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WILL MICROSCOPIC VORTICES HELP CURE CANCER?

A Simulation Case Study

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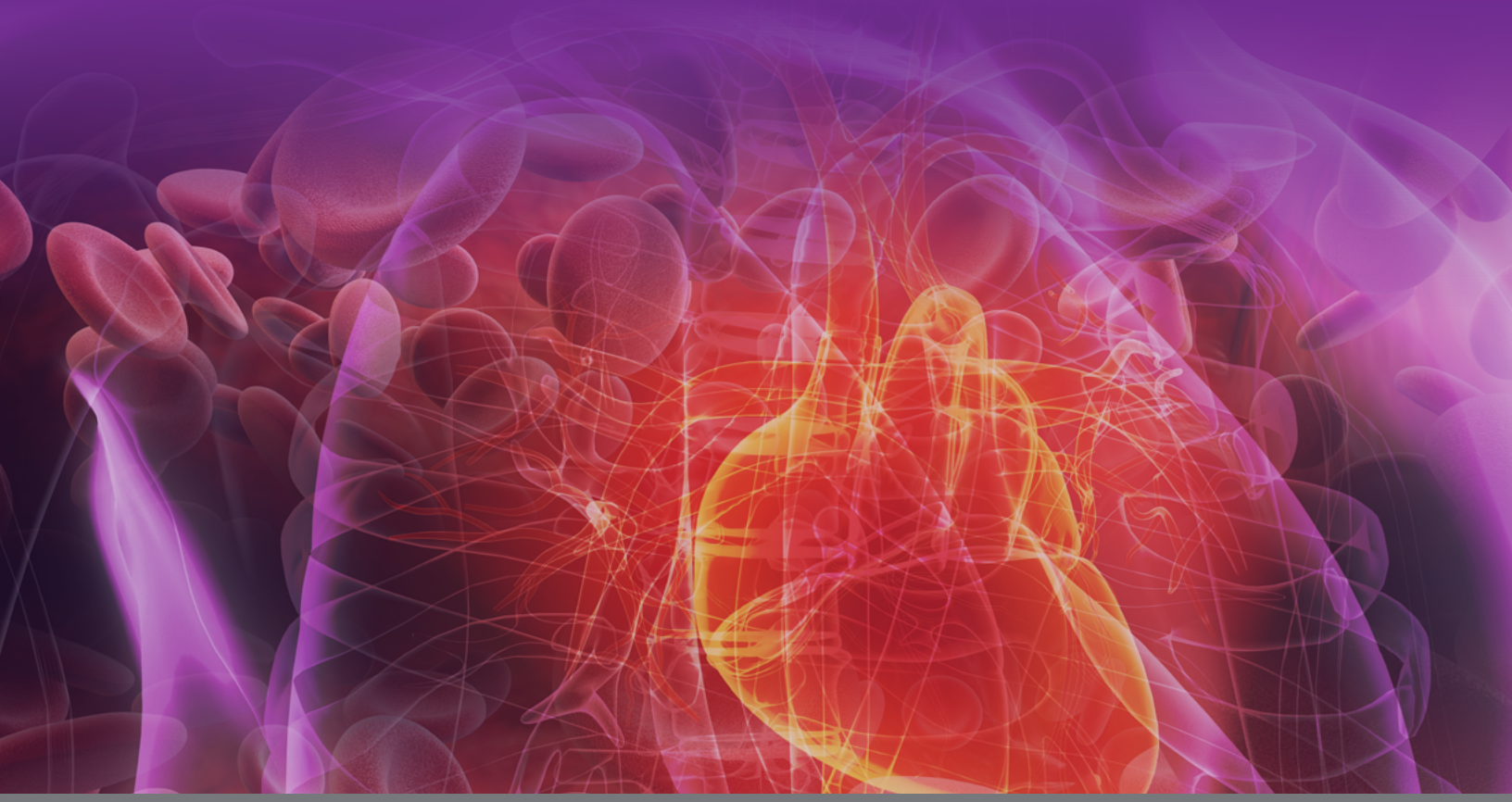
Indee Labs



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WILL MICROSCOPIC VORTICES HELP CURE CANCER?
A SIMULATION CASE STUDY

AUTHORED BY

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INDEE LABS

INTRODUCTION

Gene-modified cell therapies (GMCTs), such as chimeric antigen receptor T cells or CAR-T, represent the most effective therapeutic platform for many patients with advanced diseases such as relapsed and refractory leukemia or non-Hodgkin lymphoma. However, a host of problematic issues prevent their widespread use, most notably prohibitively high cost and an unreliable and scale-limited supply chain.

