Extracellular fluid viscosity enhances cell migration and cancer dissemination

Henry Ching, Andrew Kistner, Colten Palkon

Georgia Tech

Background

- Cells respond to physical stimuli and viscosity is a key physical cue
- Cell migration is essential for development, tissue homeostasis, immune surveillance and cancer metastasis
- Mechanical forces from cell-substrate interaction regulate cell migration
- A viscosity close to water (0.7 cP) is used for in vitro cell assays, although interstitial fluid can vary up to 3.5 cP
- Supraphysiological viscosities (>40 cP) increase the motility of carcinoma cells in 2D surfaces
- Lowered extracellular fluid viscosities are known enhance cell migration and speed

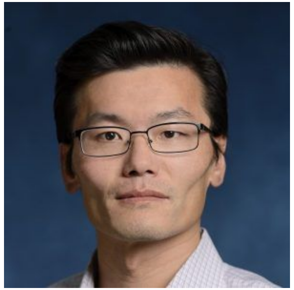
Authors & Contributors

Kaustav Bera (33 Publications)

- Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA
- Currently at University of Colorado as a post doctoral fellow
- Current research uses novel photo-responsive hydrogels with molecular biology and microscopy to study gut architecture and function of mammalian intestine

Konstantinos Konstantopoulos (325 Publications)

- Johns Hopkins Professor in the Department of Chemical and Biomolecular Engineering
- Developed a Microfluidic Invasion Network Device (MIND) for diagnosis, prognosis and precision care of cancer patients
- Konstantopoulos' group works to elucidate how vascular and tissue microenvironments regulate the dissemination of cancerous cells



Sean X. Sun (263 Publications)

- Johns Hopkins Professor in the Department of Mechanical Engineering
- His research improves on current knowledge of cell motility, molecular motors, proteins and membranes, statistical mechanics, and theoretical biomechanics, and biophysics

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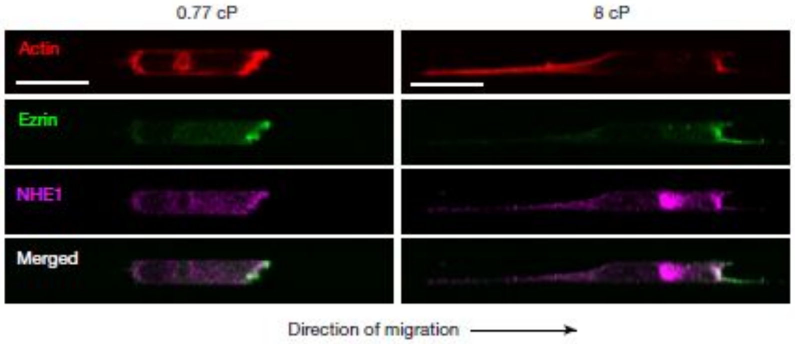
Significance

- The work in this paper is for potentially controlling the metastatic potential of breast cancer cells. This would allow for isolation of the cancer into just a single tissue.
- Cells pre-exposed to elevated viscosity acquire receptor dependent mechanical memory through transcriptional control of the Hippo pathway. This will lead to increased migration of the cancer cells.
- Elevated viscosity levels reduced wound closure time without altering cell proliferation relative to baseline viscosity. This meaning that extracellular fluid viscosity is a physical cue that can regulate motility of cells.
- More testing is needed to benefit breast cancer patients, but the study so far has promising results in mice and zebrafish.

Innovation

- Identifies **TRPV4** (Transient Receptor Potential Vanilloid 4) as a key sensor of extracellular viscosity
  - Enhanced calcium influx, RHOA activation, increased myosin II contractility, enhanced cell migration
  - Drives **viscosity-driven mechanotransduction**
- Contradicts conventional expectations and shows that **cancer cells migrate faster in more viscous environments**
- Demonstrates that cancer cells retain **memory of their mechanical environments**
- Multi-scale testing provides strong evidence



Microfluidics

Function:

1. The devices were loaded with mediums with varying viscosities/pharmacological agents before adding cells and then removed.
2. The cells resuspended in the specific mediums were added in to the cell inlet to create a pressure drive flow.
3. Cells were allowed to adhere and spread in the inlet wells, and the medium was added back in.
4. Devices were incubated at 37 C and 5% CO_2 before imaging

Cells were not exposed to chemotactic stimulus/medium flow during migration assay

Microfluidics

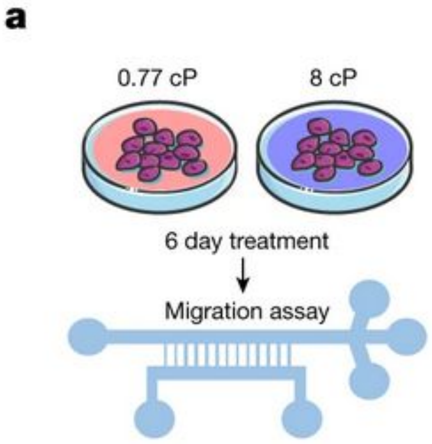
PDMS-based microfluidic created using standard multilayer photolithography containing:

