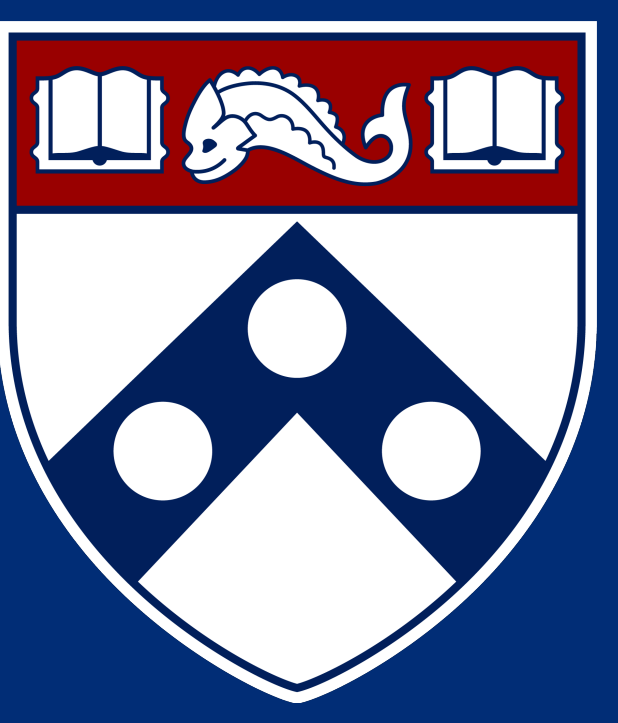


Formulating Thermosensitive Radiopaque Hydrogel for Hepatocellular Carcinoma Treatment



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Background

- Hepatocellular Carcinoma (HCC) is one of the most common cancers worldwide
- For patients whose tumor is unresectable, locoregional therapies (e.g., trans-arterial chemoembolization) are the standard of care, but the average survival rate is ~27% at 5 years
- An injectable, thermosensitive hydrogel may provide a more effective treatment by causing ischemia once it gels inside the body, thereby completely blocking blood flow to the tumor, encapsulating a drug and providing its sustained release into the tumor site to decrease tumor size, and providing X-ray contrast for direct monitoring of the hydrogel over time through gold nanoparticle (AuNP) CT contrast agents loaded in the hydrogel
- From prior work it is known that chitosan-based hydrogels are biocompatible and capable of degradation via human enzymes and that a chitosan polymer and Ammonium Hydrogen Phosphate (AHP) crosslinker mixture is liquid at room temperature and gels at the body's internal temperature of 37 °C

Therefore, in the present study, we aimed to answer the following questions:

- What ratio of chitosan to AHP gels at the ideal rate? A gelation time of 60 to 120 seconds is preferred for the in-vivo model, as a faster rate will cause the hydrogel to gel inside the catheter being used to inject it, while a slower rate will allow the hydrogel to travel inside the bloodstream
- What drug will the hydrogel be loaded with to have higher treatment efficacy?
- How will characterization of the hydrogel change as it is loaded with AuNP and the drug?

