Trapping bacteria and fungi using microfluidic design

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ABSTRACT

Escherichia coli and *Candida auris* are not easy to identify in laboratories without special technology. In this study, we have presented microfluidic designs for trapping bacteria and fungi. Two trapping chambers are designed using AutoCAD and the fluid dynamics of the bacteria and fungi are simulated using D. Schroeder's Fluid Dynamics Simulation software. The designs are modified versions of a device that is constructed and simulated with numerical predictions, which include sizes and apertures in consideration of the specified microbe. The current designs take into account the exact dimensions of *E. coli* and *C. auris* under fluid flow and passive microfluidic technique, where actuation is based on geometry, is considered. The measurements of the design ensure that the species are to be trapped due to diffusion and fluid dynamics. From the simulation, the stagnation is to be shown with its default setting, and approximation is done in its motion which is simulated in the two-dimensional space of the bacteria and fungi. The microfluidic designs will be useful during experiments in deciphering necessary information of the bacteria and fungi and will be a platform in modeling numerous biomedical assays and in the optimization of biophysical tools.

1. Introduction

Bacteria are small single-cell organisms that come in various shapes. Not all bacteria cause diseases, there are also good bacteria that help maintain and regulate the body in order to function healthily. Individual bacteria can differ significantly in their characteristic features. Cellular heterogeneity is the factor that increases their adaptation to environmental changes.

Escherichia coli (*E. coli*) are bacteria that live mostly in the lower intestines of people and animals and can be present in contaminated water and foods. It is a gram-negative rod-shaped bacterium with dimensions of a cylinder around 1.0-2.0 micrometers long, with a radius of about 0.5 micrometers. *E. coli* moves with the help of helical flagella in an aquatic environment. It is a diverse group of bacteria where most of its strains are harmless while other strains can lead to urinary tract infections, diarrhea, pneumonia, and other respiratory illnesses on any host. In a study by Patteson et al. (2015), they discussed the sudden changes of *E. coli* dynamics even a small amount of its polymer solution. The importance of direct analysis of individual *E. coli* is that researchers and scientists are able to describe the motility of the cells as they constantly navigate pore spaces and exhibits diffusive behavior when it interacts with a wall or liquids.

Fungi are more complicated organisms than bacteria. They are larger, plant-like organisms that lack chlorophyll. It is eukaryotes, which means they have cells. There are two main types of fungi: Environmental, which are yeast and mold that often live in soil and don't generally cause infection in most healthy people; and commensals, which live on and in us and generally don't hurt us.

Candida auris (C. auris) is one of the common isolated *Candida* species, which are known as drug-resistant fungi (Ben-Ami et al., 2017). It is associated with nosocomial infections and can grow on the skin. Its cells are ovoid, ellipsoidal, and 2.5-5.0 micrometers in size.

Analytical methods are important to determine the factors that may cause cellular heterogeneity, cell stages, and aging. Pilát et al. (2018) analyzed cells in various body fluids to get a better understanding of the mechanism concerning cellular physiology. An optofluidic system was developed where individual cells of *Escherichia coli* bacteria were manipulated

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and observed. It used programmable syringe pumps that supply different liquids into the microfluidic chip through microfluidic tubes. Laser tweezers were then used to transport the cells individually to the microchambers for trapping. Kim et al. (2011) constructed a microfluidic device efficient enough to trap a single bacteria cell. The concept of hydrodynamics was used to slow down the motion and trap the cells flowing near the aperture of the sieves, which are 3D rigid ellipsoids. Numerical predictions were used to approximate the motion of the bacteria cell. Liberale et al. (2013) utilized optical tweezers, Raman, and fluorescence spectroscopy in enabling on-chip manipulation and single-cell trapping. At the present, most of the trapping designs are governed by external factors, however, in our study, we are creating designs that would automatically trap *E. coli* and *C. auris* under fluid flow even without the presence of external factors like optical tweezers. The laminar flow produced by the optical tweezers has the same magnitude of hydrodynamic forces (Janča et al., 2011). The trapping execution supported by hydrodynamic concepts is achieved even in the absence of optical tweezers.