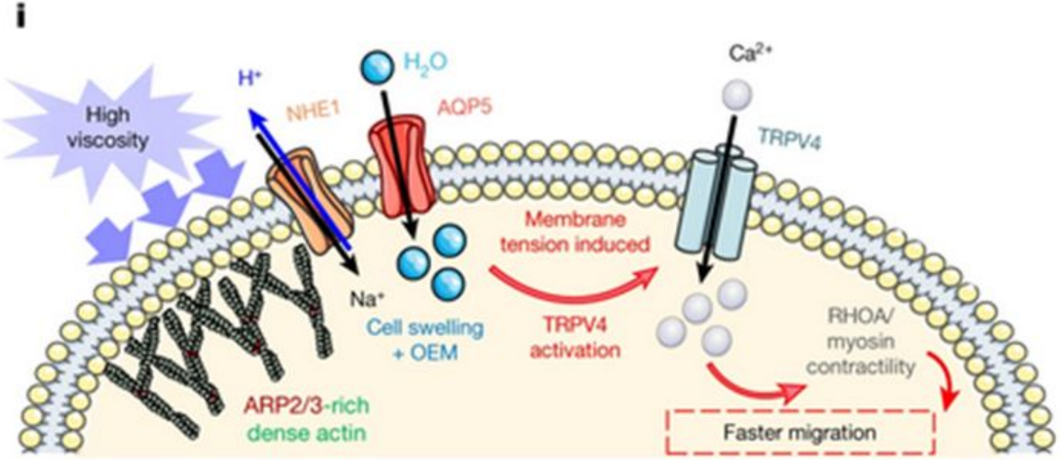
- Negative silicon wafer mold
- Array of parallel microchannels coated with collagen I
  - Soft baked SU-8 3010 negative photoresist with mask aligner
  - Baked with SU-8 developer
- Cell inlets were made with SU-8 3025 and a mask

No photos of microfluidic device provided

Approach

- Variety of cancerous and non cancerous cells
- Goal was to observe how cells respond to different viscosity mediums
- Used PDMS confining 3.5 wide μm X 10 high μm channels to test migration speed
- Cells imaged with fluorescence upon successful entry into microchannels with time lapse microscopy using fluorescein isothiocyanate filters
- Tested for viscosity sensing by cells using preconditioned viscosity in vivo

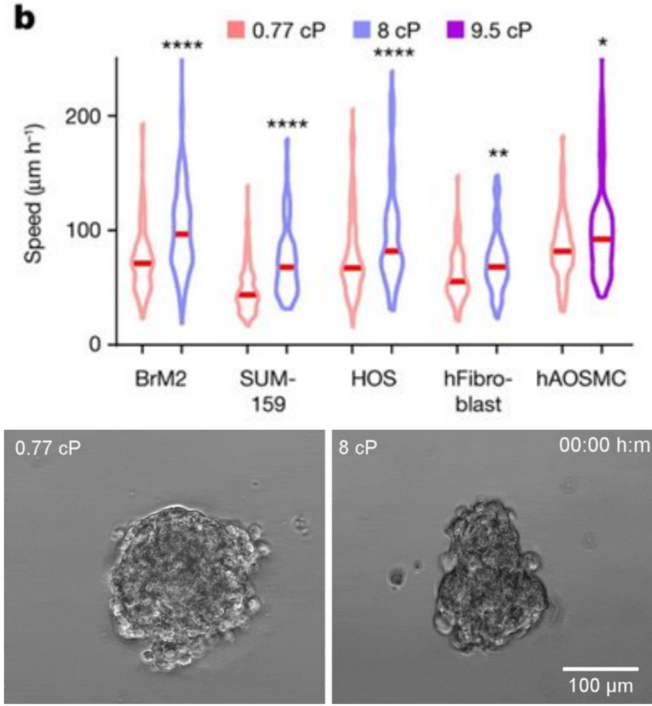




Results

Key Findings for increased extracellular viscosity

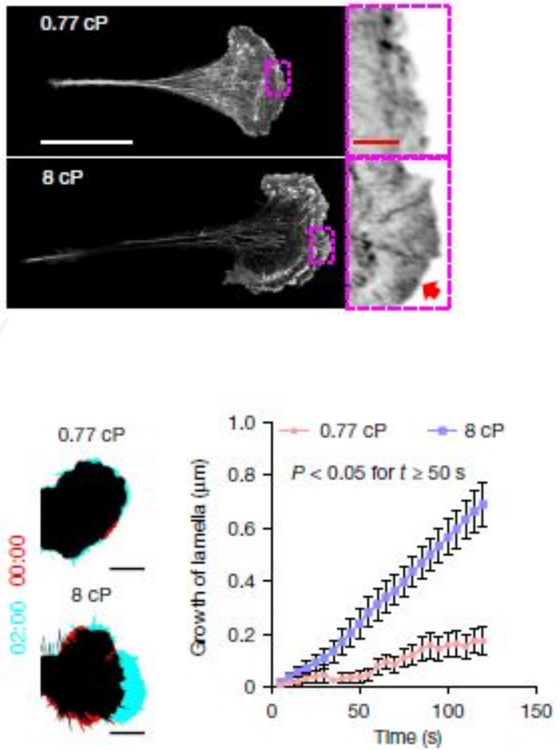
- Migration speed increase
- Accelerated cell dissociation
- Decreased wound closure time up to 50% quicker
- Increases cell volume due to NHE1- dependent cell swelling
- Induce actin remodelling (switch from blebbing to protrusive)
- Membrane tension increased similar to hypotonic solution
- Cell viscosity memory increased dissemination