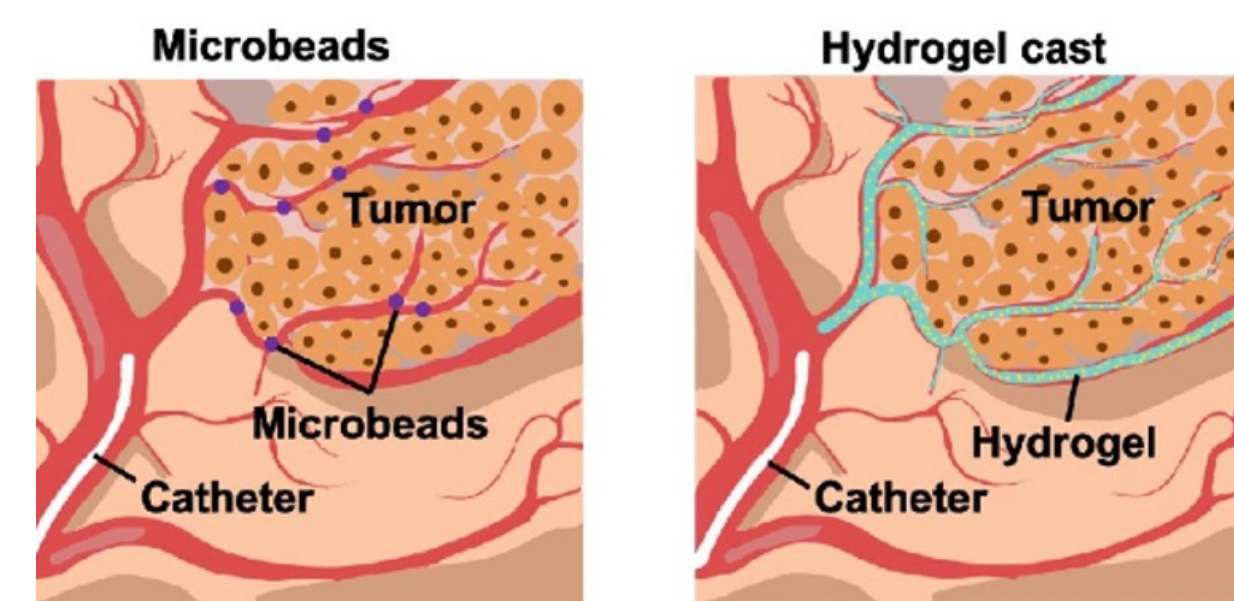


Figure 1: The image to the right shows the treatment of HCC tumors with microbeads compared to treatment of the tumor with the hydrogel.

- Incomplete reduction in blood flow
- Poor drug delivery
- Non-radiopaque
- Greater reduction in blood flow
- Better drug delivery
- Radiopaque

Methodology

- AuNP was mass produced by scaling up the lab formulation of AuNP, and the viability of the scaled AuNP was tested using UV-Vis spectroscopy
- The concentration of AuNP was found using inductively coupled plasma optical emission spectroscopy
- A search was done to determine what drug to load the hydrogel with; as HCC tumors require lactate dehydrogenase (LDH) for growth, an LDH inhibitor would be the ideal drug
- A stock solution of the drug of concentration 1 mg/mL was made
- Several trials of gelation tests in 37 °C water bath were run to characterize the gelation time of various formulations of 1 mL hydrogel, which is the amount of hydrogel that will be tested in-vivo in the healthy rat model

The formulations of hydrogels tested were as follows:

- Just AHP + chitosan – this was used to determine the ratio of AHP to Chitosan that would be used in further testing
- AHP + chitosan + AuNP – this formulation will be tested in-vivo, so the goal was for it to gel within 60 to 120 seconds
- AHP + chitosan + AuNP + drug
- To determine the AuNP and drug volumes needed in the hydrogel, their concentrations were used
- A literature search found that 8 mg AuNP and 10 uM NHI 2 (the drug chosen) are used in similar models (for contrast and treatment, respectively)
- AHP and chitosan volume was adjusted accordingly based on the volume of AuNP and the drug needed and the determined ratios from the chitosan + AHP trials

Results

- The UV-Vis spectroscopy testing of the scaled up AuNP formulation found that AuNP can be mass produced in scaled up batches and still yield viable particles
- NHI 2 was found to be an LDH inhibitor that reduced lactate production in HeLa cells, so it was the drug chosen
- As more AHP crosslinker was added to the chitosan polymer, the time the hydrogel took to gel in the in 37 °C water bath decreased, as can be seen in Figure 2
- Adding AuNP and NHI 2 did not significantly change the gelation time of the hydrogel; Table 2 shows that the addition of NHI 2 did not change the gelation time of the hydrogel when it had just chitosan, AHP, and AuNP (as in Table 1)
 - Therefore, gelation time can be increased or decreased depending on only the ratio of AHP to chitosan, with more AHP leading to a faster gelation time
- The formulation in red in Table 1 is the formulation that will be tested in-vivo in the healthy rat model as it was the only formulation that gelled between the desired 60 to 120 seconds
 - The weight ratio of chitosan to AHP in the selected formulation is 0.125

