Alginate Hydrogel Mixing:

2% Alginate Mixture

1.) Measure 2 grams of alginate powder in a balance plate using an analytical balance
2.) Pour powder into 100 mL tube
3.) Pour double distilled water into 100 mL tube until 100mL line is reached
4.) Place on rotating mixer for 48 hours

3% Alginate Mixture

5.) Measure 3 grams of alginate powder in a balance plate using an analytical balance
6.) Pour powder into 100 mL tube
7.) Pour double distilled water into 100 mL tube until 100mL line is reached
8.) Place on rotating mixer for 48 hours

5% Alginate Mixture

9.) Measure 5 grams of alginate powder in a balance plate using an analytical balance
10.) Pour powder into 100 mL tube
11.) Pour double distilled water into 100 mL tube until 100mL line is reached
12.) Place on rotating mixer for 48 hours

Nanoparticle Additions:

1.) Remove tubes from rotating mixer
2.) Divide the contents of each tube into three other 100mL tubes, leaving 25mL of the alginate solution in each (will end up with 12 100mL tubes containing 25mL of its respective solution
3.) With each tube, add the respective amount of gelatin or silica depending on the alginate concentration, multiplying the concentration by the nanoparticle amount divided by four (ex: for the tube of 2% alginate with 0.5 grams of gelatin, the gelatin amount would be 2 x (0.5/4) = 0.25 grams of gelatin added)
4.) Once added to the solution, each tube was placed back on the rotating mixer for 24 hours
5.) Repeat for remaining 11 tubes

Freeze-drying:

1.) Place samples in freezer for 24 hours to pre-freeze(makes the freeze-drying process faster)
2.) Take samples to the WPI lab where they are placed in the lyophilizer for 3 days
3.) Remove samples from lyophilizer

Compression testing:

1.) Place samples on metal plate with 200 Newton load cell
2.) Set universal testing machine to compress at 10mm/min
3.) Press "start" and watch as samples are compressed and machine stores data in Excel file
4.) Repeat for each sample

Planaria Testing:

1.) Fill each well plate with 2mL of spring water using a transferpette
2.) Wet filter paper with PBS solution and place in large petri dish
3.) Place a flatworm on a piece of filter paper, one at a time
4.) Dip scalpel blade in ethanol
5.) Use a scalpel to make incision on anterior and posterior end, using a dissecting microscope
6.) Move planaria back to well plate
7.) Using a pipette, add the alginate solution to the well plate
8.) Cover well plates with lid
9.) Wipe off scalpel each time to remove excess mucus
10.) Repeat for a total of 12 times
11.) Leave planarian in solution for 30 minutes
12.) Remove planaria with pipette and transfer to additional well plate with 2mL of spring water
13.) Observe growth of worms over a 5 day period
14.) Use dissecting microscope imaging to take photographs of the flatworm every other day to measure incision

15.) Use Fiji 2 Software to measure the area of each incision