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Technical Note

Design of a microfluidic device with a non-traditional flow profile for on-chip damage to zebrafish sensory cells

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Abstract

Hearing loss affects millions of people worldwide and often results from the death of the sensory hair cells in the inner ear, and exposure to intense noise is one of the leading causes of hair cell damage. Recently, the zebrafish lateral line system has emerged as a powerful \textit{in vivo} model for real-time studies of hair cell damage and protection. In this research, we designed a microfluidic device for inducing noise damage in hair cells of the zebrafish lateral line. As the first step, a 3D computational fluid dynamics (CFD) simulation was utilized to predict the flow pattern inside the device. An ideal flow pattern for our application should feature higher velocity near the sidewalls to over-stimulate the externally located hair cells, and minimum flow in the middle of the channel to protect the fish from high pressure on the head. Flow induced from ordinary channel geometry with a single inlet/outlet pair would not work because the parabolic velocity profile features the maximum flow speed in the middle of the channel. In order to achieve the desired flow pattern, sidewall inlet/outlet pairs were used to suppress the growth of boundary layers. CFD simulation was used to design parameters such as the dimensions of the microfluidic channel and the angle of the inlets and outlets. It was found that in the case of an empty 2.0 mm wide channel with the inlet/outlet pairs set to 45\textdegree, the flow velocity at the side of the channel would be 6.7 times faster than the velocity in the middle, approaching the optimal flow characteristics. In the case of a fish-loaded channel, simulation shows that a 1.0 mm wide channel with a 60\textdegree inlet/outlet angle creates the lowest pressure (0.3 Pa) on the fish head while maintaining a reasonably strong shear stress (1.9 Pa) on the lateral line hair cells.

Keywords: microfluidics, CFD, boundary layer, PIV, zebrafish

(Some figures may appear in colour only in the online journal)

1. Introduction

Sensory hair cells, located in the inner ear of all vertebrates, are the primary receptors for detecting sound and vestibular stimuli. These cells represent the first neural step in hearing and balance, converting mechanical energy into electrical signals that are transmitted to the central nervous system. Death of these hair cells is a major cause of hearing and balance deficits worldwide [1]. Noise-induced hearing loss represents a significant fraction of all sensorineural hearing impairment, yet our understanding of how hair cells die in response to noise and how to protect these cells from damage is far from complete. Fish and amphibians have a second hair cell hearing system called the lateral line, which helps detect water
movement and is important for behaviors such as schooling, prey detection and predator avoidance [2]. Lateral line hair cells are located in clusters along the surface of fish, offering easy accessibility for noise over-stimulation and visualization of resulting damage. Recent work demonstrates that the lateral line of larval zebrafish is a highly tractable model system for understanding drug-related hair cell damage and discovering protective compounds [3]. However, there is no comparable experimental paradigm to assess sensitivity to noise-induced damage and objectively screen for protective compounds. Such a device would be enormously beneficial to the field.

In order to induce noise damage only in hair cells without damaging the fish itself, the device has to be small enough for a larval zebrafish (4–6 mm long for 5–6 day old fish), and the flow inside the device must be precisely controlled. Therefore, a microfluidic device is recommended as an experimental platform. Previous research has used larval fish inside lab-on-chip devices to monitor organism development or for targeted delivery of chemical compounds in small fluid volumes [4–8]. However, these studies have not attempted flow-mediated sensory damage caused by noise or high shear stress. It is thus necessary to design a novel microfluidic configuration for generating the desired flow pattern in order to induce lateral line damage to a larval zebrafish in the device. The flow velocity inside the device should be higher at the sides to cause high shear stress along the lateral line of the fish and lower in the center of the channel to prevent damage to the internal organs of the animal. These requirements necessitate the manipulation of the flow pattern inside the device. It is impossible to obtain our target damage pattern with a single inlet/outlet microfluidic channel, as this design will generate maximum flow velocity at the center due to the merging of boundary layers developed from the sidewalls. Prior studies suggest that multiple inlet/outlet pairs can be used to control the depletion boundary layer inside a microfluidic channel [9, 10]. We have built on this approach to manipulate the flow inside the boundary layers using sidewall inlets and outlets.

