Teacher's Preparatory Guide

Gelatin Microfluidics

Overview: Students will create a narrow fluidic channel in gelatin, inject dye into the channel, and observe the fluid flow.

Purpose: This lab shows the importance of channel design in biotechnology and nanotechnology and the methods used to study the behavior of fluids as they flow through a channel.

Time Required: One or two 55-minute student lab period(s). See note in the *Instructional Procedure* section.

Level: High school chemistry or biology

Big Ideas in Nanoscale Science and Engineering:

- Forces and Interactions
- Models and Simulations
- Science and Technology
- Structure of Matter

Teacher Background: Understanding how fluids flow through tiny channels can simplify a doctor's job. This understanding allows scientists to create devices that can sort, identify, mix, and analyze blood, cells, DNA, or other body fluids in a quick and portable way. Essentially, we can shrink an entire laboratory down to the size of a computer chip. A lab on a chip basically works the same as a large laboratory; however, these devices allow us to use tiny amounts of fluids. For example, a tiny amount of blood can be thoroughly analyzed in a chip-sized device instead of using a large amount of blood in a regular laboratory. In order to do this, understanding how fluid flows through channels is essential[1].

A lab-on–a-chip is a micro/nano sized device that can run several biochemical analyses (tests) at one time using very small samples . They are also called Micro Total Analysis Systems - µTAS. In addition to medical applications, they can be used to detect toxins in the environment such as in water or the air. The most common area is in medical diagnostics and the most familiar ones are home pregnancy tests, drug tests, glucose monitoring, and strept tests. These devices are becoming very important as we seek ways for early disease detection and hazardous materials detection (important for Homeland Security). This interest has created a large demand for the development of easy-to-handle and inexpensive lab-on-a-chip devices that can work quickly and reliably. So why is nanotechnology important to this? It has the ability to make small devices (microfluidic channels in the micro and nanoscale dimensions) on chips capable of analyzing very small quantities of analyte.

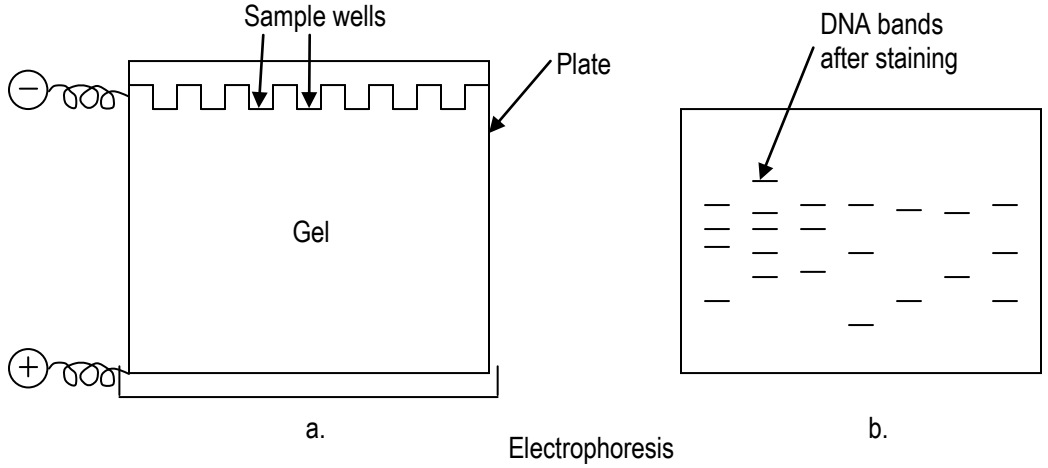
*Development and distribution partially funded by the **National Science Foundation***

NNIN Document: NNIN-1253
Rev: 06/2012

A diabetic wristwatch is a good example of a lab-on-a-chip application. These watches work by pulling a very small amount of glucose from underneath the skin using a tiny amount of current. The amounts are so small that the current actually draws blood without the need to pierce the skin. These samples are then stored in a pair of circular gel pads. Electrodes then sense and measure glucose levels, and the data are analyzed by a tiny computer chip. The user is then alerted of any changes from a previously calibrated level. The data are also stored for future analysis by the individual's doctor[2].

