Standard Operating Procedure (SOP): Making Nanoparticles

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Description of event:	Students create polymer droplets from sodium alginate and food coloring to demonstrate the production of nanoparticle-based cancer medication. The size of the alginate particles allows for the participants to visualize this production method, which normally occurs on the nanoscale.
Purpose:	To demonstrate the concept of nanoparticle synthesis to students, exposing them to the scientific fields of nanomedicine and cancer.
Length of the event:	30 - 60 minutes plus setup/cleanup.
Participants:	K-12.
Items needed:	Speakers for music, monitor/screen to show PowerPoint slides, paper towels, tables, laboratory coats, gloves, 5 mL macro centrifuge tubes, transfer pipettes, jello shot cups, food coloring, sodium alginate/calcium chloride kit (available for purchase).

Scientific background

In this activity, students will make polymeric nanoparticles with cancer medication. A polymer is a long-chain like molecule made up of many repeating units linked together. Sodium alginate (a sugar with short polymers) represents the nanoparticle material. Sodium alginate is mixed with food coloring that represents the cancer medication. When droplets of colored sodium alginate liquid make contact with the calcium chloride solution, the calcium molecules in the salt water link the short polymers together to form larger polymers. This leads to the formation of polymer droplets with a solid outside shell around a liquid interior containing cancer medication. The polymer droplets are similar to nanoparticles that are designed to bring cancer medication to tumors.

Scientific reference:

- Effective method of chitosan-coated alginate nanoparticles for target drug delivery applications. *J Biomater Appl*. 2016 Jul;31(1):3-12.

Detailed description of the event with practical notes

Set-up (1-2 h, depending on group size, the day before the activity).

1. Open the purchased package with sodium alginate (labeled as string slime liquid)/calcium chloride (labeled as activator powder).
    - Note: Refer to the table below for ordering information.
2. Take the calcium chloride bag (labeled as activator powder; 20 g total) and mix with tap water (3 g powder/240 mL water) in a container. ~ 25 mL of calcium chloride solution is required for each student.
3. Pour the calcium chloride solution into jello shot cups (one cup/student). Each jello shot cup should be half full (~ 25 mL). Seal the lid for transportation (make sure the lid is properly closed to avoid leakage).
4. Unscrew cap and remove the seal from the sodium alginate bottle (labeled as string slime liquid). Place cap back onto bottle.
5. Squeeze the bottle to dispense ~ 4 mL of sodium alginate (labeled as string slime liquid) into each 5 mL macro centrifuge tube (one tube/student).
6. Store materials for later use.

Introduction (10 min): The organizers should explain what a nanoparticle is and how it can be used to carry cancer medication to tumors. Background material will be provided for the organizers in the notes section of a PowerPoint presentation that can be shown to the students. The idea is to explain what a nanoparticle is (in terms of size compared to other objects) and the advantages of using nanomedicine to treat cancer.

First activity: Game to illustrate the formation of nanoparticles containing cancer medication (15 min)

1. Select a few students (3 - 5) to represent cancer medication.
2. The remaining students will represent small polymers. Play music for 10-20 seconds; during this time the students should freely move around the room.
3. As soon as you stop the music, the small polymer students should hold hands to attempt to encircle the cancer medication students. This demonstrates small polymers linking together to form a larger polymeric nanoparticle that encapsulates cancer medication.

- Note: This should be repeated several times by having students take turns to be the cancer medication.
- Note: This activity can be skipped for older students.