Bacteria and fungi like *E. coli* and *C. auris* respectively are not easy to identify in laboratories without special technology. It can be misidentified in standard laboratory methods that may lead to inappropriate handling of the patient, which in turn can result in the spreading of diseases. Thus, in this study creating trapping designs for *E. coli* and *C. auris* using various microfluidic devices are presented. The microfluidic designs will be useful during experiments in deciphering necessary information of the bacteria and fungi and will be a platform in modeling numerous biomedical assays and in the optimization of biophysical tools.

2. Theory and methodology

Microfluidics deals with flow behavior and precise control of fluid that is geometrically constrained to a sub-millimeter scale (Galon and Bacabac, 2019). This technology has offered numerous useful capabilities, such as the ability to use small quantities of samples or reagents, a short time for analysis, and laminar flow, which can provide the control of the chemical environment. It offers various applications in clinical research and biomedical engineering because of its ability to precisely control the cellular environment and to surely give analysis at the single-cell level.

In microfluidics, flow is predominantly laminar, creating challenges in the design of actuators such as mixers and sorters (Milne et al., 2007; Galon and Bacabac, 2019). The extremely low Reynolds numbers associated with microfluidic flows mean that inertial effects cannot be used to sort particles, yet the ability to select and sort both colloidal and biological matter is a key requirement to this

environment. Small characteristic length scale makes microfluidics especially suited for studies of the following.

a. Very low Reynolds number (Re) (Mansur et al., 2008; Galon and Bacabac, 2019):

$$R_e = \frac{inertial\ forces}{viscous\ forces} = \frac{\rho U L}{\mu} \ll 1, \qquad (1)$$

which states that flows are always laminar. Where ρ is the fluid density, U is the characteristic velocity of the fluid, L is the characteristic length, and μ is the fluid viscosity.

b. High Péclet number (Pe) (Socolofsky and Jirka, 2005):

$$P_e = \frac{convection}{diffusion} = \frac{UL}{D} \ll 1, \qquad (2)$$

which states mixing through diffusion across fluid layers is ineffective. where U is the characteristic velocity of the fluid, L is the characteristic length, and D is the diffusion coefficient.

c. Viscoelastic liquids at high elasticity (El):

$$El = \frac{elastic\ forces}{inertial\ forces} = \frac{W_i}{R_e} \ll \frac{\tau \rho \eta}{\rho L^2}, \qquad (3)$$

which states that elasticity dominates inertial effects.

Passive microfluidic techniques were taken into account in designing the trapping chambers. In this technique, actuation is based on the changes in the geometry of the microfluidics. AutoCAD 2021 software is used in designing the trapping chambers and it is created in accordance with the specified dimensions. The first trapping chamber is for *C. auris* and is based on the basic design rules of Stanford Microfluidics Foundry. Figs. 1-4 show the microfluidic device with its exact dimensions. The chamber has a diameter of 400μm and a height of 10μm, and each sieve is semicircular with an outer diameter of 75μm, with a height of 10μm, and with a width of 13μm (Fig. 1). The slit or aperture is 2μm and the close-up drawing of each sieve design is maintained at 0^0 (Fig. 2). The device exploits hydrodynamics in slowing down and in trapping cells that flow near a narrow aperture. The dimensions are adjusted according to the supposed size and shape of the fungus to be trapped.