Microfluidic devices offer the possibility of straddling both these main barriers, to unlock the promise of GMCTs at a much wider scale. Indee Labs has optimized such a device to utilize the phenomenon of microfluidic vortex shedding (*μVS*), where the fluid forces created during *μVS* are used to gently and temporarily porate the cell membrane, allowing for gene delivery in a novel manner.

This case study introduces the problems inherent in the prevailing technology, describes the discovery process for *μVS* and the benefits it provides, and how modeling and simulation provided insight into the device development - by increasing understanding of the underlying fluid dynamics, vastly reducing the time required to optimize the design; and paving the way for timely realization of this method.

PROBLEM STATEMENT

Gene-modified cell therapies (GMCTs), such as chimeric antigen receptor T cells or CAR-T, represent the most effective therapeutic platform for many patients with advanced diseases such as relapsed and refractory leukemia or non-Hodgkin lymphoma. The FDA just approved the first two CAR-T therapies in 2017. Yescarta and Kymriah provide unprecedented outcomes for certain patients; however, they cost between $325,000 and $475,000 each. Even more concerning is that nobody really knows how to reliably manufacture these CAR-T therapies at scale, and CAR-Ts come with lengthy and error-prone development timelines.

CAR-T therapies are made by drawing blood from a patient, separating out specific T cells, then modifying them with an engineered virus prior to infusing the T cells back in the patient. During the manufacturing process, the engineered viruses deliver genetic material to the cells in a gene delivery step, modifying the T cells such that they attack cancer cells.

Manufacturing cell therapies typically requires a $43M manufacturing plant with a few hundred people. That work force can then make roughly 5,000 cell therapies per year. The latest FDA approvals are for gene-modified cell therapy or cell therapies requiring a gene delivery step, typically achieved with engineered viruses. Those engineered viruses come with at least a $200M upfront fee for engineered virus development for pharmaceutical companies like Novartis, along with a royalty on therapeutic sales. Novartis purchased a $43M cell therapy manufacturing plant in 2012 and entered into those engineered virus contracts with Oxford Biomedica in 2013. As of August 2017, Novartis has been able to manufacture sufficient CAR-T for about 250 patients.

Other pharmaceutical companies are investing hundreds of millions into their own engineered virus manufacturing plants. Meaning, it costs at least $250M just to development gene-modified cell therapy manufacturing capabilities. To make things worse, lead times for engineered viruses are about 18 to 24 months, and those viruses may not work afterwards. An article in the New York Times highlights this problem.

Engineered viruses come with a whole range of additional issues, including:

(1) The FDA requires patients to be tracked for up to 15 years after infusion;
(2) lentivirus and retrovirus have payload size limits of 10 kb, limiting cell engineering capabilities;
(3) surface-presented viral antigens increase risk of immunogenicity; and
(4) additional quality control procedures are required to minimize risk of mutagenesis.

OUR SOLUTION

The numbers above and additional issues make it easy to see why the team at Indee Labs is looking to use patent-pending microfluidic devices as a replacement for viruses. The device has the potential to completely eliminate the two primary barriers to widespread GMCT: cost and scalability. The device may also reduce or even eliminate the four issues described above.

Specifically, these microfluidic devices use a tiny array of posts to induce a well-known fluid dynamics phenomenon call vortex shedding. You can observe vortex shedding in nature by watching as clouds drift past mountains or as streams flow past rocks and boulders. Microfluidic vortex shedding (*μVS*) has even been used to mix fluids for chemical reactions.

The team at Indee Labs is the first to apply *μVS* to gene delivery, where the fluid forces or hydrodynamic conditions created during *μVS* are used to gently and temporarily porate the cell membrane, allowing for gene delivery in a novel manner – Indee Labs' first patent family is starting to get accepted, and they received a clear International Preliminary Report on Patentability (IPRP) at the Patent Cooperation Treaty (PCT) stage.

