Chapter 2

Teaching Microfluidic Diagnostics Using Jell-O® Chips

Cheng Wei T. Yang and Eric T. Lagally

Abstract
Microfluidics has emerged as a versatile technology that has found many applications, including DNA chips, fuel cells, and diagnostics. As the field of microfluidic diagnostics grows, it is important to introduce the principles of this technology to young students and the general public. The objective of this project was to create a simple and effective method that could be used to teach key microfluidics concepts using easily accessible materials. Similar to the poly(dimethylsiloxane) soft lithography technique, a Jell-O® “chip” is produced by pouring a mixture of Jell-O® and gelatine solution into a mold, which is constructed using foam plate, coffee stirrers, and double-sided tape. The plate is transferred to a 4°C refrigerator for curing, and then the Jell-O® chip is peeled off for experimental demonstrations. Three types of chips have been fabricated with different molds: a JELLO mold, a Y-channel mold, and a pH-sensor mold. Using these devices, the basics of microfluidic diagnostics can be demonstrated in one or two class periods. The method described in this chapter provides teachers with a fast and inexpensive way to introduce this technology, and students with a fun and hands-on way to understand the basics of microfluidic diagnostics.

Key words: Microfluidics, Microfluidic diagnostics, Lab-on-a-chip, Microfluidics education, Teaching methods, Jell-O microfluidics

1. Introduction
Microfluidics is a multidisciplinary field that utilizes fundamentals of physics, biology, chemistry, and engineering to create miniaturized and integrated devices for various applications, including DNA chips, biological assays, and chemical synthesis (1). Because it uses small volumes of fluid samples, microfluidics has the potential to revolutionize modern biology and medicine by significantly reducing costs and reaction times associated with an analysis (2). Many types of materials have been explored for creating microfluidic channels and chips. Because it is inexpensive, optically transparent, and biocompatible, poly(dimethylsiloxane) (PDMS) elastomer has been extensively used in microfluidics (3). Soft lithography is the common technique for fabricating PDMS microfluidic chips (4).
In our laboratory, PDMS soft lithography is being used to create microfluidic chips for affinity reagent isolation (Fig. 1a) and bacterial pathogen detection (Fig. 1b). A general workflow of the soft lithography fabrication process is presented in Fig. 2.

Other extensions of microfluidics are being explored in other materials as well. For example, much effort is currently focused on producing low-cost microfluidic diagnostics for addressing the issue of global public health using both paper- and thread-based microfluidic devices (5–7). As the field of microfluidic diagnostics continues to grow and becomes an integral part of our daily lives, it is important to transfer the current research efforts and applications of this technology to young students and the general public. Recently, we have devised a set of demonstrations to illustrate the use of Jell-O® and other inexpensive materials for teaching microfluidics (8). Using these experiments, people can easily and effectively learn concepts such as microfluidic chip fabrication, laminar flow, dimensionless numbers, pH sensing, and diagnostics. These demonstrations can also serve as a bridge between nonscientists and scientists by creating a platform for discussing current microfluidics research. Moreover, these educational endeavors can help to inspire the next generation of young scientists into the field of microfluidics. This chapter describes the use of Jell-O® chips for teaching microfluidics and microfluidic diagnostics to young students and the general public.

2. Materials

2.1. General Mold Construction

1. Six 6 in. foam plates, round (see Note 1).
2. Several flat wooden coffee stirrers.
2. Scissors.
5. Personal protective equipment (gloves, lab coat, and safety goggles).

### 2.2. General Jell-O® Chip Fabrication

1. Two 85 g boxes of lemon-flavored Jell-O® jelly powder (see Note 2).
2. Two 7 g pouches of unflavored (the Original) Knox® Gelatine (see Note 3).
3. Two beakers of 120 mL of purified water for dissolving Jell-O® and Knox Gelatine.
4. One metal stirrer.
5. Hot plate.
6. Six premade molds with specific patterns.
7. PAM® Original no-stick cooking spray.
8. Some tissue paper.
9. Refrigerator with temperature of 4°C.
10. Flat 5 in. aluminum pans.
11. Personal protective equipment (gloves, lab coat, and safety goggles).