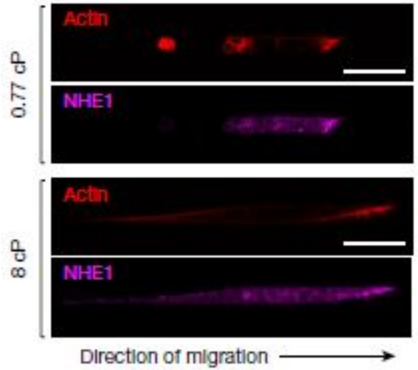


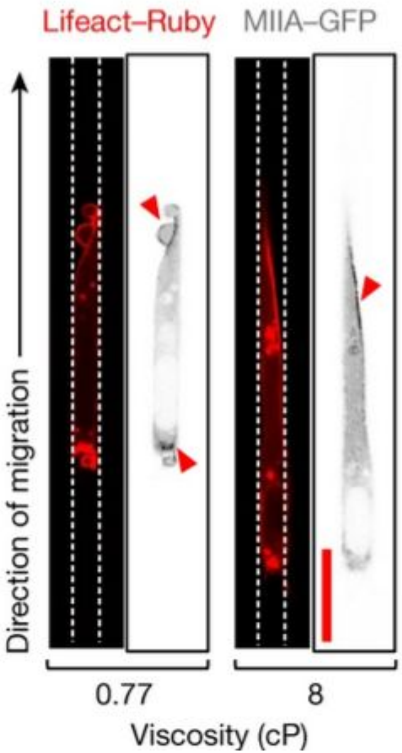
Follow up: Viscosity memory

- Metastatic potential of cancer cells
- Effects on morphogenesis

Results Continued







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Quiz Question 1

How does Increased extracellular viscosity affect tumour cell motility and dissemination?

a) Increases motility, decreases dissemination
b) Decreases motility, increases dissemination
c) Increases motility, increases dissemination
d) Decreases motility, decreases dissemination
e) Neither is affected

Quiz Question 2

What downstream effect does an increase in TRPV4 not cause?

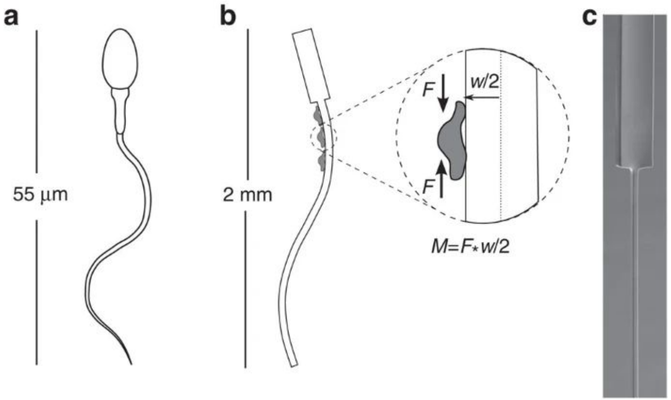
a) Enhanced cell migration
b) Enhanced calcium influx
c) RHOA activation
d) Increased myosin I contractility

A self-propelled biohybrid swimmer at low Reynolds number

Henry Ching, Andrew Kistner, Colten Palkon

Background

- Flagella are use by many biological species to swim in complex fluids
- In the microscale at low Reynolds number, viscous forces dominate inertial forces
- Spermatozoa generate complex bending patterns in the flagella by sliding microtubules relative to each other
- Bacteria rotate helix-shaped flagella to generate propulsion
- Using synthetic materials for flagellar propulsion has been achieved by using a magnetic corkscrew swimmer and a flagellar swimmer using magnetic beads connected by DNA. These swimmers are deflected by an external magnetic field to generate propulsion
- Swimmers can respond to external stimuli such as gradients, temperature, and light



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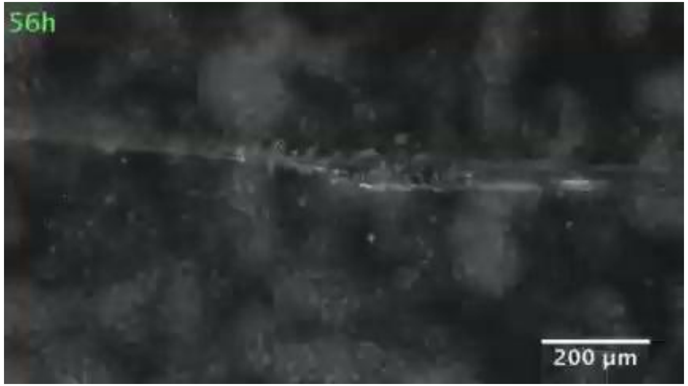
- Research includes cell mechanics, soft robotics, biomimetics, and biomechanics
- Worked in the Saif Lab

M. Taher A. Saif (117 Publications)

- Mechanical Science & Engineering Professor at University of Illinois Urbana-Champaign
- Research focuses on the mechanics of nanoscale materials and living cells. He explores the effect of size on mechanics and the role of mechanical force on cell functionality