These *μVS* gene delivery devices are made using industry-standard semiconductor processes, and about 2,500 devices fit onto a 300-mm wafer. Typical wafer-manufacturing throughput means the devices scale to a 10M+ per year without much effort – thus, *μVS* gene delivery is more than 2,000-fold more scalable than GMCTs. Plus, upfront costs for a wafer are more than 13,013-fold less than upfront costs for Novartis' viruses.

The initial research and development costs have also been minimal -- about 150-fold less than Novartis' viral development costs – and demonstrating functional T cell modification across a few modalities took less than a year.

To date, the team has only relied on a portion of their angel funding, and since getting started in February 2017 has been able to demonstrate functional modification of T cells (DNA, RNA, protein and/or various complexes) both in-house and in collaboration with a notable research institute in the mid-West as well as with a private research university in the Bay Area.

Back to another major benefit of microfluidics: reduction in wait times by orders of magnitude. Wafer lead times for Indee Labs are 36- to 48-times shorter than virus lead times, eliminating year(s) of uncertainty about whether the biology works. With access to *μVS* gene delivery devices, research and development teams do not need to wait for test results. Prototype *μVS* gene delivery devices process T cells at rates of about 2 million per second, so experiments occur in seconds rather than year(s).

μVS gene delivery has a whole host of other benefits, including:

(1) Functional modification of T cells with mRNA, DNA, protein and various complexes;
(2) Leading yield because of high efficiency, high viability and high T cell recovery;
(3) No change in T cell growth and proliferation rates;
(4) No perturbation of the T cell state as measured by up to 10 activation or exhaustion markers; and
(5) Predictable modification of certain T cell subtypes from a single donor.

The team recently started technology evaluations with a few pharmaceutical companies. Next steps involve demonstrating clinically- and commercially-relevant production of GMCTs with a 2-week turnaround time.

This will prove *μVS* gene delivery can readily replace viruses while both (1) accelerating the GMCT development cycle and (2) increasing GMCT manufacturing scales.

AHA MOMENT

The discovery happened on accident while studying cell separation in microfluidic post arrays. The initial aim was to try to process milliliter volumes of diluted blood to help translate the cell separation technology from the lab to the clinic, where milliliter-scale blood processing is required.

For a fixed microfluidic design, you only have three options for increasing throughput: (1) increase the size of the microfluidic channels with high aspect ratio features; (2) increase flow rate; or (3) do a bit of both.

We first tested high aspect ratio features to make the pipe bigger, which lead to the next question: what is the upper limit for flow rates based on cell biology or how fast can we process cells without damaging them? Some very basic proof-of-concept experiments indicated a mammalian cell model remained highly viable, while also allowing for the intracellular delivery of a membrane impermeable dye. This suggested that flowing cells through a post array at high speeds allowed for gene delivery without adversely affecting that mammalian cell model. It also indicated that the core technology could be used to develop and manufacture GMCTs such as CAR-T.

DESIGN PROGRESSION

Delivering membrane impermeable dyes to a mammalian cell model is very different than modifying human T cells. This meant investment was required to further develop the technology.

While in grad school, Ryan got a crash course in business from the NSW Health Medical Device Commercialisation Training Program. Subsequently, Indee Labs was founded upon acceptance into SOSV's IndieBio Accelerator Program in San Francisco, where the team developed and demonstrated proof-of-concept macromolecule delivery to a T cell model.

Initially, we were only able to achieve gene delivery to a relatively small number of cells with low efficiency. Some design tuning led to inlet channels that allowed for higher throughput, greater cell recovery and greater cell viability. From there, we played with the various post and array geometries to arrive at an experimentally-optimized design.

TEAM FORMATION

Immune cell model data serves as a great proof-of-concept, but the market was most interested in the functional modification of human primary T cells, and for that we needed additional investment and a Chief Scientific Officer with the right skillset.

In December 2016, Indee Labs was accepted into Y Combinator, and in February 2017 Amy Twite, PhD joined our team as Chief Scientific Officer to lead primary T cell development. We then built out our lab at QB3@953 in San Francisco and hired Katherine Lau to help speed up the biology.