Fig. 2. Scheme for producing Jell-O® chips using soft lithography. (a) A negative mold is made with desired features. (b) Liquid chip material is poured onto the mold. (c) Mold with liquid material is cured. (d) Solidified chip is peeled off and (e) placed on a rigid substrate for experiments. (Reproduced from ref. 8 with permission from American Chemical Society).
2.3. Module 1: “JELLO Chip” Demonstration

1. Jell-O® microfluidic chips, each with a continuous channel depicting the letters “JELLO.”
2. Round drinking straws.
3. One disposable transfer pipet per Jell-O® chip.
4. Food-grade color dye, green.
5. Small vials of water with a few drops of green food coloring dye (~30 mL each).
6. Personal protective equipment (gloves, lab coat, and safety goggles).

2.4. Module 2: “Y-Channel Chip” Demonstration

1. Jell-O® microfluidic chips, each with a Y-shaped channel.
2. Round drinking straws.
3. Two disposable transfer pipets per Jell-O® chip.
4. Food-grade color dye, blue.
5. Small vials of clear water (~30 mL each).
6. Small vials of water with a few drops of blue food coloring dye (~30 mL each).
7. Personal protective equipment (gloves, lab coat, and safety goggles).

2.5. Module 3: “pH-Sensor Chip” Demonstration

1. Jell-O® microfluidic chips, each with two straight channels.
2. Round drinking straws.
3. Two disposable transfer pipets per Jell-O® chip.
4. Two small pieces of acid-sensing pH paper and two small pieces of base-sensing pH paper.
5. Double-sided tape.
6. Small vial of 1 M hydrochloric acid (or cooking vinegar).
7. Small vial of 1 M sodium hydroxide (or dissolved antacid solution).
8. Personal protective equipment (gloves, lab coat, and safety goggles).

3. Methods

In general, the molds are created using foam plates, wooden coffee stirrers, and double- and single-sided tape. The coffee stirrers are first cut into various shapes and sizes, depending on the purpose of the mold, using a pair of scissors. These pieces of coffee stirrers are taped onto a foam plate using double-sided tape to create the desired mold pattern. Single-sided tape is then adhered to the junctions of the wooden sticks to reduce the gap. Three types of
molds have been constructed to illustrate the diverse concepts that can be taught using this teaching method: a “JELLO” mold, a Y-channel mold, and a pH-sensor mold. In general, the Jell-O® chips are made by pouring a liquid mixture of both Jell-O® and gelatine into the molds. These plates are left to cure in a 4°C refrigerator for about 2 days. When ready, the Jell-O® chips are removed from the refrigerator, peeled from the molds, and placed on aluminum dishes for demonstrations. The high sugar content from the Jell-O® and gelatine mixture provides a natural seal on the aluminum dishes, and the strength of the seal is suitable for the low-pressure applications presented here. A general workflow for fabricating these Jell-O® chips is shown in Fig. 3.

Instructors should allocate two 1-h class periods to conduct the demonstration(s). The first class period is dedicated to introducing microfluidics and soft-lithography, constructing the molds, preparing the Jell-O® and gelatine mixture, pouring the mixture into the molds, and moving the plates to the refrigerator (see Note 4). The second class period is focused on conducting the hands-on experiments, observing the microfluidics phenomena, elucidating the accompanying theory, and discussing some current and relevant applications. For more mature audiences (high-school, university, general public),
the learners should conduct both chip fabrication and experimentation. For younger audiences (grade-school and middle-school), the mold and the chips should be made in advance by instructors; these students would participate by manipulating the chips to form a seal on an aluminum pan, and conducting the experiments. The main learning outcomes are summarized in Table 1 below.