For design purposes, we employed computational fluid dynamics (CFD) modeling with experimental verification. In recent years, CFD has been widely used for microfluidics studies, especially for designing and optimizing devices [11–16]. It is possible to use CFD simulation to predict the flow pattern as well as the shear stress inside a microfluidic channel and to reduce unnecessary experimental tests. Since our system needed to be optimized with several parameters—such as the number of inlet/outlet pairs, the angle of the inlet/outlet and the dimensions of the device—CFD simulation serves as a useful tool to test multiple designs prior to device fabrication. In this paper, we utilized a 3D CFD simulation model to first predict the flow pattern inside an empty microfluidic device for the purpose of optimizing the flow profile. Then, we introduce a fish-shaped body into the CFD model for the purpose of predicting frontal pressure and lateral shear on the fish body.

2. Design considerations

Figure 1 shows one of our microfluidic device designs, consisting of a main channel and 12 inlet/outlet pairs. The length and depth of the main channel were set to 6 mm and 1 mm, respectively, to prevent the fish from moving too much and escaping from the fluid flow. Inlets and outlets were located along the sidewalls to suppress boundary layers. The area of each inlet/outlet was set to 0.25 mm² (0.5 mm × 0.5 mm). In order to induce shear stress to the hair cell bearing surface of the fish, we tilted the inlets/outlets as shown in figure 1(b). The inlets/outlets were arranged at the same angle, which is different from previous research, in order to save space for fabricating a more condensed channel array and to limit the length of the device. Since fish tend to stay in low-velocity areas, we chose to close both ends of the main channel to minimize the flow velocity along the center line. Two layers of inlets/outlets were arranged alternately, which is shown in figure 1(a), to eliminate the zero flow ‘dead zone’ found in a single-layer design.

3. CFD simulation

As mentioned before, in order to predict the flow pattern inside a microfluidic device, a 3D CFD technique was used. In this study, commercial software packages (FLUENT® 6.3 and GAMBIT® 1.3; FLUENT Inc., Lebanon, NH, USA) were used to build the computational domain and the models for the microfluidic device design using the finite volume method. Since the designed microfluidic device was not symmetric, especially in terms of the arrangement of inlet/outlet pairs, a 3D model was used rather than 2D. The number of cells used to discretize the fluid domain ranged from 100 000 in the case of a 1.0 mm fish-loaded channel to 500 000 in the case of an empty 2.0 mm channel. First-order schemes were
used because they are known to provide better convergence of calculations than second-order schemes, although they provide less accurate results due to the increased error in numerical calculations. The fluid was assumed to be water, and the flow was assumed to be steady, incompressible, non-isothermal and laminar for numerical calculations. Since heat transfer inside the microfluidic device was not considered, only the continuity and momentum equations were used in this study. The walls were assumed to be smooth and the standard wall conditions were applied. The total flow rate was set to 18 cc min\(^{-1}\) by assuming that the flow of each inlet was identical (1.5 cc min\(^{-1}\) each). In order to prevent merging boundary layers from each side, the same amount of flow was applied to each outlet as well.

To study the effect of inlet/outlet angles (the \(\theta\) described in figure 1), four cases were considered: 0°, 45°, 60° and 75°, with a constant 2.0 mm channel width. To explore the effect of the channel width, three cases were simulated by varying the width of the main channel to 0.5, 1.0 and 2.0 mm, while keeping the inlet/outlet angle at a constant 45°. The case of a single inlet/outlet pair at both ends of the main channel was also simulated to estimate the improvement offered by the designed device. After conducting the CFD simulation, the z-velocity, which was the velocity along the length of the main channel, was estimated along the center line and along the sidewalls (0.1 mm from the sidewall) at the height of 0.5 mm (halfway up the vertical dimension). Moreover, the effects of fish inside the designed channel were also explored using the CFD simulation. Figure 2 shows the generated mesh of a 1.0 mm straight channel containing a single simulated fish, where the shape of the modeled fish was simplified based on the shape of a zebrafish larva. The length, width and height of the modeled fish are 4, 0.5 and 0.5 mm, respectively, again based on the actual fish size for a five day-old larva. The body of the modeled fish is smooth-walled with a no-slip boundary condition, and the fish is placed in the middle of the channel. The pressure in front of the fish and the shear stress on the side body of the fish were then predicted to observe how the presence of the fish altered the flow characteristics in the channel.

