Mechanobiology and its Clinical Application

Keiji Naruse, M.D. & Ph.D.

Professor and Chair

Cardiovascular Physiology, Okayama Univ Grad Sch Med

CSO, STREX Inc.

OVERVIEW

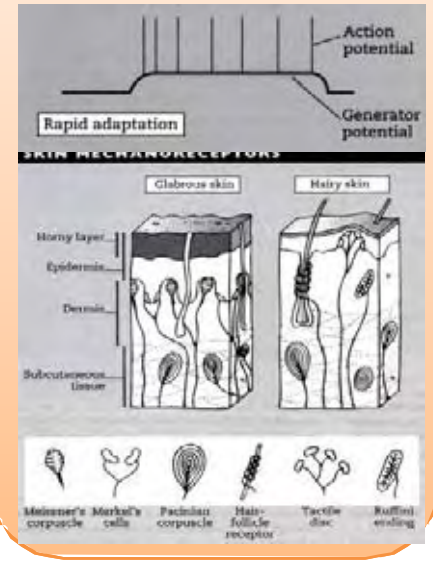
MECHANOBIOLOGY

MECHANOTRANSDUCTION

REPRODUCTIVE MEDICINE

We are continually exposed to mechanical stimuli

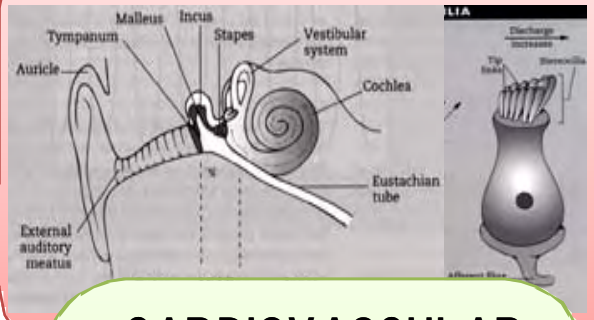
TOUCH



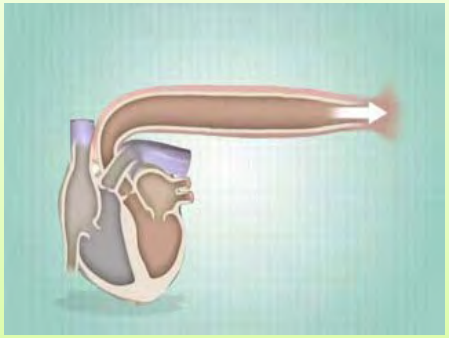
TEETH

periodontal ligament cells
Bone

HEARING