3.1. General Mold Construction

1. “JELLO” Chip: Cut the coffee stirrers into rectangular shapes of various lengths, according to the letters “JELLO.” Using double-sided tape, attach these small pieces of wooden sticks onto a foam plate to form a continuous channel depicting the letters “JELLO.” Use small pieces of single-sided tape to cover

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Module I</th>
<th>Module II</th>
<th>Module III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target learners</td>
<td>Grade-school science students</td>
<td>High school science students</td>
<td>High school science students</td>
</tr>
<tr>
<td>Mold fabrication difficulty</td>
<td>Medium</td>
<td>Medium</td>
<td>Easy</td>
</tr>
<tr>
<td>Experimental difficulty</td>
<td>Easy</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Learning objectives</td>
<td>Basics of microfluidic fabrication</td>
<td>Visualization of laminar flow</td>
<td>Differences between acids and bases</td>
</tr>
<tr>
<td></td>
<td>Soft lithography</td>
<td>Differences between laminar flow and turbulent flow</td>
<td>Fundamentals of pH sensing</td>
</tr>
<tr>
<td></td>
<td>Concept of pressure-driven flow</td>
<td>Significance of dimensionless parameters</td>
<td>Concept of parallelization</td>
</tr>
<tr>
<td></td>
<td>Diversity, complexity, and flexibility of designs</td>
<td>Current microfluidic applications of laminar flow</td>
<td>Current microfluidic parallelization applications</td>
</tr>
<tr>
<td>Questions to be answered</td>
<td>What is microfluidics and how are microfluidic chips made?</td>
<td>Why do the two solutions not mix in this Jell-O chip?</td>
<td>What are acids and bases?</td>
</tr>
<tr>
<td></td>
<td>How are channels formed in microfluidic chips?</td>
<td>What is the difference between turbulent flow and laminar flow?</td>
<td>How can we determine the pH of solutions using pH papers?</td>
</tr>
<tr>
<td></td>
<td>How do liquids flow in microfluidic chips?</td>
<td>What are dimensionless numbers?</td>
<td>What is parallelization?</td>
</tr>
<tr>
<td></td>
<td>Can fluid be passed through the chip with only one inlet and no outlet?</td>
<td>How can dimensionless numbers help us to build our devices?</td>
<td>What are current microfluidic applications of parallelization?</td>
</tr>
</tbody>
</table>
the junctions of coffee sticks to ensure a smooth overall mold surface (see Note 5).

2. Y-Channel Chip: Two pieces of coffee stirrers are needed for forming one Y-channel mold. Cut the first coffee stirrer at both ends using a pair of scissors to obtain a long rectangular-shaped wooden stick of ~3 in. long. One end of this stick should be flat (outlet) and the other end should be further cut into a dagger shape. Cut the second coffee stirrer to obtain two smaller rectangular-shaped sticks of the same length (~1 in. long) (see Note 6). Using double-sided tape, tape the longer stick near the bottom half of a foam plate. Similarly, tape the two smaller sticks at the dagger-shaped end of the longer stick to form a mold with the letter “Y” (see Note 5). Use small pieces of single-sided tape to cover the junctions of coffee sticks to ensure a smooth overall mold surface.

3. pH-Sensor Chip: Two pieces of coffee stirrers are required for forming the pH-sensor mold. Cut both of the coffee stirrers to obtain two long rectangular-shaped wooden sticks of the same length (~3 in. long). Using double-sided tape, attach these sticks to the middle of the foam plate at ~1 in. apart.

1. After constructing the mold plates (see Note 7), mix two pouches of Jell-O® jelly powder in 120 mL of purified water in one beaker (using a metal stirrer). Mix two pouches of Knox Gelatine powder in another beaker with the same amount of water (see Note 8).