4. Micro-PIV experiment

In order to validate the CFD simulation, micro-particle image velocimetry (PIV) experiments were conducted. A microfluidic channel was fabricated in an acrylic plate with a micro-milling machine (Mini Pro 3, Minitech). The size of the main channel was 0.3 mm wide and 0.4 mm long, which was built to fit the maximum field-of-view of our PIV system. The microfluidic device had two inlets/outlets to reduce fabrication complexity, and \(\theta\) was set to 45° as a representative case for the micro-PIV validation experiments. The fabricated microfluidic channel was installed in a test loop, which was operated by a single syringe pump. The system used two syringes: one that was compressed for inflow and one that was expanded for outflow. The pump simultaneously drove the two syringes at the same rate throughout the experiments. The pump provided distilled water, which was seeded with tracer particles for micro-PIV imaging. The particles were 1 \(\mu\)m polystyrene spheres coated with fluorescent dye, which absorbs and emits light at differing wavelengths. This coating preferentially absorbed light at 532 nm, corresponding to the micro-PIV illumination, while it emitted at 612 nm. This response permitted the filtering of incident light from the particle images. The particle concentration was approximately 0.05%, selected for optimal particle visibility without saturation.

The tracer motion was studied using a commercial micro-PIV system (TSI, Inc.). This used a dual-head, frequency-doubled Nd:YAG laser, which provided two 50 mJ pulses of light at a wavelength of 532 nm. The laser was attached to a liquid light guide that directed the beam into an inverted microscope. The light passed through a 10X microscope objective with a numerical aperture of 0.25, which focused it in a plane located inside the test device. The resulting particle emissions passed back through the objective to a filter cube, which removed any incident reflections. The remaining emitted light was then directed to a 1.4 megapixel digital camera to capture the tracer images. The laser and camera were operated using a synchronizer with 200 ns resolution, permitting fine control of the time delay between images.

5. Results and discussion

5.1. Validation of CFD modeling

Figure 3 shows the images obtained by the micro-PIV experiment and CFD simulation under the same conditions. Similar velocity distribution was seen in both cases. We can clearly see that flows from both inlets were bent toward the outlets. Note that in this test size, both flows merged a little in the middle of the microfluidic channel. According to the simulation results, this merging is not expected to happen in a fish-sized device. Nevertheless, the PIV results indicate that CFD simulation can be applied for the device design considered above.

5.2. Optimization of the flow profile in the microfluidic device

After the validation of our numerical model, we started our design process by simulating a case with a 2.0 mm wide main
channel and 60° of the sidewall inlet/outlet pairs. This case is used to help us understand the basic flow characteristics in this kind of microchannel geometry. Figure 4 shows the predicted flow pattern inside the designed microfluidic device. Since a flow rate was applied to both the inlet and outlet, water injected into the main channel escaped through the nearest outlet. That caused rounding of the flow pattern along the sidewalls and prevented merging of boundary layers at the center. It was shown that the ‘dead zone’, which meant the area where the velocity was zero, was eliminated by two layers of flow that were arranged alternately. Each layer had a role to supplement the ‘dead zone’ of the other layer.

Figure 5 compares the flow pattern for a single inlet/outlet pair (straight channel) and multiple inlet/outlet pairs (designed channel). It was found in our preliminary trials that a 2.0 mm wide channel is suitable for demonstrating the effects of the sidewall inlet/outlet pairs on the flow field. Therefore, the channel width in figure 5 was fixed at 2.0 mm. In the case of the straight channel, the boundary layers from the sidewalls merge at the center, such that the velocity is maximal at the center of the channel. The z-velocity at the center was 0.224 m s⁻¹, which was 96.5% higher than that along the side (0.114 m s⁻¹). In contrast, in the case of the designed channel with multiple inlet/outlet pairs, it was shown that the highest velocity was along the sidewall (0.054 m s⁻¹), which was about 6.7 times faster than the z-velocity at the center (0.008 m s⁻¹). This latter arrangement closely approximates our desired channel characteristics.

Figure 6 shows the predicted flow pattern when the inlet/outlet angle (θ) is varied, and figure 7 shows the average z-velocity at the center and side for each case, calculated by the software based on values in each node. The channel width was set to 2.0 mm. It is shown that the z-velocity along the sidewall increases as the inlet/outlet angle increases. When the angle was 75°, the z-velocity increased approximately 20 times as compared to 0° (orthogonal to the sidewall). In contrast, z-velocity at the center decreased as the angle increased, since the water escaped before the merging of the boundary layers. Interestingly, the center z-velocity increased in the case of the 60° inlet/outlet angle, because the z-velocity at the sidewall was fast enough to reach the end of the main channel, resulting
in the flow being reflected to the center. This problem could be solved by extending the length of the main channel. However, the fish may move too much if the length of the main channel is too long.