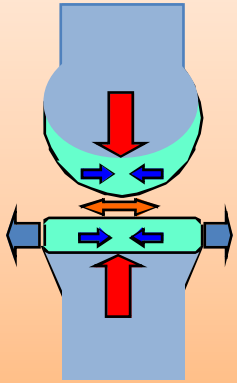


CARDIOVASCULAR



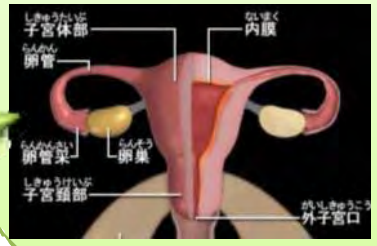
MECHANOBIOLOGY

JOINTS



1xG

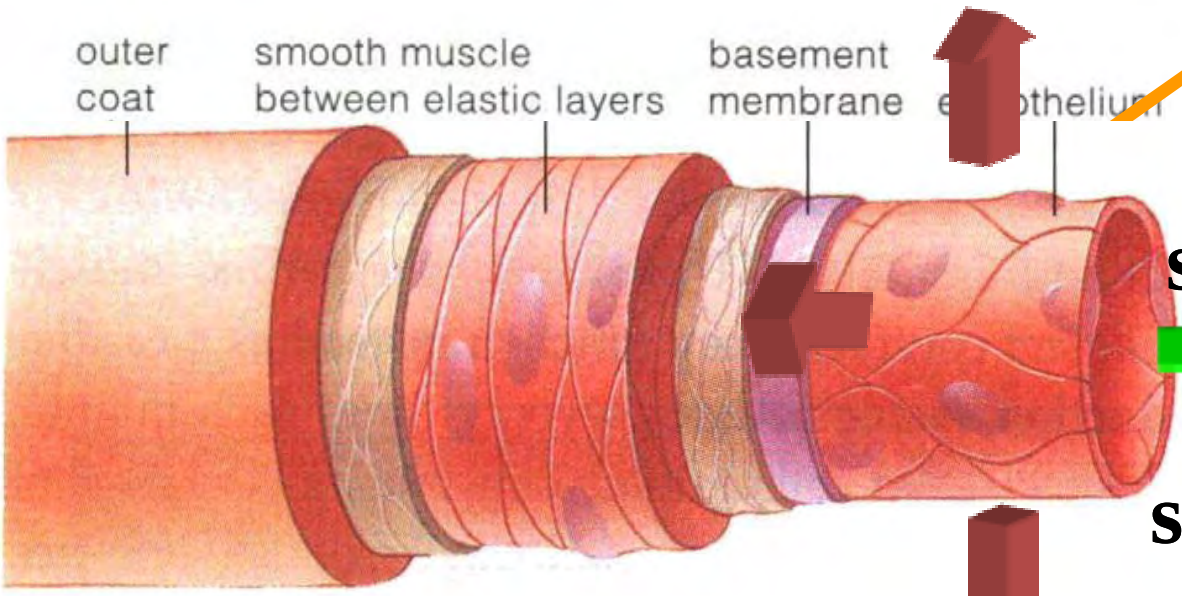
REPRODUCTION



Modified from
"ICURUS"
by HENRY MATISSE

Hemodynamic forces

Endothelial cells



shear stress

stretch

pressure

NO, ET1 etc.
Gene expression
Morphological changes

STRETCH-ACTIVATED ION CHANNEL

Mechanobiology

Biomedical

MEMS

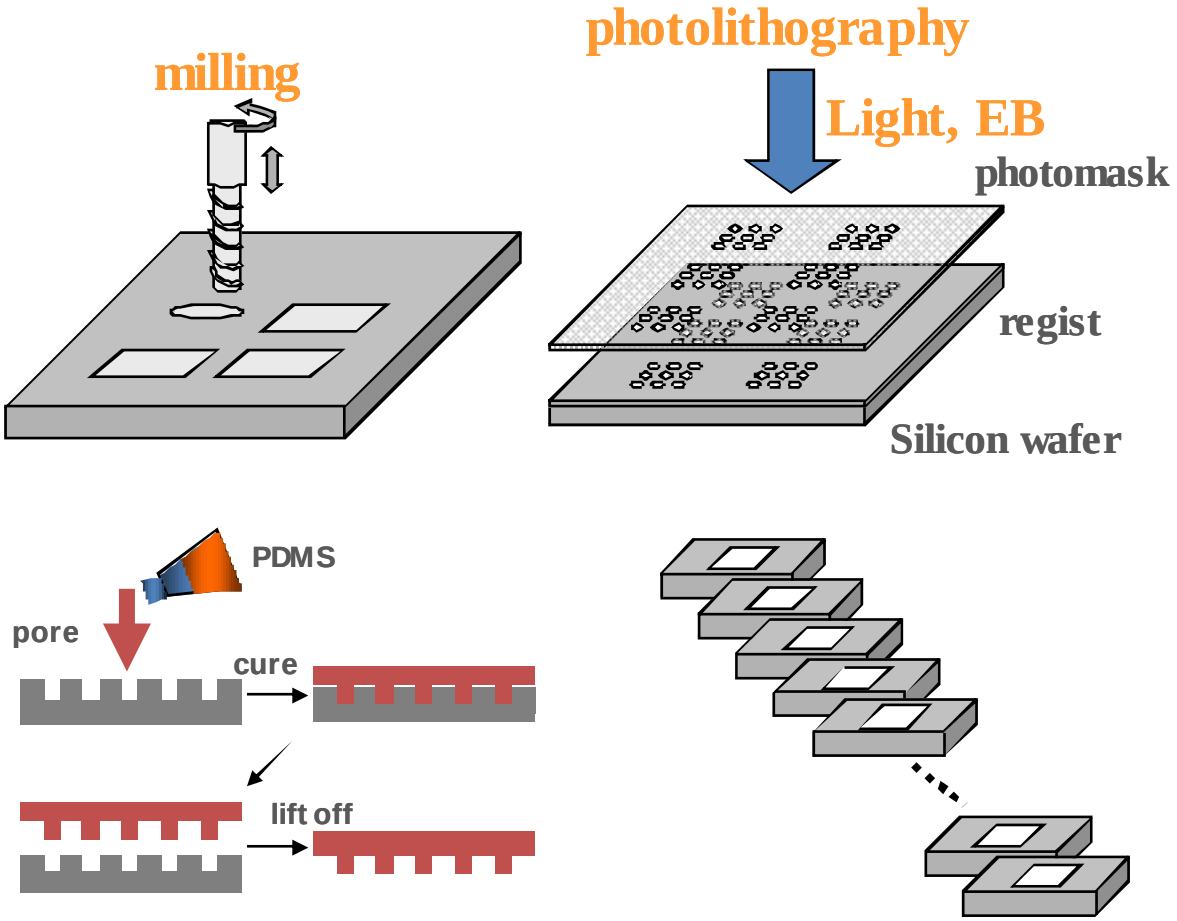