ROLE OF SIMULATION

For most of this effort, devices were designed through the more traditional, iterative process of hypothesis followed by experiment. Our chips are made using semiconductor processes, which meant each set of design iterations would require 6 to 8 weeks for prototyping and biological testing (We've since reduced this to about 2 weeks).

This worked, but optimal data was ultimately going to come from optimal design and simulation through computational fluid dynamics (CFD), the only way to get there. We brought Moein Kashani, PhD on to use CFD to simulate the flow conditions in our devices and further optimize device design.

ANSYS had recently initiated their Startup Program, which includes their CFD software and HPC packs, allowing us to access mission critical software in a startup-friendly manner. The software, however, was only half of the battle. We needed unique scientific talent and powerful computing software. Serendipitously, that ended up being rather easy.

Industry-experienced computational scientists like Moein are not found on the shelf at your local hardware store. Luckily, we'd been collaborating with the Australian National Fabrication Facility in South Australia and the University of South Australia's Future Industry Institute on various projects for 3 or 4 years, such that we had a long-standing working relationship. Moein ran the first few simulations on a project basis for us before joining the team.

The first simulations were of simplified two-dimensional geometries, which is a good proof-of-concept that allows for meaningful insight. Eventually, we needed device-scale simulation and in three-dimensions.

Rescale's startup program allowed us to utilize the AWS cloud computing credits we got through Y Combinator and SOSV/IndieBio on Rescale's cloud simulation platform. The ability to run simulations on the cloud enabled Moein to run transient and device-scale models three times faster than available through a desktop computer purpose-built for simulation.

To put things into perspective, we need to analyze human immune cells flowing through our microfluidic device. The entire device is about the size of your pinky nail, and the immune cells are about 10-times smaller than the thickness of a human hair. What makes things even more difficult is that the cells are moving about 10 to 20 meters per second or up to 50 miles per hour. Plus, the *μVS* that causes gene delivery occurs at a time scale of about 0.2 milliseconds, or roughly 70-times faster than a hummingbird beats its wings. The only way to really see what is going on in the chips is through simulation. Simulation also allows us to look at different flow conditions and device designs without having to build and test them. Simulation both speeds up the iterative design process while reducing development costs.

HOW SIMULATION LED TO A SOLUTION

Simulation allowed us to answer a lot of lingering questions like: (1) how long does it take for the flow condition to develop? and (2) are all cells being processed in a uniform matter? Simulation also allows us to check these variables for different design ideas in a manner that is not available through experimentation, leading to a pre-optimized design that can then be tested by Amy and Katherine in our lab to determine if these simulated designs that improve the fluid dynamics also improve the biology.

VERIFICATION & VALIDATION OF THE SOLUTION

Simulation provides greater insight into our technology, but that insight is only useful if the simulation is accurate and that accuracy can be assessed. Thus, we've taken steps to maximize the accuracy of our simulations within the typical startup constraints. This includes comparing the simulations with similar bits of literature, engineering correlations and checking things like the experimental fluid viscosity at representative shear rates then inputting those experimental numbers into the simulation.

All gene delivery devices have relied on cylindrical posts to generate flow conditions thought to induce vortex shedding. In practice and prior to computational analysis of full device-scale, we started benchmarking with simulation of flow around an isolated micro-cylindrical post, results shown in **Fig. 1,** to study the parameters that affect the microfluidic vortex shedding. We used a 2D spatial unit geometry to minimize model development time and computational requirements. This provides us with additional insight and reasonable approximations to aid in the development process. **Fig. 1** indicates how the flow conditions develop within the microchannel and over time, and how vortex shedding can be identified by the soft curvature of streamline in the wake.