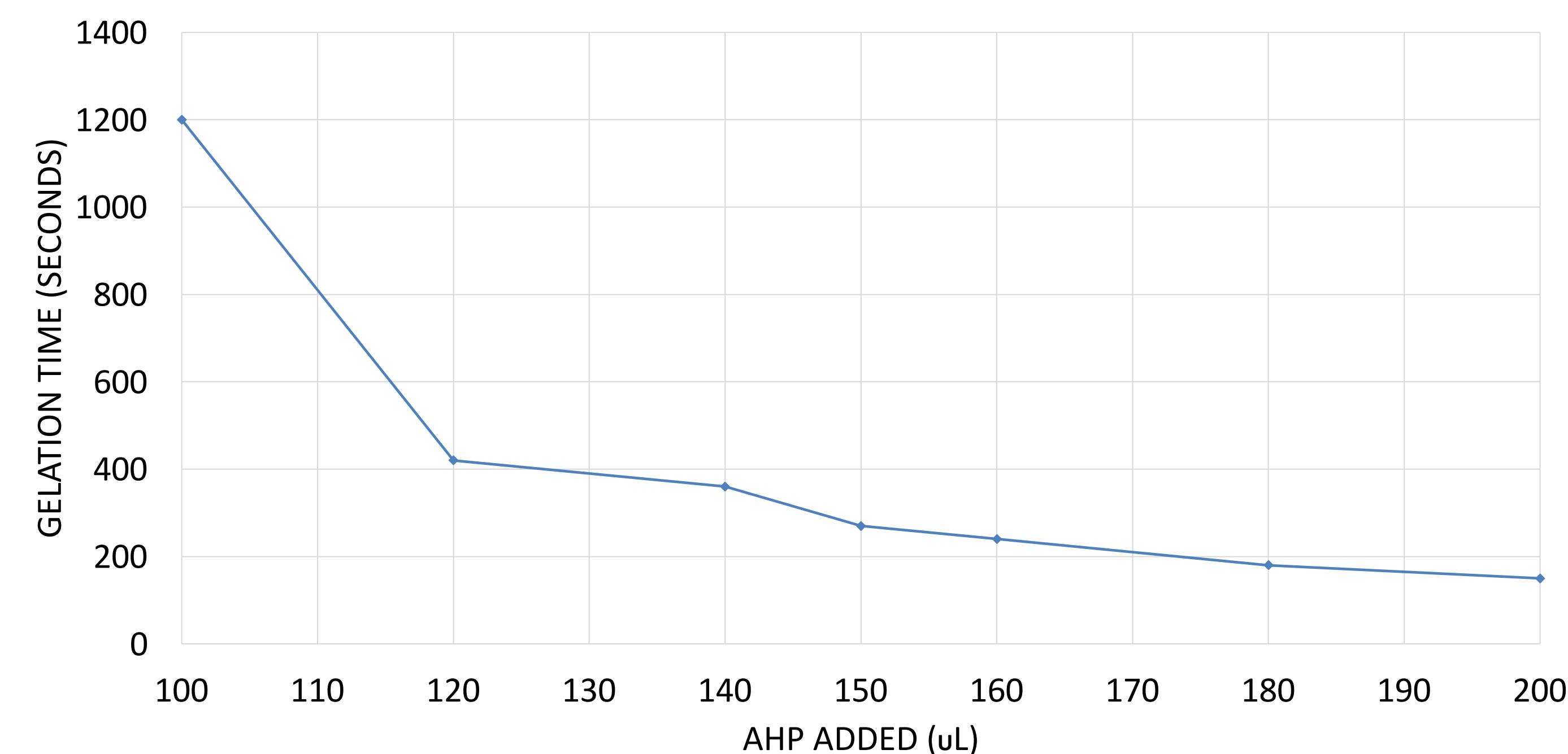


Figure 2: Trial 1 showing the gelation time of the hydrogel decreases with the addition of increased amounts of AHP to 1 mL of the chitosan polymer.

Table 1: Gelation times of different formulations of 1 mL hydrogel with chitosan, AHP, and AuNP

Chitosan (uL)	AHP (uL)	AuNP (uL) (68.014 mg/mL)	Average Gelation Time (seconds)
769	107.6	120	150 ± 0
759	121.4	120	103 ± 12
746	134.2	120	50 ± 0
733	146.6	120	40 ± 10

Table 2: Gelation time of a formulation of 1 mL hydrogel with chitosan, AHP, AuNP, and NHI 2

Chitosan (uL)	AHP (uL)	AuNP (uL) (68.014 mg/mL)	NHI 2 (uL) (1 mg/mL)	Average Gelation Time (seconds)
756	121	120	3.35	103 ± 12