2. Place the first beaker (containing partially dissolved Jell-O®) on a hot plate and heat the solution to a boil (see Note 9). Remove beaker from the heat and pour the content of the second beaker (containing partially dissolved gelatine) into the first beaker. Reheat the mixture of Jell-O® and gelatine solution to a boil on the hot plate, and finally remove this beaker from the heat.

3. Apply a small amount of cooking spray onto the inside rim of the foam plate (with tissue paper) to facilitate the peeling of the Jell-O® chips after curing. Pour the mixture of Jell-O® and gelatine solution into the molds (an amount that can adequately cover the wooden sticks). Approximately six mold plates can be filled with the amount of solution prepared.

4. Transfer the molds with liquid mixture to a 4°C refrigerator for curing (see Note 10) and cure the chips for about 2 days to obtain more robust Jell-O® chips.

5. When ready for demonstration, carefully peel the cured Jell-O® chips off of the mold. Bending the foam plate at the rim may help with the peeling process. Be careful when peeling the Jell-O® chip near the wooden sticks to prevent any tears, which can result in leakage in the chip.
6. Determine the side of the chip with hollow channel(s). Place this side against an aluminum pan to create a natural and reversible seal, and to form an enclosed channel. Make sure to eliminate all visible air bubbles between the chip and aluminum pan (see Note 11).

3.3. “JELLO Chip” Demonstration

1. These instructions assume that chips with a continuous channel depicting the letters “JELLO” are cured and ready to be used for demonstration.

2. To motivate this demonstration, questions including “What is microfluidics?,” “How are microfluidic chips made?,” and “How are channels formed in microfluidic chips?” can be presented to the class. It may be helpful to use Figs. 1 and 2 to facilitate the discussion.

3. After class discussion, peel the “JELLO” chips from their foam plates and place them on aluminum pans as described in Subheading 3.2, then proceed with experimental demonstration. These instructions explain the procedure for working with one chip (see Note 7).

4. Using a round drinking straw, puncture an inlet hole at the tip of letter “J” and an outlet hole at the end of letter “O” with a gentle twisting motion.

5. Add a few drops of green food coloring dye into a small vial of water, and load the green water into a disposable transfer pipet.

6. By gently squeezing the pipet bulb, inject the green water into the channel via the inlet hole. The resulting fluid flow can be directly visualized without the use of any imaging apparatus. An example of the results produced is shown in Fig. 4.

7. Learning Objectives including “Basics of microfluidic fabrication” and “Soft lithography” can be easily taught using the “JELLO” demonstration presented above. After conducting the hands-on experiment, the similarities and differences between
Jell-O® chip and PDMS chip fabrication can be highlighted. For example, negative molds for PDMS chip fabrication are produced using photolithography; the PDMS pre-polymer is cured in an oven at 60°C; and the feature sizes of PDMS microfluidic chips are usually a few micrometers in width (4). However, the general fabrication concepts and PDMS soft lithography can be easily explained using this demonstration.

8. The concept of pressure-driven flow can also be explained using this demonstration. Questions including “How do liquids flow in microfluidic chips?” and “Can fluid be passed through the chip with only one inlet and no outlet?” can be used to motivate the class discussion. When pressure is applied to the pipet bulb, a pressure is applied to the colored water in the transfer pipet, and a fluid flow is observed. The fluid flow stops as soon as the pressure from the pipet bulb is released. In contrast, if there is only one inlet and no outlet, then the fluid cannot flow through the channel. This phenomenon occurs because the air present in the channel has no place to escape. Furthermore, if a large enough pressure is exerted on the fluid in this inlet-only system, then the reversible seal between chip and aluminum pan would break. The outlet provides a path for the air inside the channel to escape, therefore allowing the fluid to flow.

9. Finally, this demonstration can be used to illustrate the level of creativity that can occur in designing microfluidic chips. Depending on our specific needs, we can fabricate molds and chips with varying flexibility, diversity, and complexity. Currently, microfluidic chips are being designed to address specific problems in microfluidic diagnostics.