According to Kim et al. (2011), all sieves would be able to maintain in trapping the fungi. The U-type sieves are to keep the fungi trapped in place once they enter. Kim et al. (2011) also claimed that the probability of trapping a non-spherical cell is smaller than the probability of trapping a spherical cell. The design to trap the fungi *C. auris* has a similar approach of entrapment. The measurements are modified in accordance to the size of the fungus.
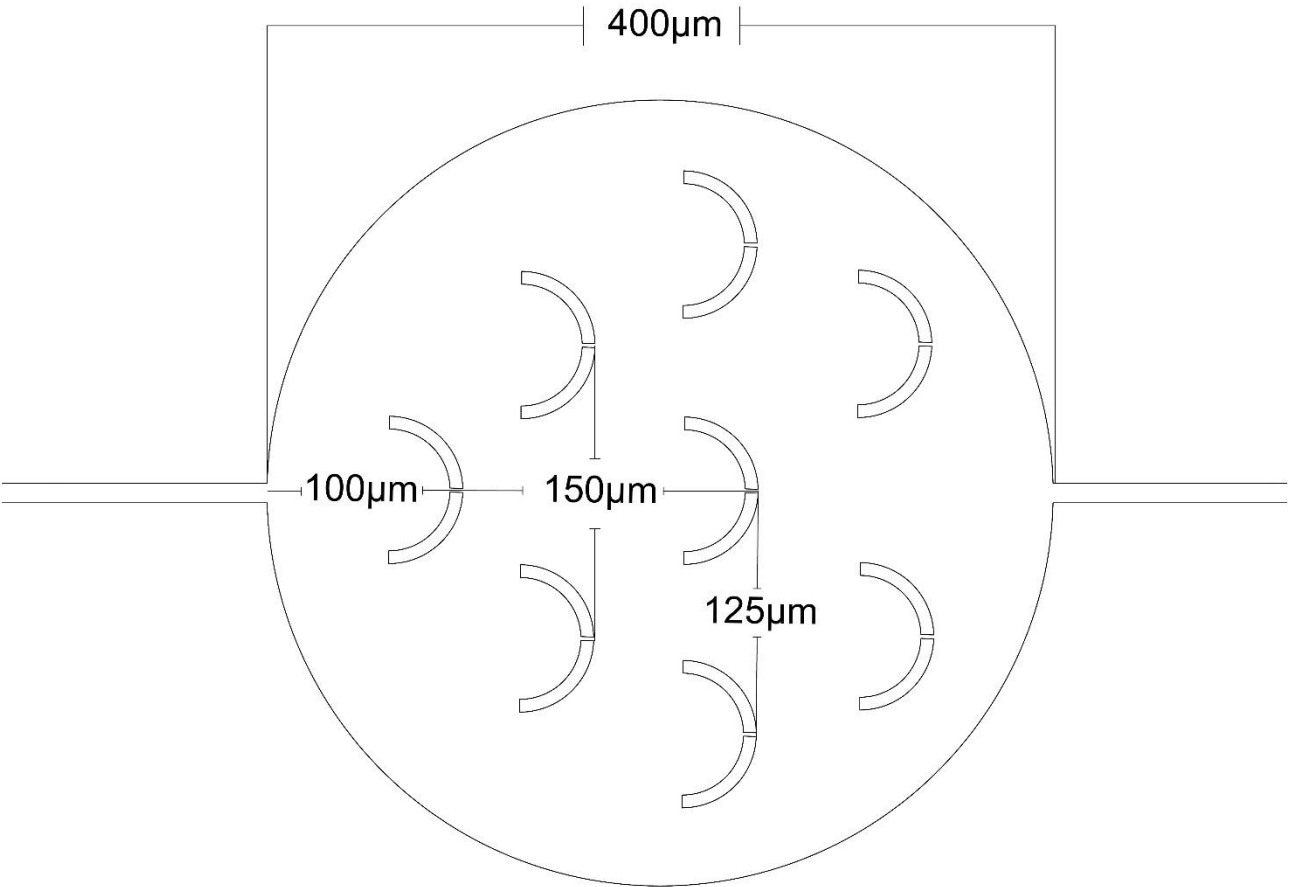
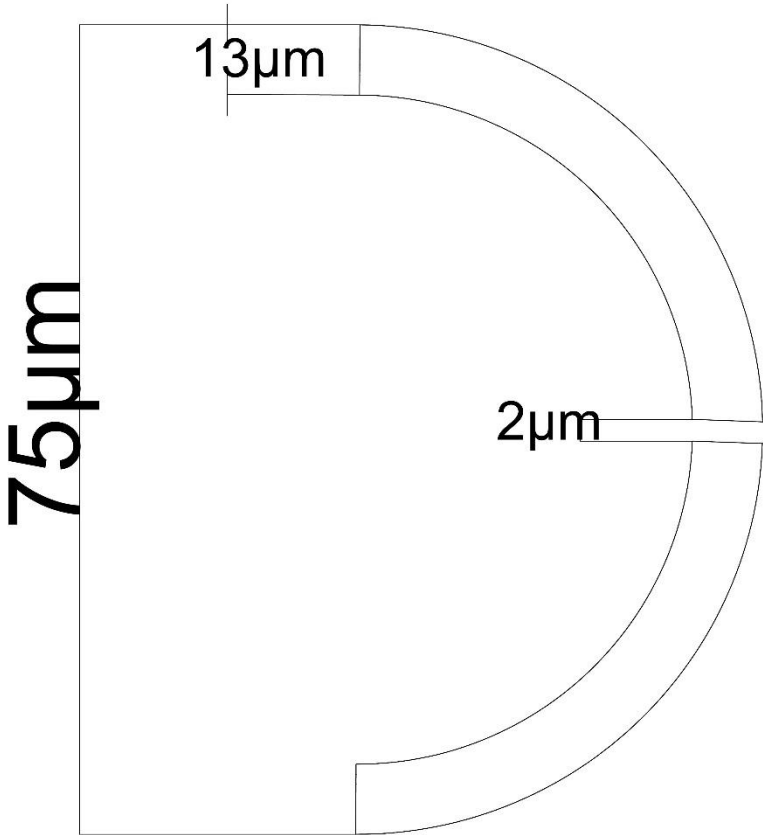**Fig. 1:** Chamber measurement for *C. auris* trapping chamber