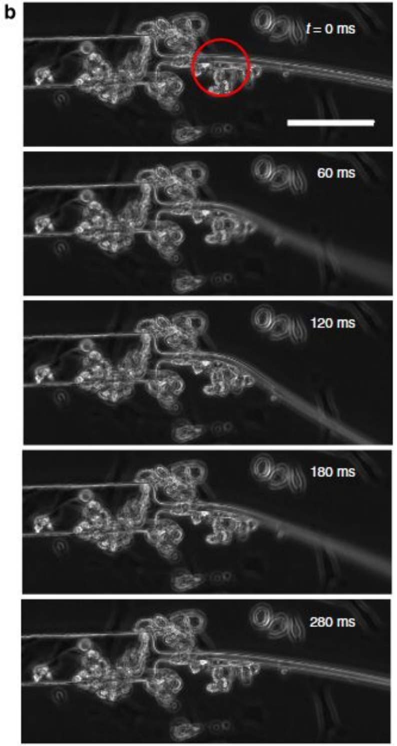
Significance

- The feasibility of an elementary flagellar swimmer actuated by contractile cells
- The characterization and exploitation of nonlinear interaction shown may facilitate the development of more complex biological machines
- Other homotypic cell types could be used instead of the cardiomyocytes as the contractile cells
- This study provides a foundation for a new class of self propelled, batch-fabricable, soft biological swimming machines



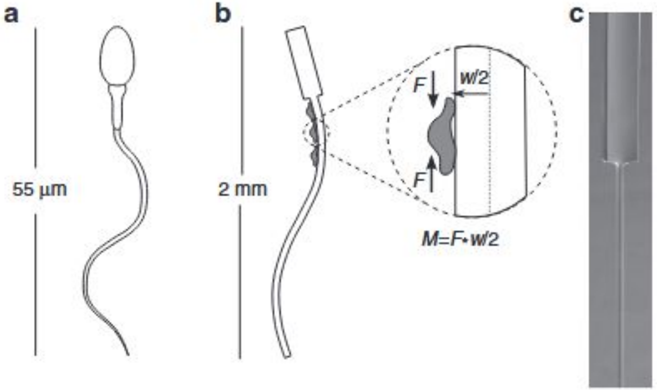
Innovation

- Developed a **biohybrid model** machine using a **PDMS filament** and controlled **cardiomyocyte seeding**
- Introduced a **self-propelled filament** without the use of external control/stimuli (i.e. magnetic, light-based)
- Accompanied with a successful demonstration of movement with a single-tailed and two-tailed model
- Created an elastohydrodynamic model to predict swimming behavior
- Used a combination of soft lithography and micromolding



Microfluidics

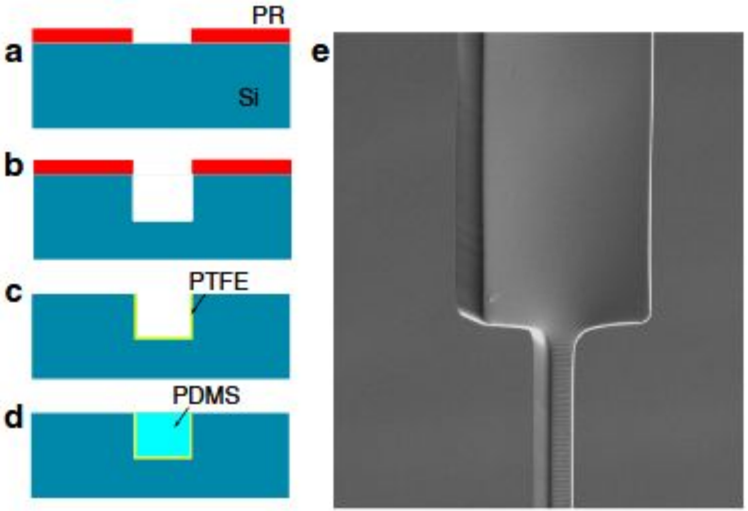
- The biohybrid swimmer consists of:
  - A small, rigid head
  - A long, flexible tail
  - A small cluster of cardiomyocytes
- Functions through the **contraction of the cardiomyocytes** (each produce 1-10 µN)
- Move through the creation of **time-irreversible deformations** of the tail
- Single-tail models moved 5-10 microns/s, while two-tailed models moved 81 microns/s
- Multiple actuation sites or tails can possibly steer the filament



Microfluidics

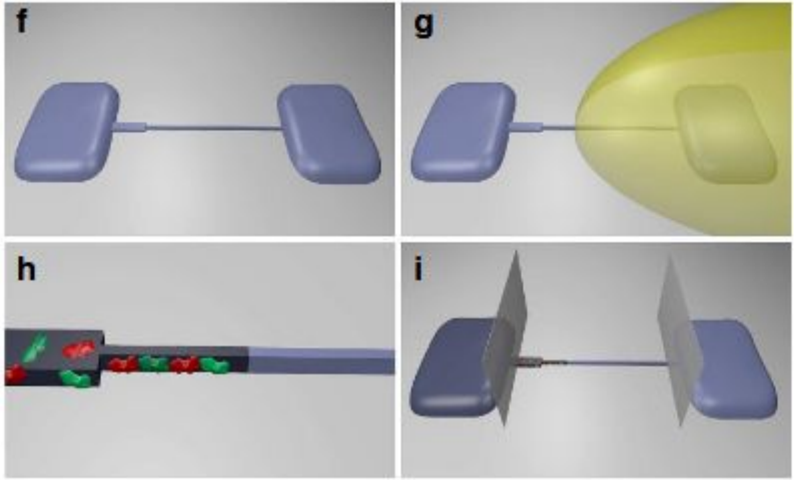
The fabrication process:

1. Carve a channel on a silicon wafer by dry etching to desired depth
2. Coat wafer with PTFE to reduce stiction with cured PDMS filament
   a. Cross-section of the channel determines the shape of the filament
   b. Study went with a rectangular cross-section