3.4. “Y-Channel Chip” Demonstration

1. These instructions assume that chips with a continuous channel depicting the letter “Y” are cured and ready to be used for demonstration.

2. To motivate this demonstration, pose the question “What would happen when you pour clear water and blue water in a cup?” to the class. Evidently, the students would expect mixing of the two solutions. Subsequently, pose the question “What would happen when you inject clear water and blue water in the ‘Y-channel’ chip?” The majority of the students would most likely answer that mixing of the fluids would occur. Without revealing the answer, obtain the aluminum pans with “Y-channel” chips and proceed with experimental demonstration. These instructions explain the procedure for working with one chip (see Note 7).

3. Using a round drinking straw, puncture two inlet holes at the top of letter “Y” and an outlet hole at the bottom of letter “Y” with a gentle twisting motion.
4. Prepare two small vials of purified water, and add a few drops of blue food coloring dye to one of the vials. Obtain two disposable transfer pipets: load one pipet with blue water and the other one with clear water.

5. Simultaneously inject clear water and blue water into left channel and right channel, respectively (see Note 12). The resulting laminar fluid profile can be directly visualized without the use of a microscope (see Note 13). An example of the results produced is shown in Fig. 5.

6. Furthermore, the flow rate of one solution can be changed (by changing the pressure applied to the pipet bulb), and the shifting of the interface between clear and colored water can be observed. It is counterintuitive to see that the two fluids do not mix in the Y-channel chip, so this demonstration provides a convenient starting point for discussing the differences between turbulent flow and laminar flow.

7. Students who are more mathematically advanced can be introduced to dimensionless parameters or numbers for a more comprehensive understanding (see Note 14). For examples, dimensionless parameters including the Reynolds number ($Re$) (see Note 15) and Péclet number ($Pe$) (see Note 16) can be discussed and calculated in class. To gain a better understanding of $Pe$ numbers, diffusion and diffusion coefficient ($D$) may also need to be discussed (see Note 17).

8. The significance of dimensionless numbers can also be highlighted, because learning how to use dimensionless numbers is an important skill for scientists and engineers. For example, dominant forces in the fabricated microfluidic devices can be calculated using dimensionless numbers. Reynolds number can be used to determine whether laminar or turbulent flow dominates; Péclet number can be used to determine whether

![Fig. 5. (a) A Jell-O® Y-channel chip with a Reynolds number of 30. The injection of colored water to one inlet and clear water to the second results in the classic laminar flow profile, in which both streams remain separate and mix solely by diffusion along the length of the channel. (b) Diagram of laminar flow diffusive mixing occurring at the interface between two different fluids along the channel length. This phenomenon is governed by the Péclet number (reproduced from ref. 8 with permission from American Chemical Society).](image-url)
convective mass transfer or diffusion dominates. Conversely, the value of a parameter can also be changed to switch between the analytical regimes, and dimensionless numbers can facilitate in the designing of microfluidic chips.

9. In these Jell-O® Y-channel chips, reliable separation of analytes based solely on diffusion or molecular size cannot be easily achieved (Fig. 5a); however, this result can be achieved in smaller microfluidic systems (Fig. 5b). For example, diffusive mixing has been used in microfluidic T-sensors for chemical concentrations measurements (9) and for rapid determination of diffusion coefficients for molecules of varying sizes (10).

10. After discussing the theory behind the Y-channel chip, some current applications of this device can be highlighted. In addition to the two T-sensor examples discussed above, laminar flow can also be used to separate the anode and cathode streams in Y-shaped fuel cells, without the use of a polymer electrolyte membrane (11–13).

### 3.5. “pH-Sensor Chip” Demonstration

1. These instructions assume that “pH-sensor” chips are cured and ready to be used for demonstration.

2. To motivate this demonstration, questions such as “What are acids and bases?” and “How can we determine the pH of solutions using pH papers?” can be presented to the class. Obtain aluminum pans with “pH-sensor” chips and proceed with experimental demonstration. These instructions explain the procedure for working with one chip (see Note 7).