In order to make a lab-on-a-chip for other applications, researchers are investigating how small volumes of fluids behave. *Microfluidics* is a field that studies the behavior of fluids in volumes that are 1,000 times smaller than a falling raindrop[3] (having volumes in micrometers). A *micrometer* is one millionth of a meter. The *microscale* ranges from 1–100 µm. *Nanofluidics* is a field that studies the behavior of fluids in volumes that are 1 million times smaller than a drop of water (having volumes in nanometers). A *nanometer* (abbreviated nm) is one billionth of a meter. The *nanoscale* ranges from 1–100 nm[4].

Using microfluidics and nanofluidics, engineers also craft systems—called micro-electro-mechanical-systems (MEMS) or nano-electro-mechanical-systems (NEMS)—that can sequence DNA and separate or identify proteins[5]. One way to do this is through a process called *electrophoresis.* Electrophoresis takes advantage of the fact that molecules are negatively or positively charged and are of different sizes. Researchers use an electrically charged gel to sort molecules by size. One end of the gel is positively charged and the other is negatively charged. The molecules are charged as they flow through the channel. For example, DNA is negatively charged due to its phosphate end. When a charge is applied along the channel, DNA will move toward the positively charged end of the gel. Larger molecules will move through the gel slower than smaller ones as they encounter more obstruction. Researchers can identify unknown molecules by comparing the distribution path that a protein or DNA leaves in the gel with the distribution path of a known sample. Single strands of DNA are not visible (2.5nm wideand 1.5-3mlong when straightened), but similar strands clump together and appear as bands on the gel. A dye is then added to make the bands of DNA visible[6]. See the following figure.



a. Different samples are placed at the top of the wells. An electrical charge is then applied and the samples move down the gel according to their size.

b. A dye is then added making clumps of DNA visible on the gel. Similar DNA or proteins will clump and appear as bands on the gel[6].

Sources

1. Booker R., Boysen E., *Nanotechnology for Dummies*. Wiley Publishing, Inc., 2005.
2. U.S. Dept. of Health & Human Services. "GlucoWatch® Automatic Glucose Biographer." (accessed July, 2010) http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cftopic/pma/pma.cfm?num=p990026
3. U.S. Geological Service. "Why raindrops are different sizes." (accessed July, 2010) http://ga.water.usgs.gov/edu/raindropsizes.html
4. Rogers B., Pennathur S., Adams J., *Nanotechnology: Understanding Small Systems*. CRC Press, 2008.
5. Senturia, S.D., *Microsystem Design*. Springer Science, 2001.
6. Donlan DNA Learning Center. "Gel Electrophoresis." (accessed July, 2010) http://www.dnalc.org/resources/animations/gelelectrophoresis.html

Materials per class

- 4 envelopes (7.2 g) Knox unflavored gelatin
- 250 ml water (fills about 40 squares to desired amount)
- hot plate
- small pot to heat and dissolve gelatin
- wood stirring sticks (tongue depressors)
- pair of oven mitts
- yellow food coloring
- blue food coloring
- hammer, soldering iron (http://www.itcelectronics.com, part number 12-055), *optional*
- Helping Hands with magnifier (http://www.amazon.com), *optional*
- clock (a classroom clock will do)

Materials per student group

- 4 large metal paper clips
- wire cutters
- needle nose pliers
- a square mold cut from a 24-square cavity silicone mold (e.g., gelatin mold, or bite-size brownie square baking mold, Wilton brand, part 2105-4923 found here: http://www.wilton.com/store/site/product.cfm?id=AF414343-1E0B-C910-EAF4100780D53DC6
- laser transparency sheets cut to fit the bottom of the silicone mold squares (*NOT inkjet transparency; it contains a film that forms on the gelatin*)
- gelatin (unflavored)
- masking tape
- 2 microscope glass slides
- 6–8 pipette tips (e.g., part #21-277-2A from http://www.fishersci.com), depending on student's design (3–4 to *create* inlet/outlet points; 3–4 new, *unclogged* ones for directing flow of dye)
- 2 pipettes, micro-graduated disposable (e.g., part #13-711-9AM from http://www.fishersci.com), one for each color dye
- small cup with water and a few drops of yellow food coloring
- small cup with water and a few drops of blue food coloring
- microscope

Development and distribution partially funded by the ***National Science Foundation***

NNIN Document: NNIN-1253

Rev: 06/2012

- pencil/pen

Advance Preparation: Purchase gelatin and food coloring at a grocery store. We purchased the silicone baking squares from Michaels, which is an arts and crafts store. Wooden tongue depressors can also be purchased at an arts and crafts store.