Microfluidics

3. 3 mm squares are etched onto either end of the channel to act as a reservoir to fill the channel
4. Liquid PDMS fills the channel through capillary draw
5. Cure for 12 hours at 60°C
6. Inundate with ethanol to release the PDMS from the channel
7. Selective seeding is achieved by functionalizing the PDMS with an extracellular matrix (ECM) and the rest of the tail is covered with de-adherent
   a. Cardiomyocytes were extracted from 2 to 4-day-old Sprague-Dawley rats



Approach

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- Test swimmers with cardiomyocytes at varying locations
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- Test 2 tailed swimmers for direction control and velocity
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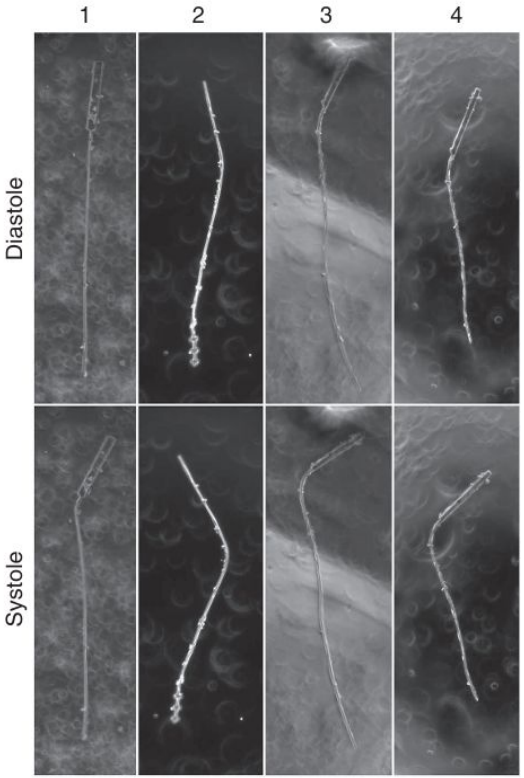
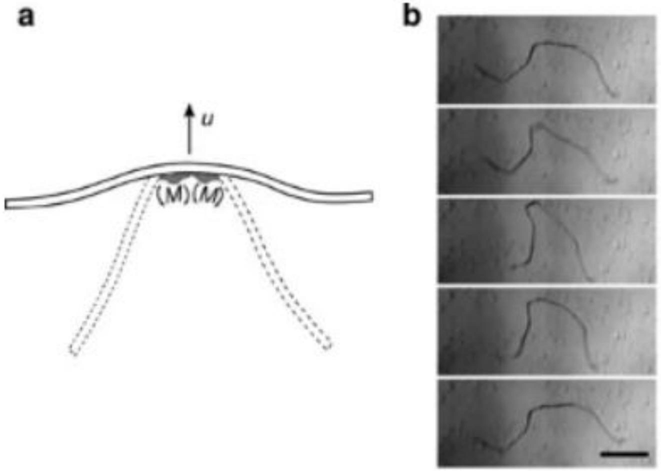


Figure 6: Two-tailed swimmer.



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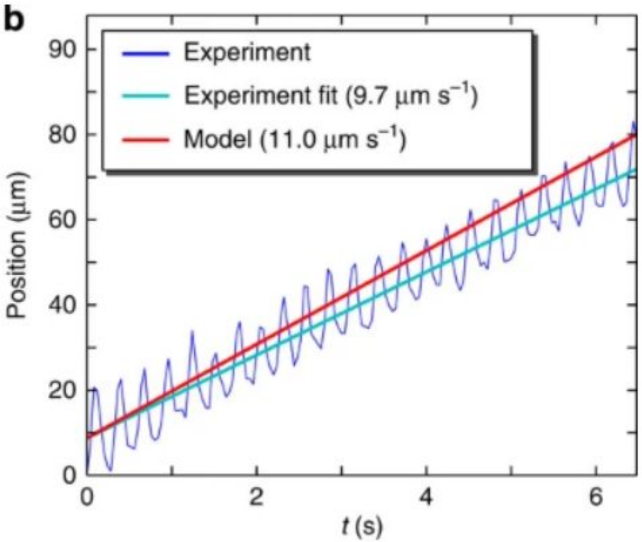

- Biohybrid design follows models in low viscosity
- 2 tailed swimmers eliminate head movement which results in more efficient swimming
- Biohybrid design is much slower than natural
- Natural's advantage is continuous deformations along flagella and higher frequency actuations

Natural spermatozoon (70um length)	1 tailed biohybrid swimmer (2mm)	2 tailed biohybrid swimmer
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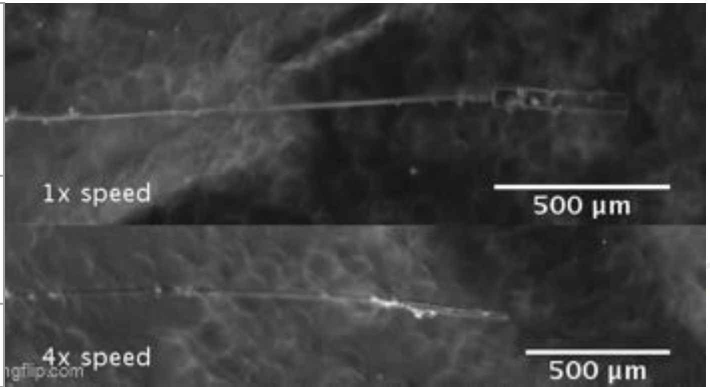