Second activity: Experiment to make nanoparticles with cancer medication (15 - 20 min):

1. Set up a space for each student to do the experiment. This should be on a table and students can sit or stand next to each other.
2. For each student, lay out the necessary items: One pair of gloves, one lab coat, one transfer pipette, one 5 mL macro centrifuge tube with sodium alginate, one opened jello shot cup with calcium chloride solution (leave the lid next to the cup), and paper towels.
3. Instruct the students to put on gloves and lab coats.
4. Distribute the cancer medication (food coloring) among the students (some will need to share).
5. Instruct the students to drop one drop of cancer medication (food coloring) into their 5 mL macro centrifuge tube with sodium alginate (small polymers).
6. Instruct the students to use the transfer pipette to mix the sodium alginate (small polymers) with the cancer medication (food coloring).
    - Note: Tell the students that the sodium alginate solution is very thick; pipette the solution slowly to properly mix with food coloring.
7. Instruct the students to pipette small drops of the mixture into the calcium chloride solution to create larger polymers that form nanoparticles with cancer medication inside.
    - Note: Tell the students not to touch the pipette tip in the calcium chloride solution. This will cause the alginate to solidify in the transfer pipette, creating a clog.
8. Give the students compliments on the nanoparticles they have made.
    - Note: At this point, the droplets are solidified into spheres due to the reaction between sodium alginate and calcium chloride. Sometimes other shapes are formed depending on how the solution is dropped (these shapes should also be encouraged, as nanoparticles come in various shapes).
9. Instruct the students to touch and examine the polymeric nanoparticles.
    - Note: Tell the students to observe that the food coloring does not leave the droplets (some food coloring might spread into the calcium chloride solution, but most of it will be contained).
    - Note: This represents a nanoparticle (alginate droplets) carrying the cancer medication (food coloring).
10. Instruct the students to squeeze the droplets and observe what happens.

- Note: This is the messy step. Have paper towels ready. The solid droplets will burst open and cause the food coloring to spill out. This represents the release of cancer medication from the nanoparticle upon reaching the tumor. Explain how this is advantageous ("The tumor targeting properties of nanoparticles allow for the release of cancer medication to the site of the tumor. This helps to prevent the side effects of cancer medication reacting with various cells/tissues in the body when administered intravenously").

11. Instruct the students to carefully seal the lead of the jello shot cup. Tell the students that they can keep the nanoparticles.
    - Note: Tell the students that the nanoparticles and calcium chloride solution are none edible and should never be consumed (all materials are non-toxic but not suitable for consumption).

Step-by-step preparation for the event

Step	Preparation and check-list	Time needed
1.	Obtain items needed for the experiment: - **Speaker for music** - **Monitor/screen for PowerPoint presentation** - **Paper towels** - **Tables** - **Laboratory coats (one/student).** - **Gloves (one pair/student)** obtain from a laboratory or purchase on Amazon (100/box) (https://www.amazon.com/AMMEX-Nitrile-Gloves-Disposable-Powder/dp/B007N8WQDG) - **5 mL macro centrifuge tubes (one/student)** obtain from a laboratory or purchase on Amazon (100/pack) (https://www.amazon.com/Celltreat-229449-Macro-Centrifuge-Sterile/dp/B014AFYOAW). - **Transfer pipettes (one/student)** obtain from a laboratory or purchase on Amazon (150/case) (https://www.amazon.com/Teenitor-Plastic-Transfer-Pipettes-Dropper/dp/B01G82SJRY) - **Jello shot cups (one/student)** purchase on Amazon (125/package) (https://www.amazon.com/Polar-Ice-PI125200CT-Plastic-2-Ounce/dp/B00OFX5DSM) - **Food coloring (one package/50 students)** purchase from the grocery store or Amazon (https://www.amazon.com/Purple-4-pack-Assorted-Yellow-MCCORMICK/dp/B00TOYK2U6) - **Sodium alginate/calcium chloride kit (one/50 students)** purchase from Steve Spangler (select the **clear color**; sodium alginate is labeled as string slime liquid and calcium chloride is labeled as activator powder) (https://www.stevespanglerscience.com/store/spanglers-string-slime.html)	At least 1 month prior to the event (depends on how long it takes for products to ship)
2.	Try the experiment yourself. It is recommended that the organizers try the experiment at least once to get a better understanding of the process and to address any potential problems that may occur.	2-3 weeks prior to the activity
3.	Set up the materials for students to use	1 week - 1 day prior to the activity