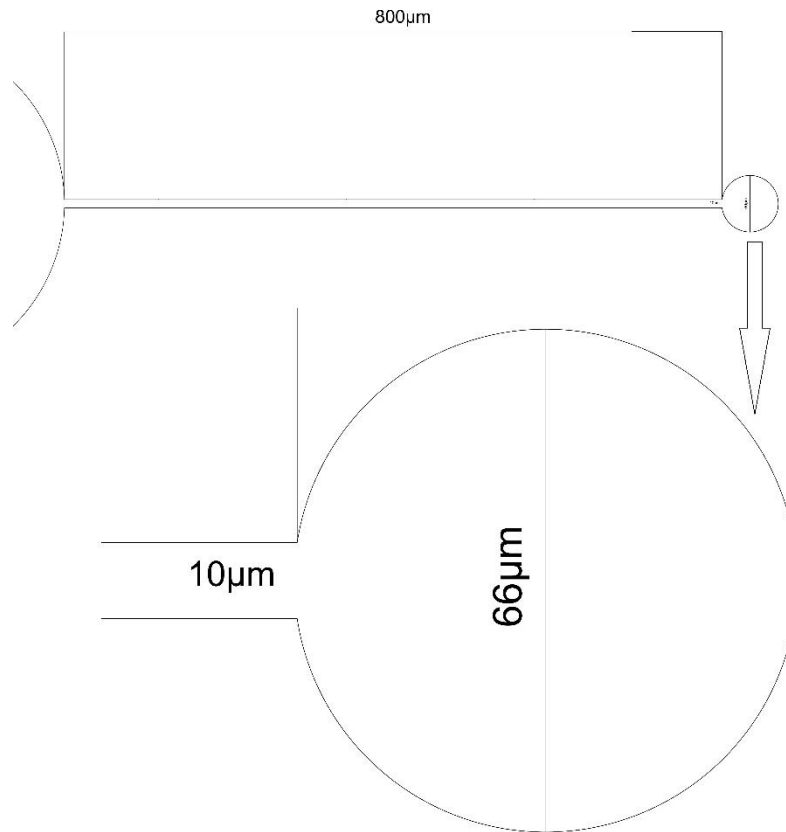**Fig. 2**: Sieve measurement for *C. auris* trapping chamber

