Producing and Measuring Oscillatory Shear in a Novel Microfluidic Chip

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MOTIVATION

- Understanding endothelial mechanobiology requires tools that expose endothelial cells (EC) to controlled mechanical stimuli and maintain ECs in vitro culture [1].
- Physical forces on ECs alters gene expression, influence vessel morphology, and contribute to vascular diseases [2].
- Limited instruments deliver a controlled mechanical stimulus to cell cultures under live microscopy. Microfluidic devices enable optimized dynamic cell culture conditions while reducing reagent consumption [2].
- Applying oscillatory flow within microfluidic channels is difficult due to dampening by the tubing external.
- Developing an oscillatory flow chip will enable studying how the shear stress level at different frequencies affects cells differently, as well as a comparison of the effect of unidirectional pulsatile flow and bi-directional oscillatory shear [1].

OBJECTIVES

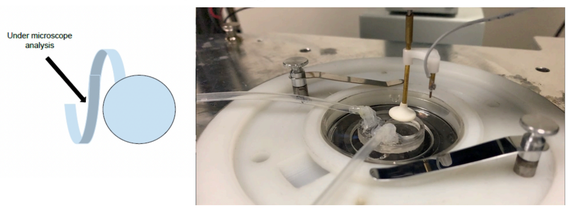
Cell biologists require instruments that can deliver oscillatory shear stress in a controlled manner. The primary objective of this project is:

Produce and measure oscillatory flow and shear stress in a novel microfluidic device, using particle image velocimetry (PIV), to evaluate the reproducibility and accuracy of the flow conditions.

METHODS

INSTRUMENT SETUP

- Oscillatory flow is induced by a plunger connected to a vibrating speaker (Fig. 1b). Frequencies ranging from 5 to 60 Hz and voltages of 5-10 V peak-to-peak are applied. Constant fluid flow rate (Q) can be applied via a syringe pump.
- Fluorescent 1.1 um particles seeded in distilled water were excited using a broad-spectrum light source and emission was recorded using a high-speed CMOS camera using frame rates of 800-1200 Hz depending on maximum fluid velocity.



a) b)

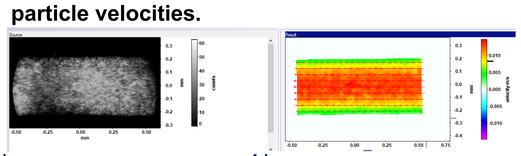
Fig. 1: a) Microfluidic flow channel consisting of a cylindrical reservoir preceding a rectangular channel (nominally 0.5 mm x 0.3 mm).

b) Microfluidic chip setup where plunger shaft connected to a speaker sits in a cylindrical well and compresses the surface membrane of the reservoir to impose oscillations with a range of frequencies and amplitudes.

METHODS (CONT'D)

PARTICLE IMAGE VELOCIMETRY (PIV)

- PIV processing (DaVis 8.4, LaVision Inc.) uses a cross-correlation algorithm between consecutive images to measure particle displacement between frames and determine particle velocities.



a) b)

Fig. 2: a) particle seeded fluid containing distilled water and 1.1 um microspheres fluoresce under a light source, allows for tracking velocities.

b) PIV uses cross-correlation to determine velocity vectors.

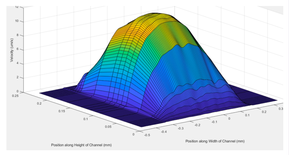


Fig. 3: Mean velocity profiles for laminar flow stacked from 21 planes across channel height, demonstrates fully developed paraboloid flow profile.

DATA ANALYSIS

- Matlab was used to translate velocity vector data into a 3D paraboloid to demonstrate laminar flow profiles and calculate shear stress (Fig. 3).
- Experimental channel dimensions were confirmed by applying fluid at a known flow rate, measuring the mean velocity and width of particle flow, and determining cross-sectional area and channel height.
- Average profile found from vector lines along the channel for each phase of flow cycle.
- Oscillatory flow was visualized by plotting average velocity profiles over course of at least one oscillatory cycle and extracting the central axis velocity vectors across the profiles, which were fit to a sinusoidal function.
- Maximum velocity amplitude from sinusoidal fits was determined for each frequency and applied voltage to determine parameters needed to deliver desired shear stress for future cell experiments.

RESULTS

SINUSOIDAL FLOW WAVEFORMS

- Channel dimensions were determined to be 491+/-7 mm wide by 282+/-5 mm high.
- Fig 6. of oscillatory velocity amplitude as a function of applied voltage for frequencies 10-60 Hz demonstrates a linear dependence on voltage.
- Velocity profiles are used to determine the wall shear stress values at the floor of the device where a cell monolayer is formed in cell experiments.