Figures

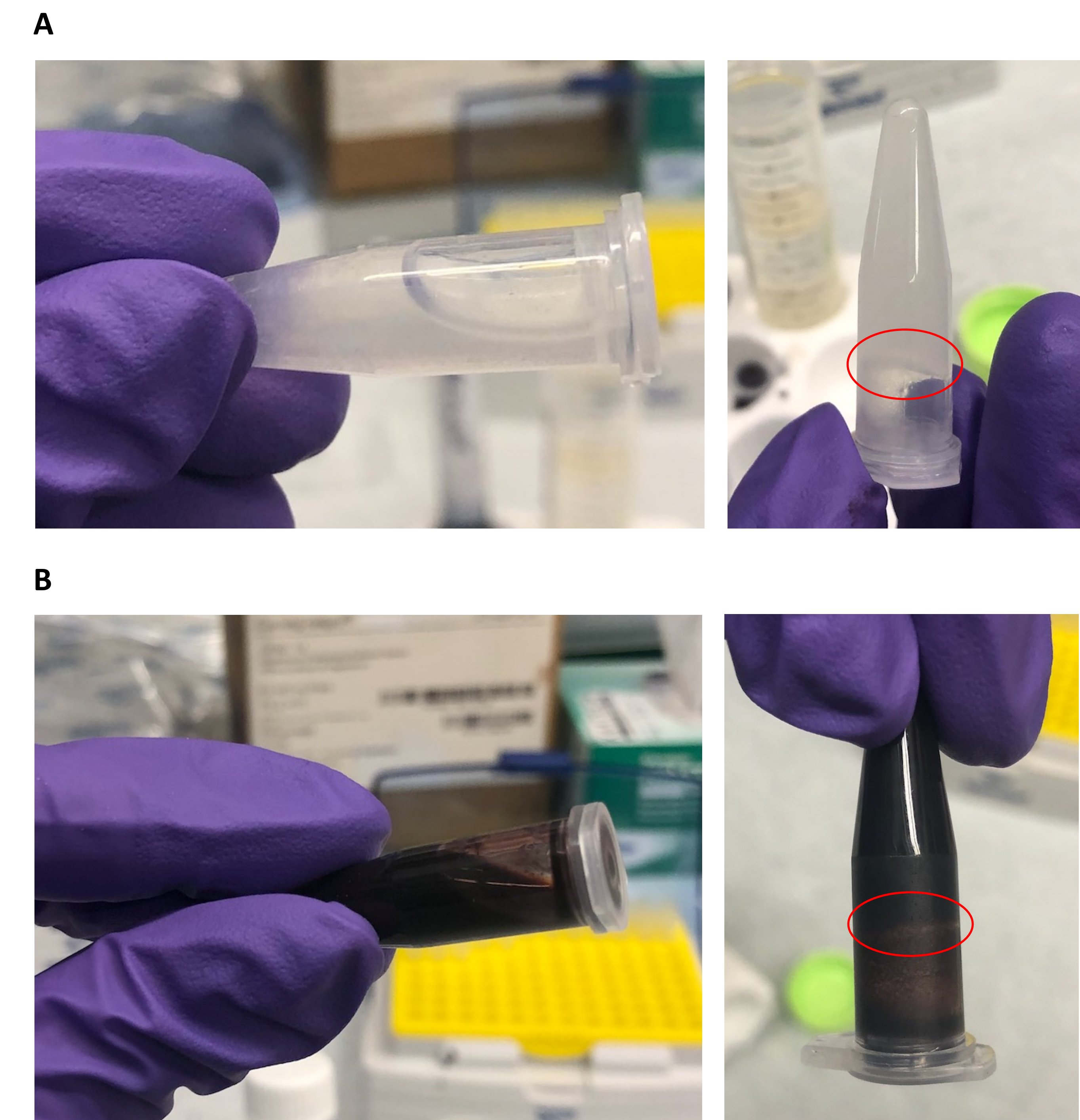


Figure 3: A) The chitosan and AHP hydrogel on the left is liquid before it is put into the 37 °C water bath and solidifies after being taken out of the water bath (as shown on the right). The red circle distinguishes where the hydrogel begins. B) The chitosan, AHP, and AuNP hydrogel exhibits the same behavior as the chitosan and AHP hydrogel: it is liquid outside of the water bath and solid once put in the water bath and allowed to gel. The addition of AuNP gives the hydrogel a dark purple color.

Future Work

- Further testing will be done to confirm there is no significant effect on gelation time when AuNP and NHI 2 are added to the hydrogel
- A cell viability assay on the HUH7 cell line, a liver cell line, will be done to test the cytotoxicity of NHI 2 to those cells
- In-vivo testing in the healthy rat model with the AHP + chitosan + AuNP hydrogel formulation that was developed will be done in order to:
 - See the hydrogel's contrast capabilities
 - Confirm the gelation time holds up inside the vasculature
- Different in-vivo groups with different formulations of the hydrogel will be tested to see if a longer or shorter gelation time is needed in-vivo
- Follow up testing in a diseased rat model will be conducted
 - Those hydrogels will have the AHP + chitosan + AuNP + NHI 2 hydrogel formulation that was developed
 - Release testing of the drug will also be done at this time