Cut the silicone mold to separate each square so each group gets their own square. Cut pieces of laser transparency sheets into 3.4 × 3.4 cm squares. Patterns may be made ahead of time to facilitate and expedite the lab. Designs must be flat (hammer to flatten) and they must fit inside the baking square. Prepare the gelatin while students make their designs. Refrigerate the gelatin in the mold if it is warm outside or if you will do the lab on more than one day.

Safety Information: Hot plates and soldering irons can burn skin. Use oven mitts when handling the hot plate and use care when using the soldering iron.

Instructional Procedure:

Time	Activity
5 min	Read the introduction section of the *Student Worksheet.* Watch "Do the fluids mix?" video demonstration (it's the 9th video down) at http://mrsec.wisc.edu/Edetc/nanolab/PDMS_fluidics/index.html Ask students: Do the two dyes mix? *No.* Why? *Laminar flow causes fluids to flow parallel to each other when there is no turbulence present.* What would you have to do to get them to mix? *One would have to introduce a pattern that causes turbulence in the flow.*
15 min	Students design and create their channels (steps 1–7 of the *Student Worksheet*). Meanwhile, make the gelatin mixture. *Note: If students need more time with this (if they choose to solder, etc), you can divide this lab into 2 days by stopping the lab at step 7, after putting the gelatin in the refrigerator.*
15 min	While gelatin is in the refrigerator, students define terms (in the *Guided Dialog* section). Explain why channels are important using videos and/or the PowerPoint in the *Resources* section.
15 min	Students complete and test their channels and finish the *Student Worksheet*.
5 min	Clean up.
Homework	Students answer conclusion questions. Students also write a one-page description of DNA electrophoresis, DNA finger printing, or protein identification.

Teaching Strategies: This lab is best done individually or in groups of two students. One 55-minute period is recommended.

Guided Dialog: *Before* beginning the lab, review the meaning of these terms:

Channel *Any canal that can move gases or fluids from one place to another.*
Micrometer *One millionth of a meter.*
Nanometer *One billionth of a meter.*
Microscale *The microscale ranges from 1–100 µm.*
Nanoscale *The nanoscale ranges from 1–100 nm.*
Microfluidics *The study of the behavior of fluids at the microscale.*

Nanofluidics *The study of the behavior of fluids at the nanoscale.*
Lab-on-a-chip *A tiny device used to analyze body fluids, such as a sample of blood or DNA.*
Electrophoresis *The use of an electrically charged gel to separate DNA and proteins.*
Biotechnology *The use of living things in engineering, medicine, and technology.*

Procedure:

1. **Place transparency square in square mold.**
   Place a transparency square in the bottom of a square mold.

2. ***Optional:* Create pieces of channel patterns with paper clips.**
   If you do this ahead of time, make an array of V, I, snaking, and zig-zag shapes for students to connect together to make their design. Or, allow *plenty* of time for students to do this themselves. Twist a paper clip piece into the shape of your channel with pliers. Students can join pieces either by placing them very close together in the mold (be sure there are no gaps between the metal pieces, or the experiment will fail) or by soldering 2–3 metal pieces together (a "third hand-Helping Hand" makes this easy and is ~ $5). Use wire cutters to cut the design to fit the bottom of the silicone square. Be sure that the pattern is very flat. If not, pound it flat with a hammer. Place the pattern in the mold atop the transparency sheet.

3. **Prepare the gelatin.**
   Place the pan on the hot plate. Pour 250 ml of water into the pan and turn the hot plate to low. (More gelatin can be made but be sure to maintain the appropriate ratio.) Slowly pour 4 envelopes of unflavored gelatin into your pan as you stir gently with a tongue depressor. Stir continually and slowly raise the temperature of the water until all gelatin has dissolved. The gelatin should be clear of bubbles and clumps.