800μm

10μm

66μm

Fig. 3: Inlets, Outlets and flow channel measurement for *C. auris* trapping chamber
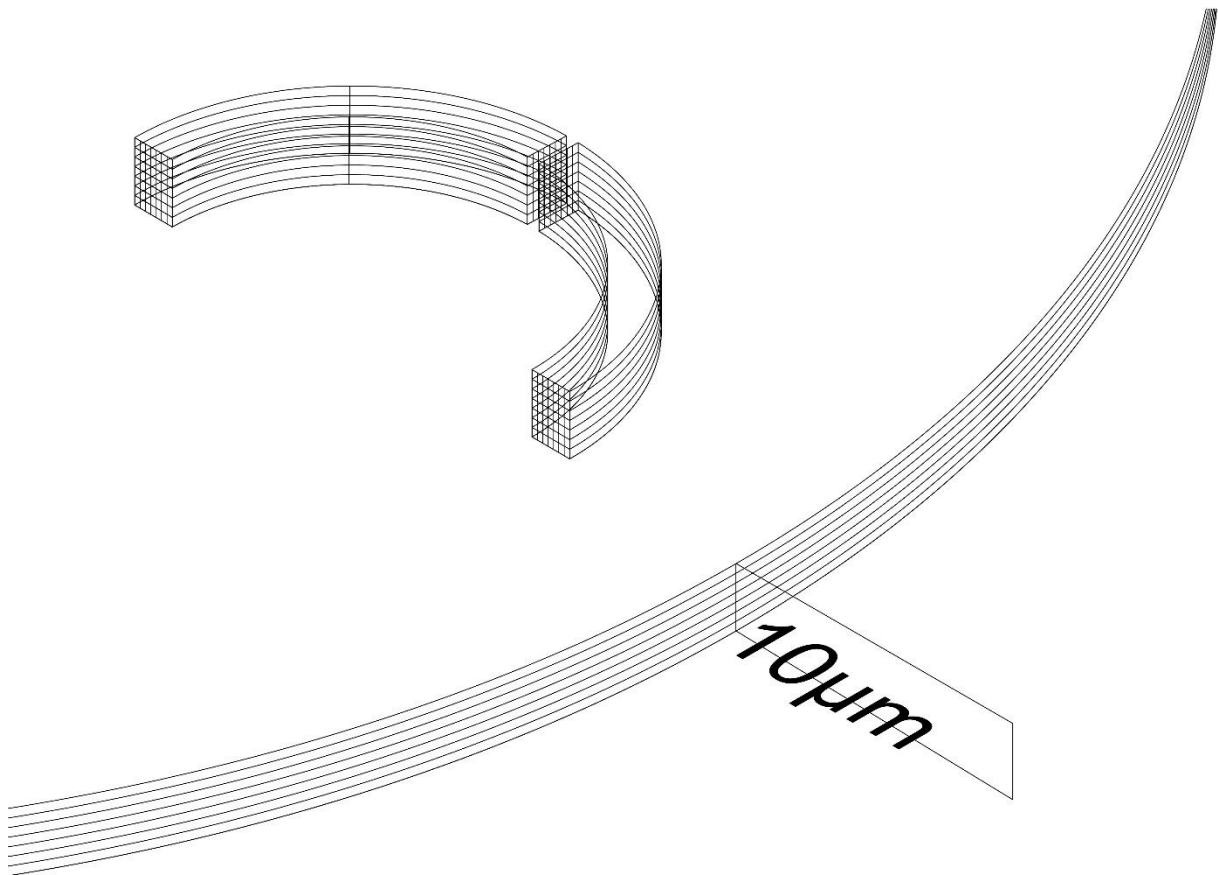
10μm