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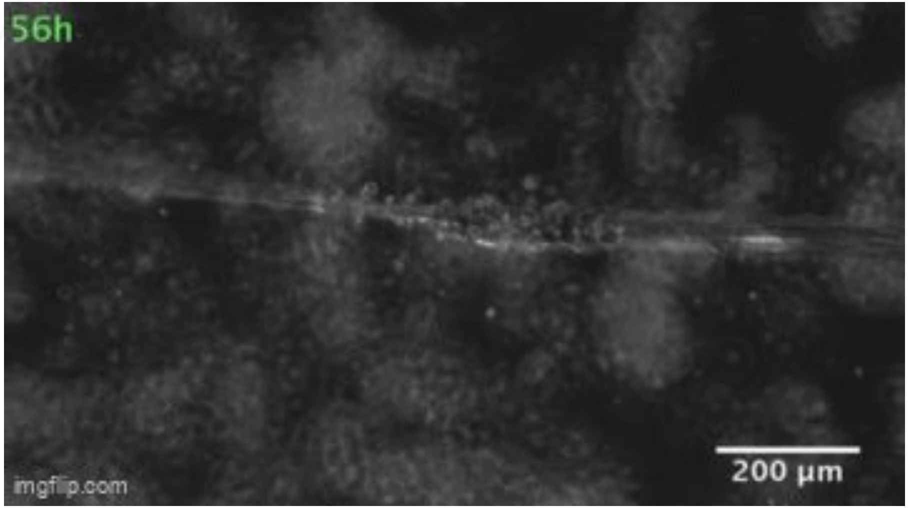

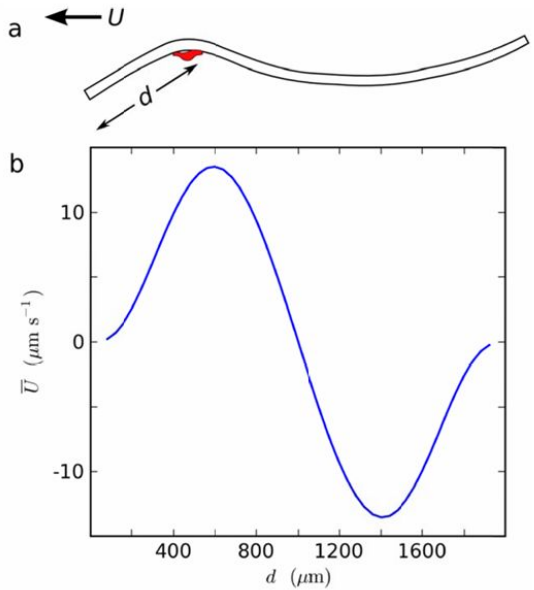

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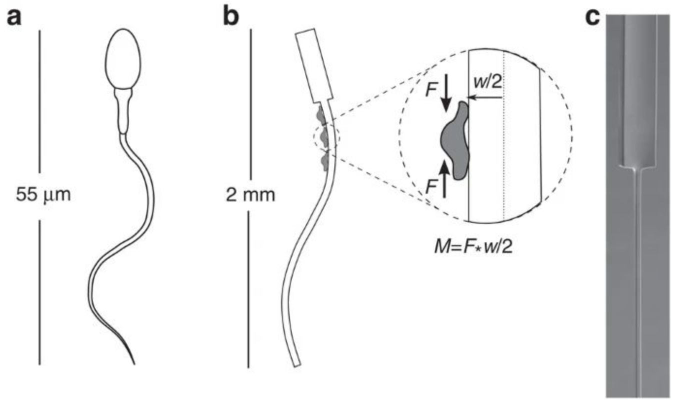

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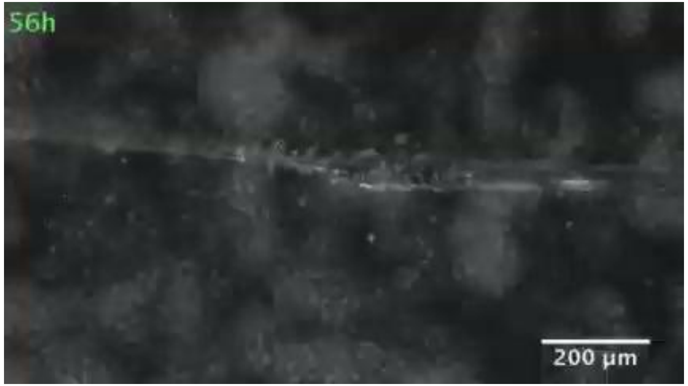

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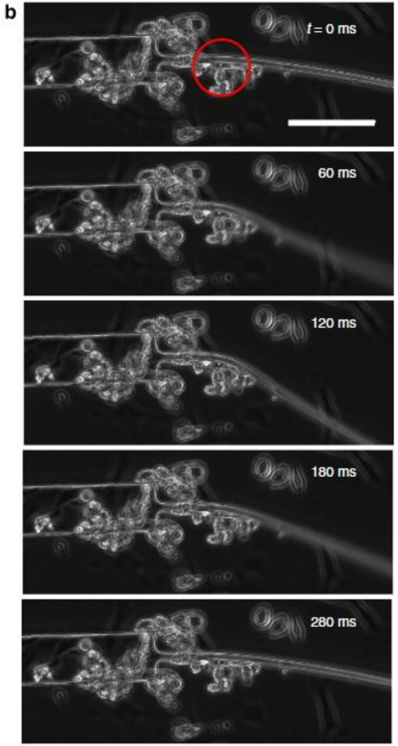