3. Using a round drinking straw, puncture two inlet holes at the top of both channels, and two outlet holes at the bottom of both channels with a gentle twisting motion (to form two separate channels).

4. Use double-sided tape to attach the small pieces of pH paper onto the aluminum pan, within the boundaries of the channels. In both of the channels, tape a piece of acid-sensing pH paper near the inlet and tape a piece of base-sensing pH paper near the outlet.

5. Carefully prepare small vials of 1 M HCl and 1 M NaOH (each with volume of ~30 mL) (see Note 18). Obtain two disposable transfer pipets, and load one with NaOH and the other with HCl.

6. Simultaneously inject NaOH into the left channel and HCl into the right channel, and directly visualize the resulting color changes in the pH papers. An example of the results produced is shown in Fig. 6.

7. Alternatively, safer acidic or basic solutions, such as cooking vinegar and dissolved antacid solution, could be used for this experiment (see Note 19).
8. When 1 M NaOH is injected into the channel on the left, the base-sensing pH paper nearest the channel outlet turns blue. In contrast, when 1 M HCl is injected into the channel on the right, the acid-sensing pH paper nearest the channel inlet turns purple. As a result, the differences between acids and bases, and the fundamentals of pH sensing can be explained.

9. Optionally, this demonstration can be combined with the Y-channel demonstration to form a more integrated learning activity. For example, a weak acid and a weak base can be injected over a pH paper placed within the Y-channel chip. Therefore, distinct pattern of color change can be observed on the same pH-sensing strip due to laminar flow, diffusive mixing, and neutralization.

10. The concepts of parallel analysis and microfluidic diagnostics can also be introduced with this demonstration. As an example of parallel analysis, first assume that the indicator strips near the channel inlets represent areas of the microfluidic chip that contain a type of ligand (capturing agent) that detects analyte A. Similarly, assume that the indicator strips near the channel outlets
represent a second type of ligand that detects analyte B. When two unknown fluid samples are introduced into the channels, the presence of analyte A or B in each solution can be determined. Therefore, we are running two separate tests in parallel.

11. This concept of parallel analysis can be further extended. For example, first assume that the channels have been modified with four different ligands. Consequently, four independent tests can be conducted on one fluid sample in parallel. Therefore, current efforts in microfluidic diagnostics to conduct hundreds of tests in parallel can be easily explained using this simple demonstration (14, 15).

4. Notes

1. To be more environmentally sustainable, flat paper plates may be used instead of foam plates.

2. Lemon-flavored Jell-O® jelly powder was used because it created chips with the best optical transparency. Other flavors could also be used, as long as fluid flow can still be seen through the chips.

3. This is a modification of the initial protocol, which used only one pouch of Knox® Gelatine. It has been found that this new approach shortened the curing time and also increased the robustness of the chips.

4. Since 2 days of curing time is recommended, it is ideal to make these chips on Friday and continue with the demonstration on Monday.

5. During the taping of the sticks onto the foam plate, the sticks need to be pressed against each other well to reduce the gaps between them. It may also help to wear gloves during the taping process since tape becomes less sticky when oils from the fingers are transferred to the tape.

6. The length for the two sides of the dagger-shaped end should be the same as the width of the smaller coffee sticks to ensure a continuous and smooth Y-channel geometry.

7. The amount of Jell-O® and gelatine mixture produced in this protocol is enough to completely cover six mold plates. Thicker chips can be made by reducing the number of molds made (to three or four). However, increasing the thickness of the chips will most likely affect and lengthen the curing time. The number of Jell-O® chips needed for a presentation or a workshop depends on the number students present. From our experience, a ratio of 2–3 students per chip produced the best learning experience.
8. Jell-O® and gelatine powder are not readily soluble in cold water. Therefore, the solution in this beaker will remain opaque until heated to a boil in the following steps.