Fig. 4: Width measurement of microchip for *C. auris*

Fig. 5 shows the second trapping chamber design which is for *E. coli*. The outside channel diameter is 460μm, the channel width is 30μm, has a chamber volume of 155pL, and has a trap gap size of 1μm. The measurements ensure that the bacteria are trapped and could not escape easily due to diffusion. Narrow

channels may limit the movement of bacteria to reorient during the laminar flow from the main channel to the area of trapping. This instance could be addressed by changing the geometry or measurement of the microchip to control the dispersal of the cells which could change the cell motility pattern.
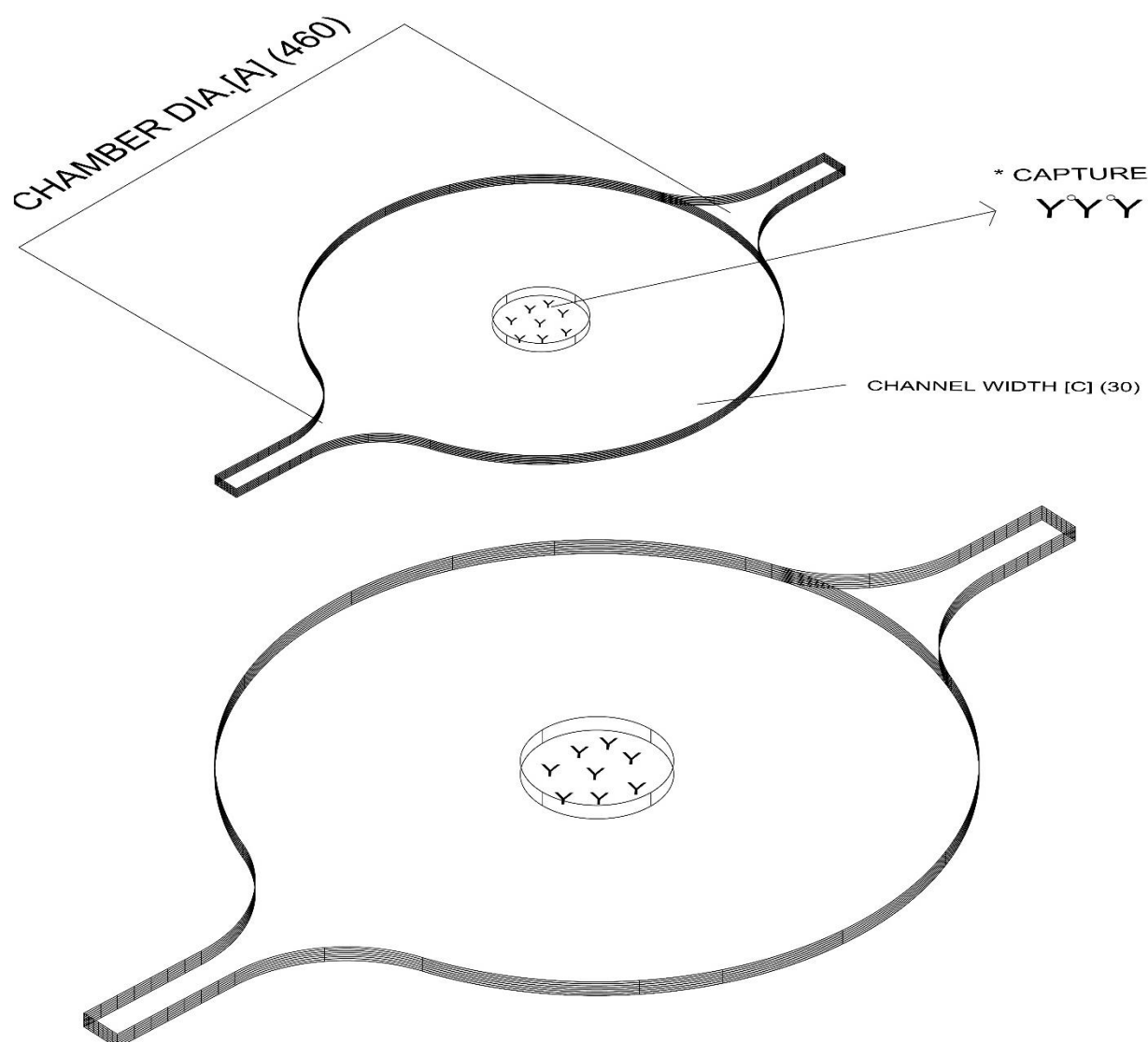