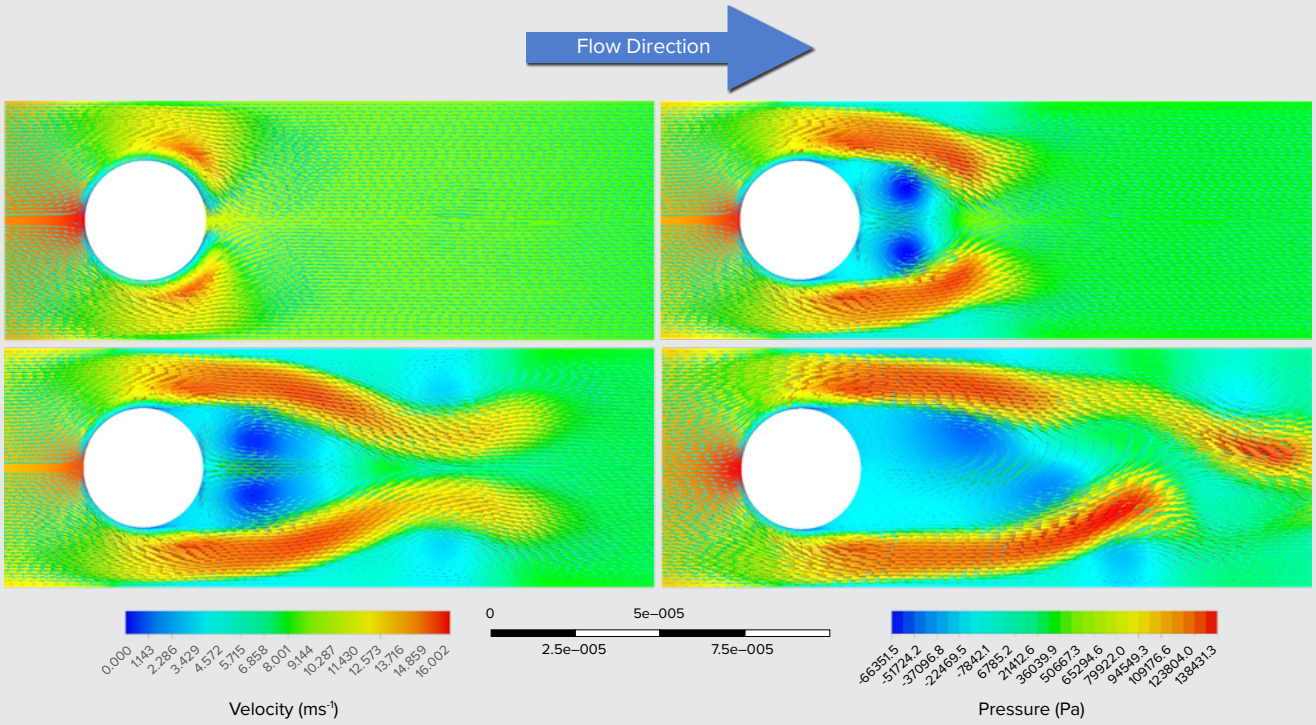

Figure 1:
Velocity vectors and pressure contours in microchannel and around the micro-cylindrical post

In the next step and to simulate a part of full device-scale, simulation was conducted for micro-cylindrical posts in a post array, to study the boundary effect and influence of vortices on the flow regime and turbulence. **Fig. 2** shows the velocity contours of this simulation and flow condition progress over time from initial condition to a developed condition.