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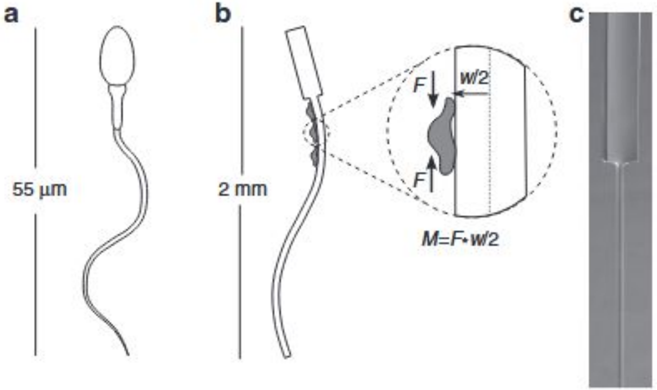

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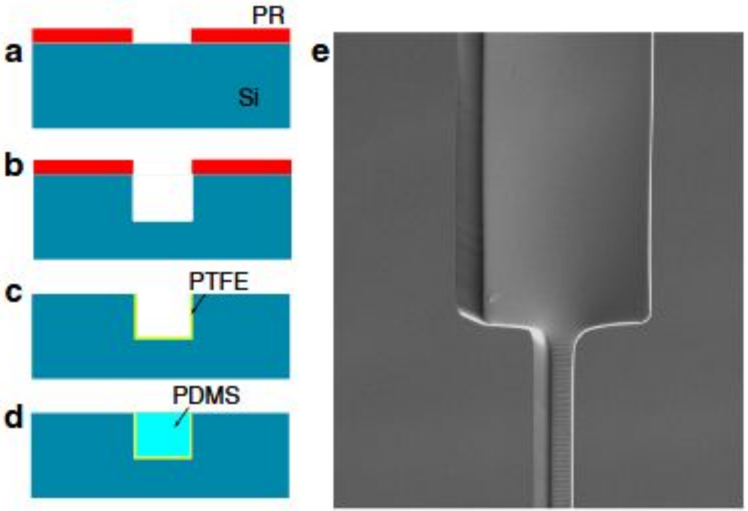

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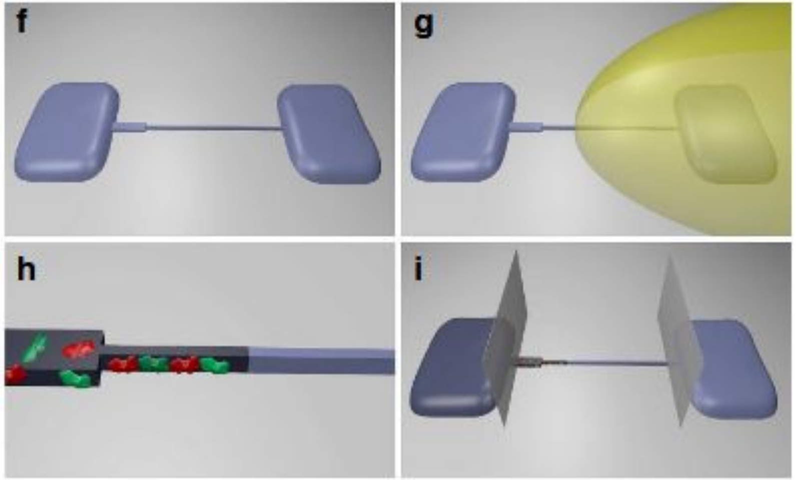

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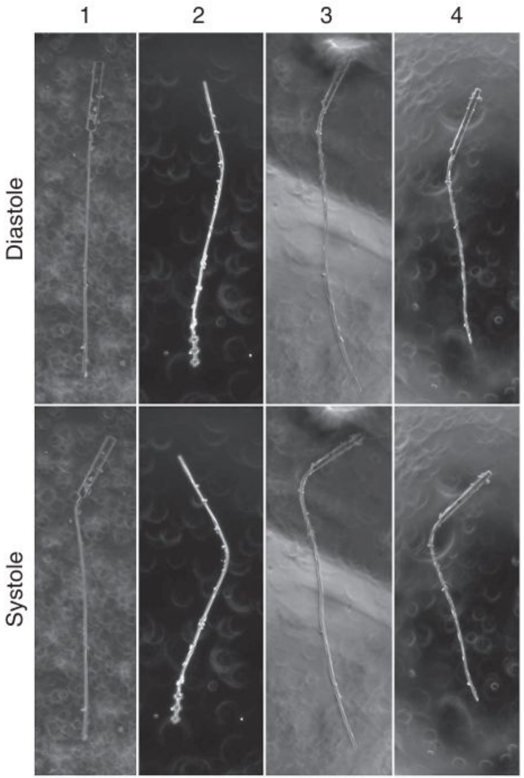