Figure 8 shows the predicted flow pattern versus the width of the main channel, and figure 9 shows the average z-velocity at the center and side for each case. The inlet/outlet angle was set to 45°, since it gives the largest side/center velocity ratio, according to figure 7. The results show that the velocity at the sidewall decreased 20% when the width was increased from 0.5 to 2.0 mm. However, the z-velocity at the center was reduced by 50% (near 0) with a 2.0 mm channel width, as the flows from each sidewall merged together as described in section 4. Therefore, a wider channel might show better performance in terms of achieving an optimized flow profile. However, for our purpose, the channel size should be compatible with the fish size (about 0.8 mm wide at the head and gradually tapering to near 0 at the tail). Too small a channel will not be able to accommodate the fish, and too wide a channel will leave too much room for the fish to swim around and escape the fluid flow.

5.3. The effects of the fish

The results shown above demonstrate that we can manipulate the flow pattern inside a microfluidic device using multiple inlets/outlets. This technique can be applied to any applications that require spatial flow control in a microchannel. In this study, we focus on the application of damaging fish lateral line hair cells while maintaining the viability of the fish. We performed further simulations to explore the effects of the fish in the design channel. In these simulations, the 1.0 mm wide channel was used because it is about the size of the fish head. Therefore, the fish will be kept relatively stable in the channel. Figures 10 and 11 show the simulated contours
of the pressure field and shear stress in a fish-loaded channel. The numerical values of the fish frontal pressure and lateral shear stress are reported in Table 1.

From Table 1, we can see that a straight channel will induce a very large pressure on the fish, while our design—especially the 60° inlet/outlet case—will reduce that pressure to less than 0.1% of the straight channel. The trade-off is that the shear stress on the fish lateral line is also significantly reduced (5.6 times). Since maintaining the viability of the fish is critical for our experiments, we determined that the 60° inlet/outlet design is the most suitable for initial testing with live fish larvae.

Note that there will also be a net lateral force exerted on the fish body. We expect that the swimming force of the fish will be able to overcome this force. If the fish gets pushed toward one end of the main channel with large body deformation, then new simulation is needed to assess its effect on the flow.

Table 1. Average fish frontal pressure and lateral shear stress for a 1.0 mm wide channel with variable inlet/outlet angles.

<table>
<thead>
<tr>
<th>Case</th>
<th>Frontal pressure (Pa)</th>
<th>Lateral shear stress (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straight channel</td>
<td>418.9</td>
<td>10.6</td>
</tr>
<tr>
<td>45°</td>
<td>6.5</td>
<td>2.3</td>
</tr>
<tr>
<td>60°</td>
<td>0.3</td>
<td>1.9</td>
</tr>
<tr>
<td>75°</td>
<td>9.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Meanwhile, the viability of the fish may be another concern. In a previous study [8], zebrafish were pushed against a solid microchannel restriction and were immobilized for at least 24 h without any noticeable adverse effect on the fish. Experiments in adult zebrafish demonstrate that 36 h of noise exposure is sufficient to achieve significant damage to inner ear hair cells, suggesting that similar time scales may induce lateral line damage in larvae [17]. Future experiments will objectively identify the optimum flow speed and exposure time to achieve maximum hair cell damage with minimum damage to the rest of the animal.

6. Conclusions

In summary, we have used 3D CFD simulation to design a microfluidic device for inducing noise (fluid movement) damage to hair cells of the zebrafish lateral line. The device was designed to have 12 inlet/outlet pairs with two layers of arrangement. Simulation results indicate that the pressure on the head of the fish and the shear stress on the lateral line of the fish are greatly affected by design parameters, specifically the inlet/outlet angle and channel size. We determined that a 1.0 mm channel with 60° inlet/outlet pairs along the sidewalls of the channel is the most suitable for our fish experiments. Overall, we have shown that CFD simulation is a useful tool to optimize multiple parameters in our design.
significantly reducing trial and error in experiments and saving both cost and time.

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References