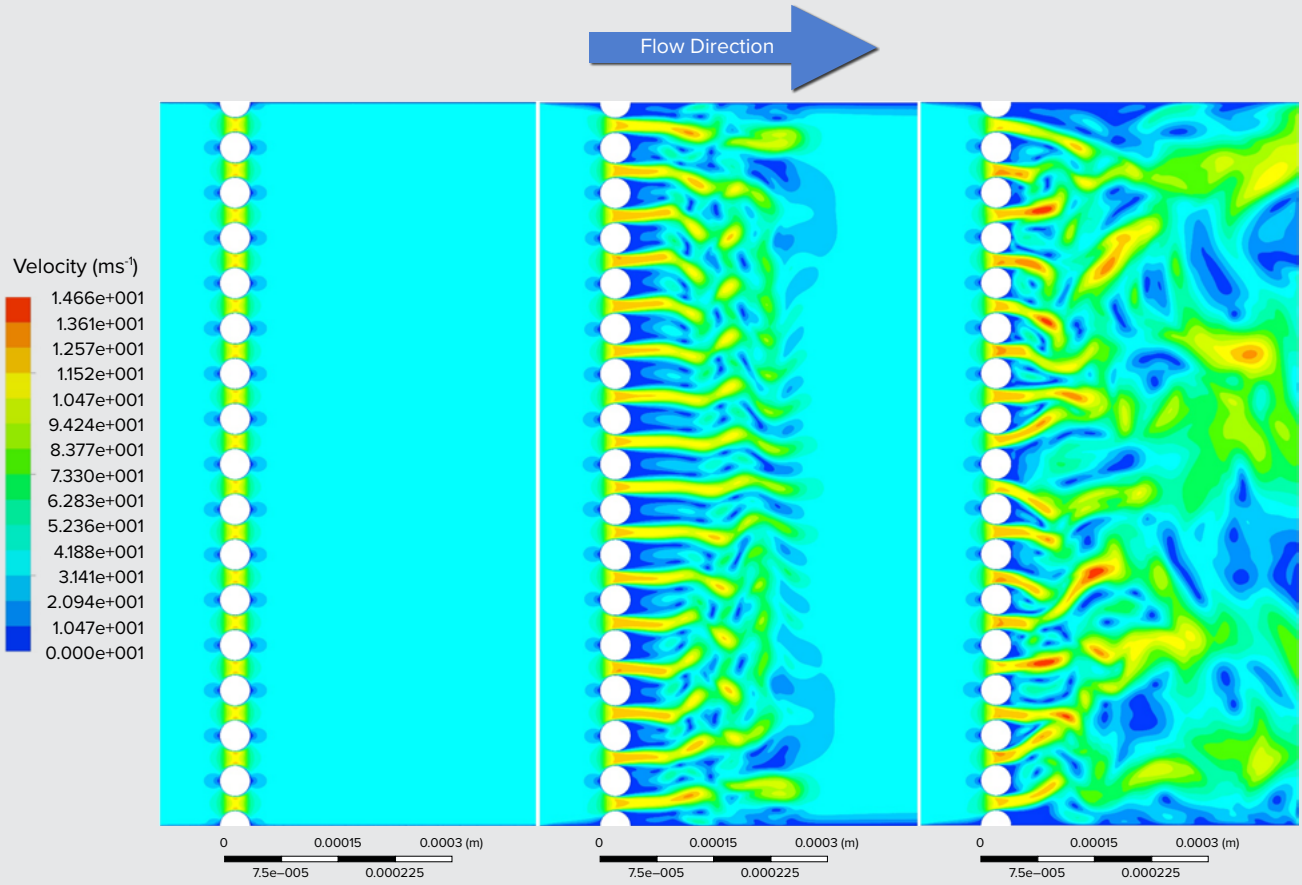

Figure 2:
Velocity contours around the micro-cylindrical posts in a post array

In the final step, we improved the earlier flow domain to run 2D device-scale simulation. The flow condition development within the device and over time can be seen in **Fig. 3**, where the last contour shows developed flow condition. This figure reveals a recirculation region at the inlet and different flow velocities between the middle of the device flow cell and edges of the device flow cell, which would not have been possible to grasp without doing the simulation made possible by the support from ANSYS, AWS, SOSV/IndieBio, Y Combinator and Rescale.