Fig. 5: Trapping chamber for *E. coli*. The outside channel diameter is 460μm, the channel width is 30 μm, has a chamber volume of 155pL, and has a trap gap size of 1μm. The Y-like traps are valves that could trap *E. coli* by opening and closing which is possible through a pressure control system

The microfluidic designs are to be simulated using the Fluid Dynamic Simulation. The Fluid Dynamic Simulation is a free two-dimensional simulator that uses a Lattice-Boltzmann algorithm, which is coded in JavaScript. The simulation has a limitation to model fluids at constant temperature and the speed is less than the speed of sound. The unit that is used in this simulation is arbitrary. From the simulation, the stagnation is to be shown with its default setting, and approximation is done in its motion which is simulated in the two-dimensional space of the bacteria and fungi.

3. Results and discussion

Microfluidic devices were designed using computer-aided design software. There are many ways to test a microchip design, one of which is through simulation. A simulation in fluid dynamics made by Schroeder (2019) of Weber State University, which is available online, was used to test the design. For the trapping design of *Candida auris*, the chamber was ought to trap a cell size of 2.5-5.0 micrometers with a shape of ovoid, ellipsoidal to elongate. There are variations in apertures that are used in a similar study which is to determine the correlation between the trapping performance and hydrodynamic resistance associated with the shape of the aperture (Kim et al., 2011). In this study, the first trapping design was a U-shaped sieve as it fits the form of *C. auris* to be entrapped. The aperture degree 0^0, which was chosen, has a streamline passing through that matched the trajectory of a cell of an ellipsoidal microbe. Although the stagnation point could vary, the chamber was built specifically for *C. auris*. Fig. 6 shows the flow that describes all the sieves which are placed in the designed chamber.

The simulation is a two-dimensional fluid. The fluid is initially flowing from left to right as shown in the direction of the arrow in Fig. 6. The barrier, which is the sieve that is depicted on screen, creates vortices and diverts fluid. The colors indicate curl or the local rotational motion of the fluid, which means there are two motions described as there are two various shades of colors. The darker the shade the stronger the motion of the curl. The slit or the aperture is where the bacteria is supposed to be trapped. The shade shown at the slit is light, which makes the motion of the fluid to be faint or weak.

Figs. 7-10 show the simulation for the trapping design of *E. coli*. The fluid flows from left to right and the colors indicate the local rotational motion of the fluid. The trapping of the bacteria is depicted in the tracers.
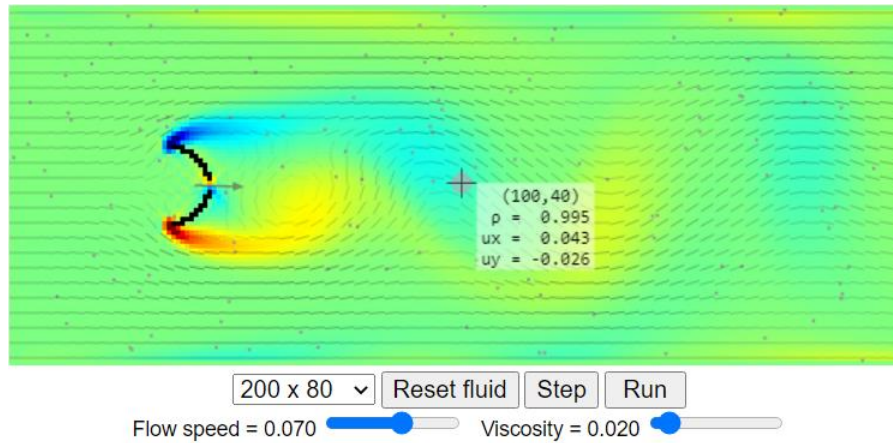