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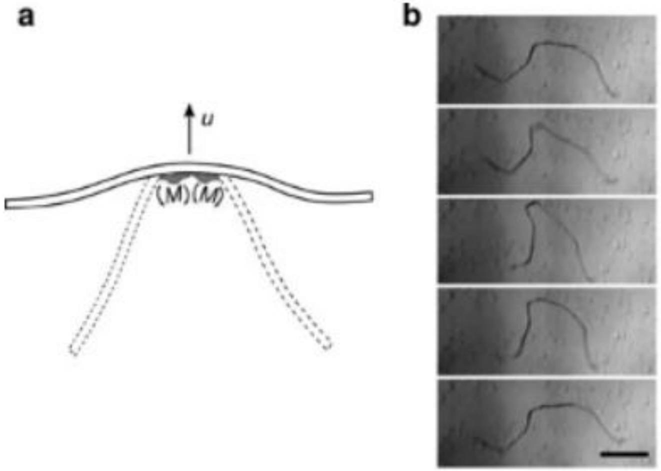

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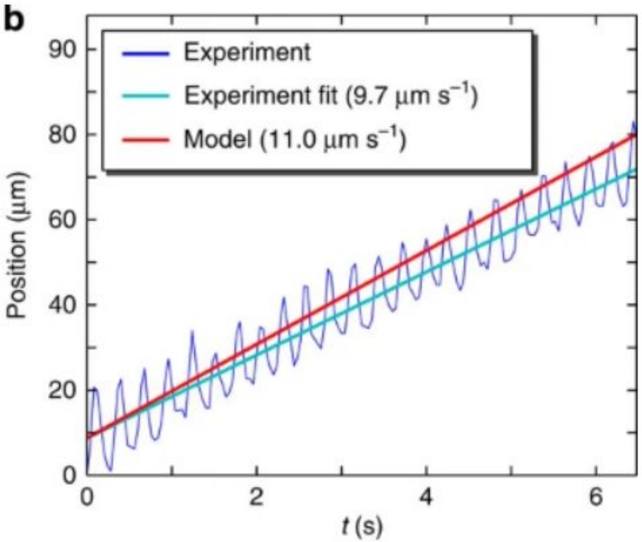

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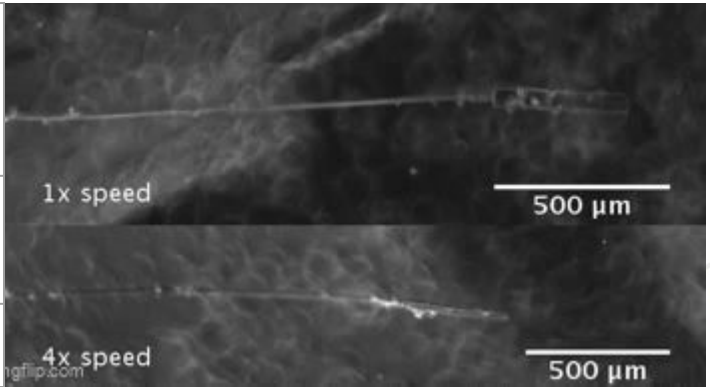

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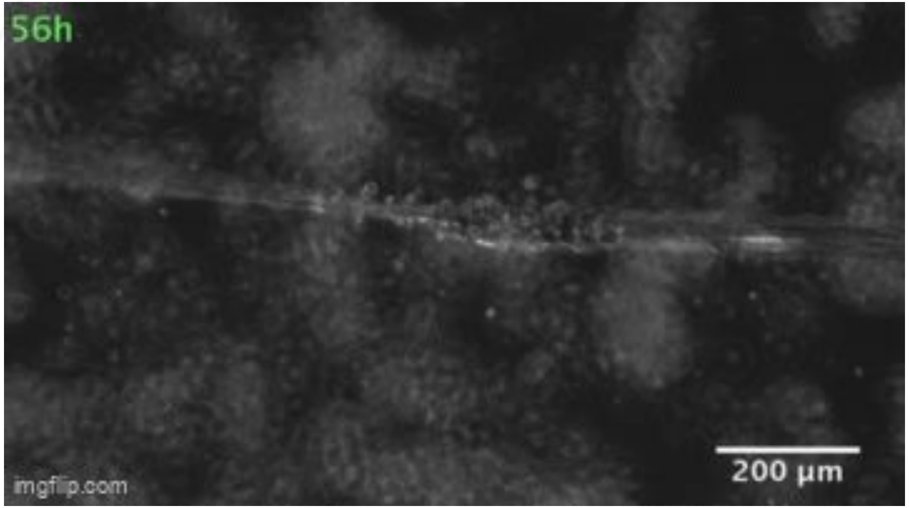

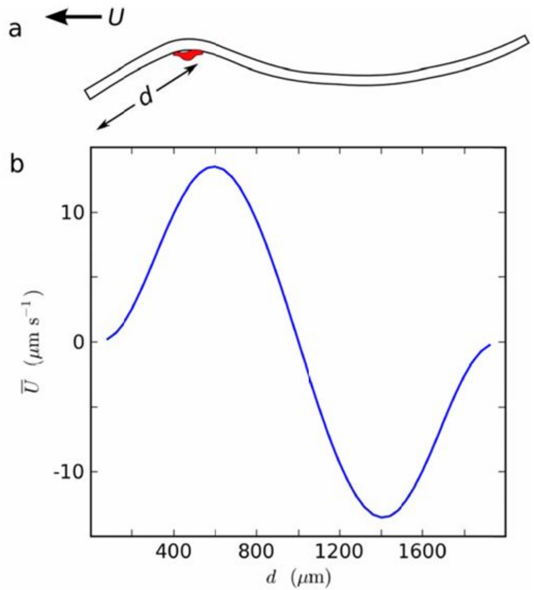

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