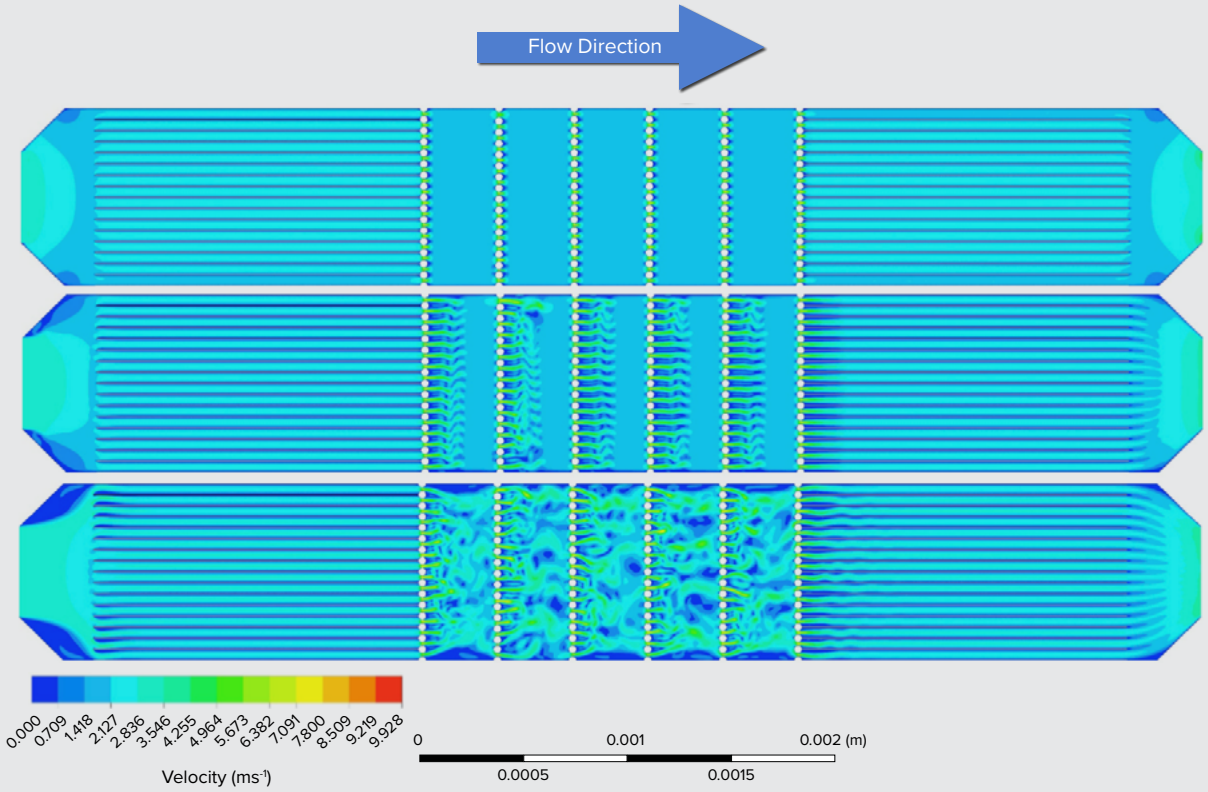

Figure 3:
Velocity contours in 2D device-scale simulation

Simulation adds a vast value in our work to design, test and optimize designs without having to fabricate the devices. We can design the next generation of devices to minimize both recirculation and maximize flow uniformity, to have more uniform cell processing while also reducing any uncertainty because of the simulated fluid dynamics. To continue the simulation and extend it to 3D spatial unit geometry, we are leveraging Rescale's cloud-based simulation and optimization platform to access massive-scale computing resources and run the simulation on hundreds of cores.

Moving forward, we are considering direct imaging of cells flowing through the chips using high speed optical microscopy and subsequent image analysis or computer vision. Plus, we can experimentally analyze vibrations within the chips through methods like non-contact vibrometry. This data could then be compared to the simulated data to further validate the accuracy of our computational models.

Ultimately, we will look to comprehensively validate our simulations using these methods. In the startup environment, however it is important to balance speed and resources with scientific rigor. We balance speed with scientific rigor by collaborating with well-regarded scientists and subjecting our science to peer-review both through our scientific advisors and through the more formal peer-reviewed publication process. We just got our first manuscript back from peer review!

SCALING THE DEVICES

Semiconductor processes have been well developed by the computing industry, meaning that transitioning from prototyping to scalable manufacturing is simple and straightforward.

The *μVS* gene delivery devices are made using a lot of the same processes that are used to make the processors found in every computer, smart phone and tablet. The semiconductor industry has made billions of these processors. We really aren't that concerned with scaling, which is a serious issue for GMCTs made with viruses. Plus, we already have automated computer vision software and other proprietary technology around microfluidic quality control. These technologies pre-emptively address the microfluidic quality control issues that have been a major barrier-to-entry for commercial microfluidics.

In more detail, the *μVS* gene delivery devices have micrometer-scale features with relatively simple geometries, whereas the computing industry requires nano-scale geometries with more complex geometries. Thus, it is reasonable to assume that (1) we will be able to manufacture our chips at the same scales as computer chips, and (2) it will be easier because the computing industry has already scaled computer chips with more complex structures.

NEXT STEPS FOR COMMERCIALIZATION

Our commercialization strategy is simple, focused, iterative and potentially lucrative. It relies on optimizing a patent-pending kit consisting of devices, reagents and protocol for a single application where there is substantial market pull (e.g., GMCT development and manufacturing). The vision is to then partner that specific kit with a pharmaceutical company. Subsequently, we iterate onto the next kit and then partner that kit.