Fig. 6: The fluid dynamic simulation of one sieve with 0° aperture. It has a flow speed of 0.070 and a viscosity of 0.020
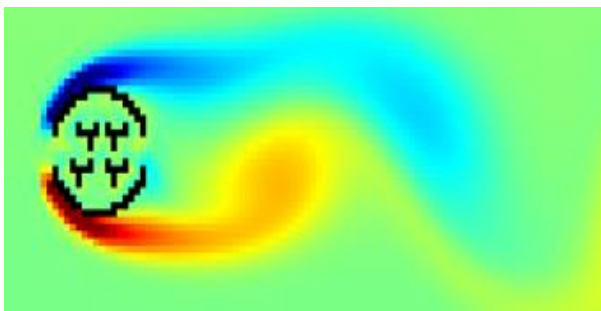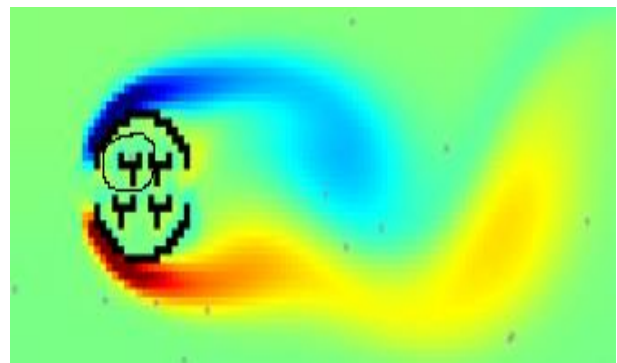

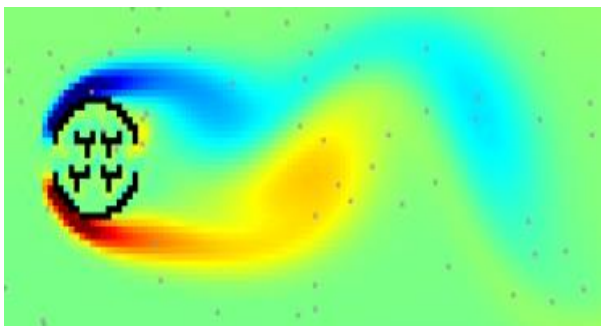**Fig. 7:** The barriers divert the fluid and create vortices

Fig. 8: The tracers denote as the bacteria. This shows how it enters the trapping chamber

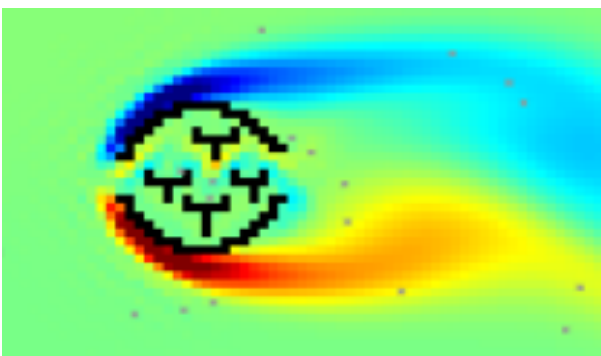**Fig. 9:** Tracers, which are the bacteria, are trapped

Fig. 10: Encircled are bacteria that are trapped

4. Conclusion

This study successfully shows trapping designs for *Candida auris* (*C. auris*) fungi and *Escherichia coli* (*E. coli*) bacteria using microfluidics. The approach in these designs is for the materials to be inexpensive, simple, and accessible in laboratories. The proposed designs are not simulated in a numerical approach. The designs are modified versions of a device that is constructed and simulated with numerical predictions, which include sizes and apertures in consideration of the specified microbe. The microfluidic designs will be useful during experiments in deciphering necessary information of the bacteria and fungi and will be a platform in modeling numerous biomedical assays and in the optimization of biophysical tools.

Recommendation

It is recommended that the microfluidic chambers will be fabricated and will be tested using

Candida auris (*C. auris*) fungi and *Escherichia coli* (*E. coli*) bacteria.

Compliance with ethical standards

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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