4. **Pour the gelatin.**
   Using oven mitts, pour the gelatin into the mold on top of the patterns to about 5 mm in height. If the patterns move, reposition them with a paper clip. Refrigerate for about 15 minutes (or overnight).

5. **Peel and place the gelatin.**
   Once the gelatin has solidified, remove it from the mold. **Do not rip the gelatin.** You may use a paper clip to scoop and lift the gelatin or you may pop it out by turning the mold inside out. Peel off the transparency film. Carefully remove the paper clip design. Carefully place the gelatin on a glass slide and press it so that the channel (where the paper clip was) makes a smooth bond with the glass. Press to remove any air bubbles. Trim the edges to fit the slide (another glass slide works well for this).

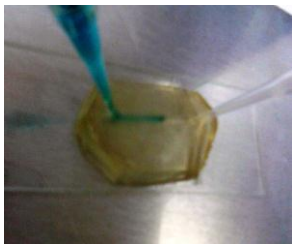
6. **Cut inlet and outlet holes.**
   Insert a pipette tip at the inlet and at the outlet points of your design. **Do not push the tips all the way to the glass, as this may break the seal**. Remove these pipette tips, for they will be clogged with gelatin now. Insert new pipette tips and leave them in place as shown in the image to the right. Congratulations! You have made a microfluidic device!

Development and distribution partially funded by the ***National Science Foundation***

NNIN Document: NNIN-1253

Rev: 06/2012

7. **Observe the movement of a fluid through a channel.**
   Now that the microfluidic device has been created, place the device on a microscope stage. Use a micropipette to inject a small bit of dye into the channel. Observe and analyze.



Cleanup: Once the gelatin is poured, immediately fill the pan with water to prevent the gelatin from hardening in the pan. If it does harden, the pan can be cleaned by repeated washing with warm water. Really hard, sticky gelatin can be re-watered and reheated to remove it easily.

Going Further: Students who have a good grasp of the content of the lab can be further challenged with these questions:

1. Proteins are molecules that often have a (+) or (–) charge. How do you think channels can be used to separate proteins? *Proteins can be separated according to their size and charge by charging the walls of the channel.*
2. How could these channels be used to sequence DNA (Deoxyribonucleic Acid)? *DNA could be spliced (cut into smaller pieces that code for a protein part) and tagged (attached with markers or dyes) then sent through the channels to be sequenced.*

Assessment:

The student will be able to (a) build a channel in gelatin using a mold he or she has made (b) inject fluid into the channel (c) describe the behavior of fluid flow in a channel (d) state the importance of using microfluidic and nanofluidic channels in nanotechnology and in biotechnology.

Resources:

- YouTube. "BioBytes 101 - What is Biotechnology?" (accessed July, 2010) http://www.youtube.com/watch?v=Wm6_BBS_KE4
- George Lisensky. Beloit College, University of Wisconsin. "Fluidics." (accessed July, 2010) http://mrsec.wisc.edu/Edetc/nanolab/PDMS_fluidics/index.html
- Cheng Wei T. Yang, Eric Ouellet, and Eric T. Lagally, "Using Inexpensive Jell-O Chips for Hands-On Microfluidics Education." *Analytical Chemistry* 82(13) (2010): pp 5408–5414.
- Low-cost prototyping of flexible microfluidic devices using a desktop digital craft cutter Po Ki Yuen* and Vasiliy N. Goral http://www.10gigfiber.org/assets/0/15/19/65/83/2927/A149E5DD-99D3-464A-AAED-040C12414E98.pdf
- Wikipedia – Microfluidics: http://en.wikipedia.org/wiki/Microfluidics

National Science Education Standards (Grades 9–12)

Content Standard E: Science and Technology

- Understandings about science and technology

Content Standard B: Physical Science

- Structure and properties of matter
- Chemical reactions

Development and distribution partially funded by the ***National Science Foundation***

NNIN Document: NNIN-1253

Rev: 06/2012