9. Before using the hot plate, it is helpful to cover the hot plate with some aluminum foil to prevent any spills from drying up on hot plate surface and to facilitate the cleanup procedure. During the heating process, the solution should be stirred often.

10. If a large quantity of Jell-O® chips is required for the demonstration/workshop, it is helpful to place the foam plates on a cart with wheels, and then pour the Jell-O® and gelatine mixture into the plates. Subsequently, the cart can be wheeled into and stored in a 4°C cold room for curing the chips.

11. From experience, it is important to store the Jell-O® in the refrigerator until immediately prior to the demonstrations. If left at room temperature for too long, the chips may lose their robustness and the peeling process may fail.

12. To prevent leakage from the inlets, the transfer pipets should be held at a 45° angle to the surface, and pointed into and parallel to the inlets of the Y-channel. Both clear and dyed water should be injected evenly and slowly into the channels in order to create the laminar flow profile.

13. If Y-channel chips are too difficult to fabricate, simpler T-channel chips can also produce similar results. Two rectangular-shaped coffee stirrers can be combined to make the letter “T” on a foam plate. Puncture the two inlet holes and one outlet hole as before. Laminar flow profile can still be observed using the T-channel chips.

14. Dimensionless parameters do not have a physical unit, and they are usually defined as a ratio of two properties (these properties may have units individually, but the units cancel out when they are combined) (16).

15. Reynolds number (Re) is a dimensionless parameter that relates inertial to viscous forces in fluid flows: \( Re = \frac{\rho U_0 L_0}{\eta} \), where \( \rho \) is the fluid density, \( U_0 \) is the characteristic velocity, \( L_0 \) is the typical length scale, and \( \eta \) is the shear viscosity (16, 17). A high Re implies that inertial forces are dominant; and a low Re implies that viscous forces are dominant. Therefore, Re is used to differentiate between laminar and turbulent flows. Using water as the working fluid (\( \rho = 1.0 \text{ g/cm}^3 \) and \( \eta = 0.010 \text{ g/cm s} \)) with a velocity of 1.0 cm/s and a channel radius of 0.30 cm yields \( Re = 30 \). Typically, the transition from laminar to turbulent flow in round pipes occurs at \( Re = 2,000–3,000 \) (16), so \( Re = 30 \) indicates that the flow is within the laminar flow regime.
16. Péclet number ($Pe$) is another dimensionless parameter that is defined as $Pe = U_0 L_0 / D$, where $U_0$ is the characteristic velocity, $L_0$ is the typical length scale, and $D$ is the diffusion coefficient.

17. Understanding the implications of $Pe$ requires an understanding of diffusion. Elementary diffusion explanations can be initiated using the Jell-O® chips described here, as laminar flow is an ideal flow regime in which to demonstrate the effects of diffusion. For a molecule with an unknown diffusivity, the diffusion coefficient can be modeled as a spherical solute: $D \approx k_B T / 6 \pi \eta a$, where $k_B$ is the Boltzmann constant, $T$ is the temperature, $\eta$ is the shear viscosity, and $a$ is the molecule size (16). The diffusion coefficient of typical food coloring dye is about $200 \, \mu m^2/s$ (18); therefore, calculation yields $Pe = 150,000$ in our Jell-O® chips. This large $Pe$ indicates that convective mass transfer dominates and little diffusion along the length of the channel occurs (Fig. 5a). However, as the typical length scale decreases (such as in the case of research microfluidic systems), intermediate $Pe$ is achieved, and the differences in solute diffusion rates will become more pronounced (Fig. 5b).

18. Be extremely careful when working with high concentrations of acids and bases: Proper protective clothing including lab coat, gloves, and goggles must be worn at all times. This step may not be suitable for younger students.

19. Household solutions that are safer than 1 M HCl and NaOH may be used. Common acidic solutions include vinegar and lemon juice; common basic solutions include dissolved antacid solutions and soapy water.

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