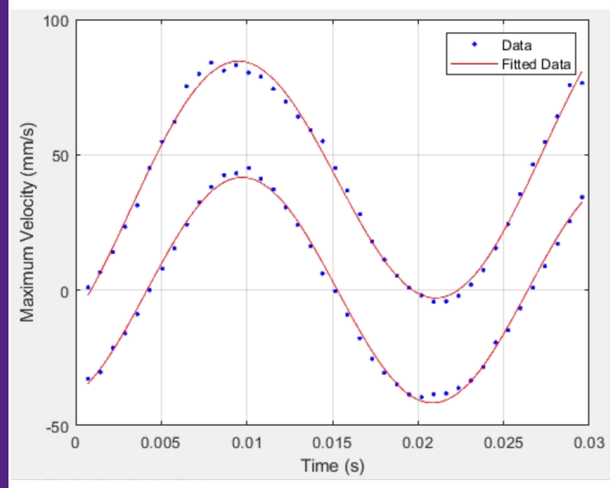


Fig. 4: Maximum velocities were extracted by plotting maximum velocities located along the middle of the channel width into a sinusoidal figure. Unidirectional pulsatile flow (above sinusoidal) was obtained by applying a theoretically calculated flow rate needed to cause DC flow.

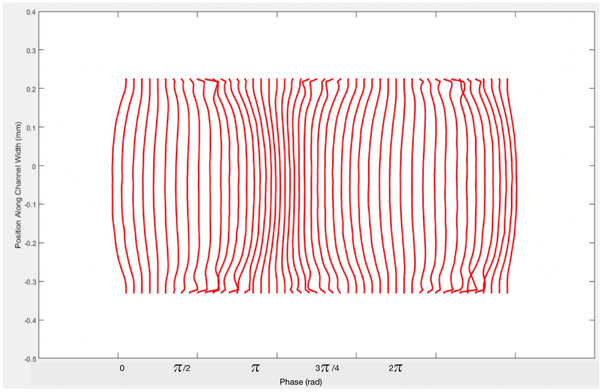


Fig. 5: Midplane was determined through locating the fastest-moving particles using PIV. Velocity profiles were plotted through averaging velocity vectors across length of channel for at least one oscillatory cycle.

LINEAR VOLTAGE DEPENDENCE

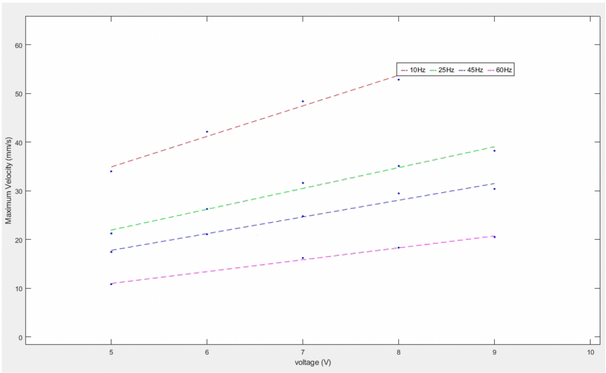


Fig. 6: Maximum velocity amplitudes are plotted for each frequency and voltage, providing a guide for cell biologists needing to know the necessary parameters to apply desired shear stress. Maximum velocity values are used to determine shear stress magnitude on walls of channel.

SUMMARY & DISCUSSION

- The microfluidic oscillatory flow chip demonstrated the ability to effectively and accurately deliver oscillatory flow between 10–60 Hz using 5–10 V, delivering a wide variety of shear stress conditions.
- Velocity amplitude as a function of applied voltage demonstrates overlap in resulting peak flow velocity, allowing analysis of EC response under fixed shear stress magnitude with varying frequencies.

FUTURE WORK

- Repeated measurements will enable determining intra-device and inter-device reproducibility to show the reliability of the microfluidic chip for cell experiments.
- A method for reproducible placement of the plunger at mid-channel depth without the use of fluorescent particles will be explored.
- Expanding the range of frequencies and voltages will be explored to offer a broader range for cell experiments if needed.

CONCLUSIONS

- Our analysis demonstrates that this microfluidic chip is able to execute controlled shear stress conditions to test how endothelial cells respond to oscillatory shear.
- Device shows potential in applying a range of pulsatile-flow frequencies and amplitudes, as well as unique waveforms.

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REFERENCES

[1] Lorusso, D.,Nikolov H. N., Soon, K., et al., Dynamic mechanostimulation of live cells with high-frequency oscillatory fluid shear during realtime microscopy

[2] Young EWK, Simmons CA. Macro- and microscale fluid flow systems for endothelial cell biology. Lab on a chip. 2019;10(2):143-60