This will be done in parallel with instrument development to generate revenues through (1) upfront, milestone and royalty licensing fees, (2) instrument sales and servicing, and (3) recurring kit sales.

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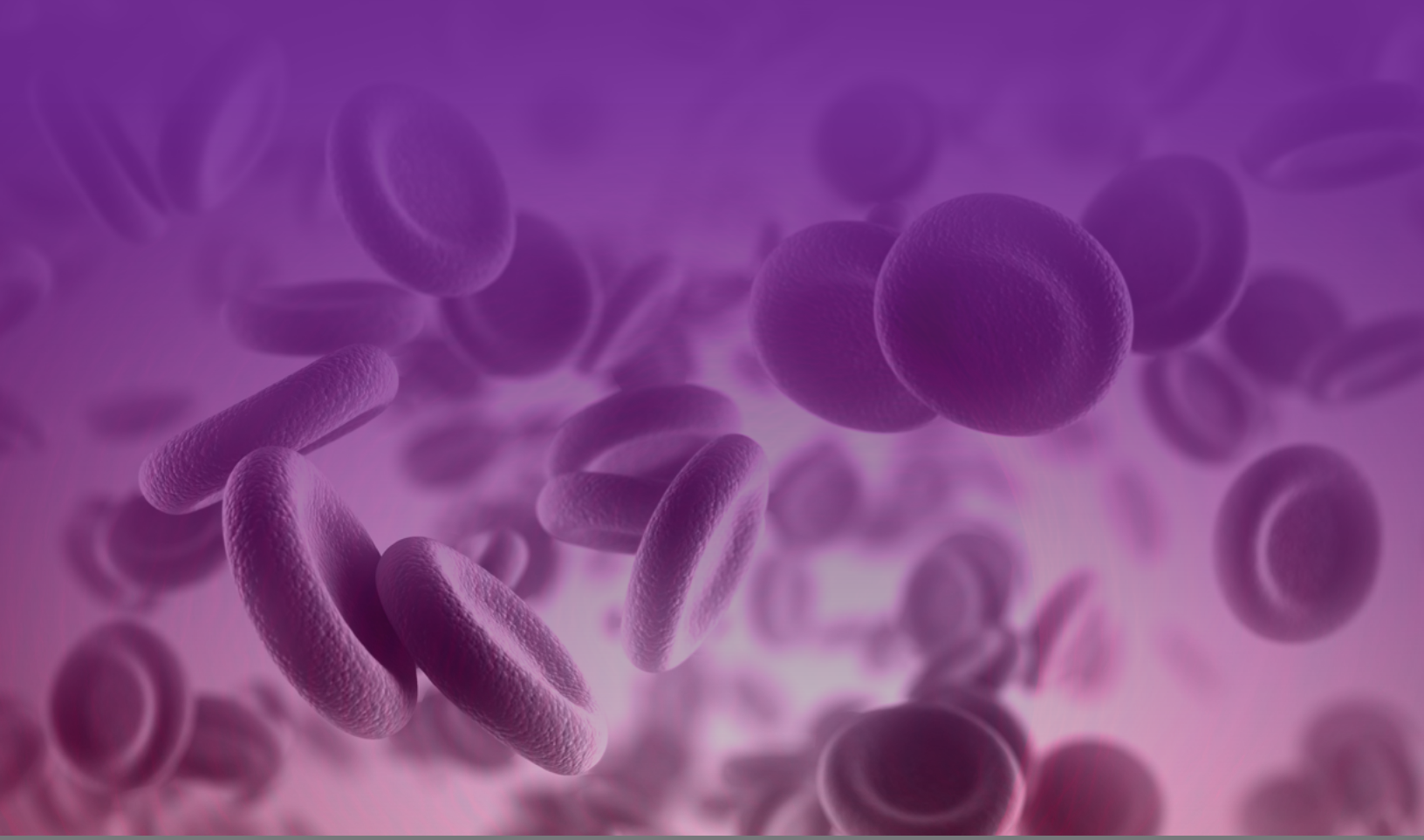

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