Biomedical Engineering

SOFT LITHOGRAPHY

- **MEMS(micro electro mechanical systems)**

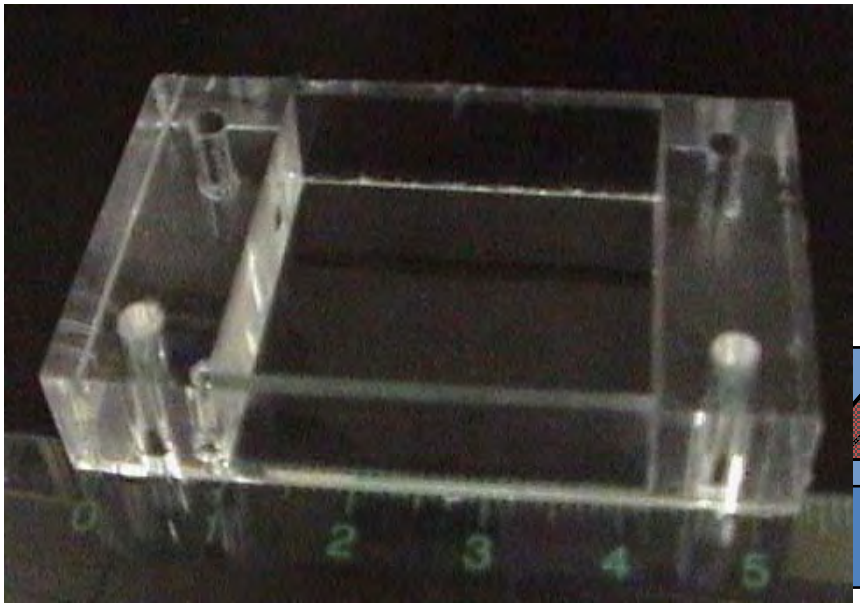
Soft Lithography

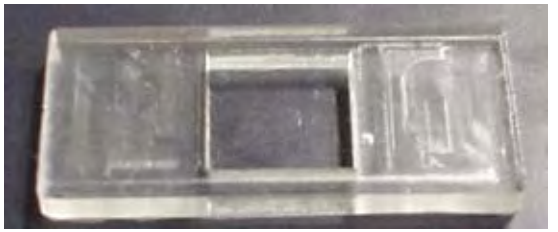
- PDMS (polydimethylsiloxane)
- nm-mm
- Merits
  - Transparent
  - Stable
  - Flexible
  - High throughput
  - Inexpensive

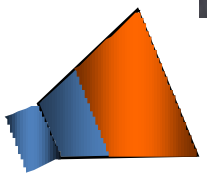


Stretch Chamber

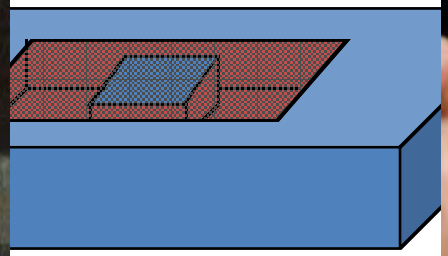
Stretch chamber



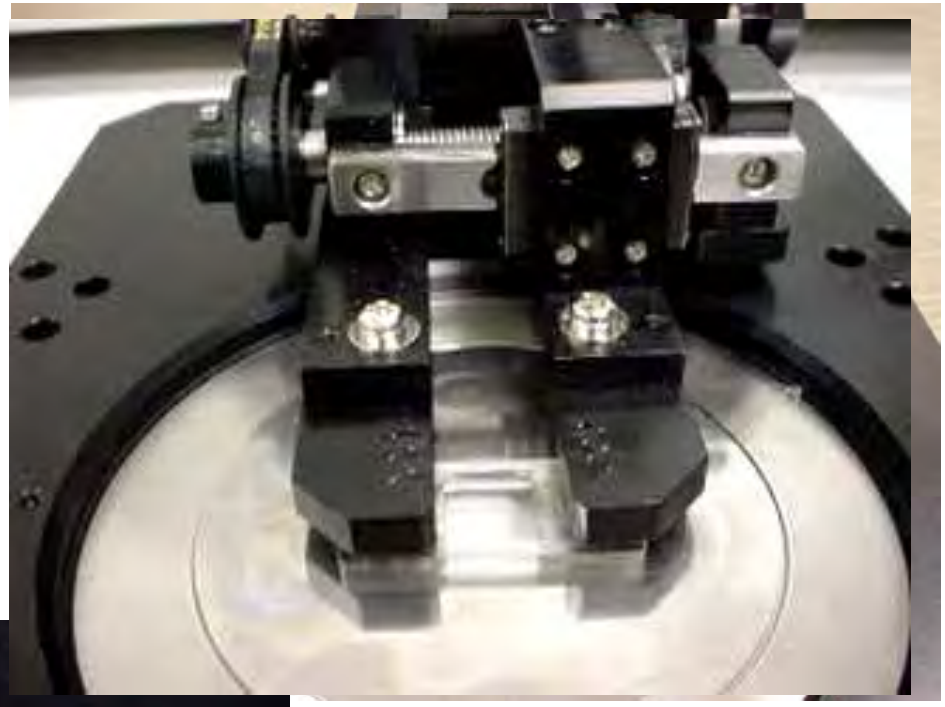




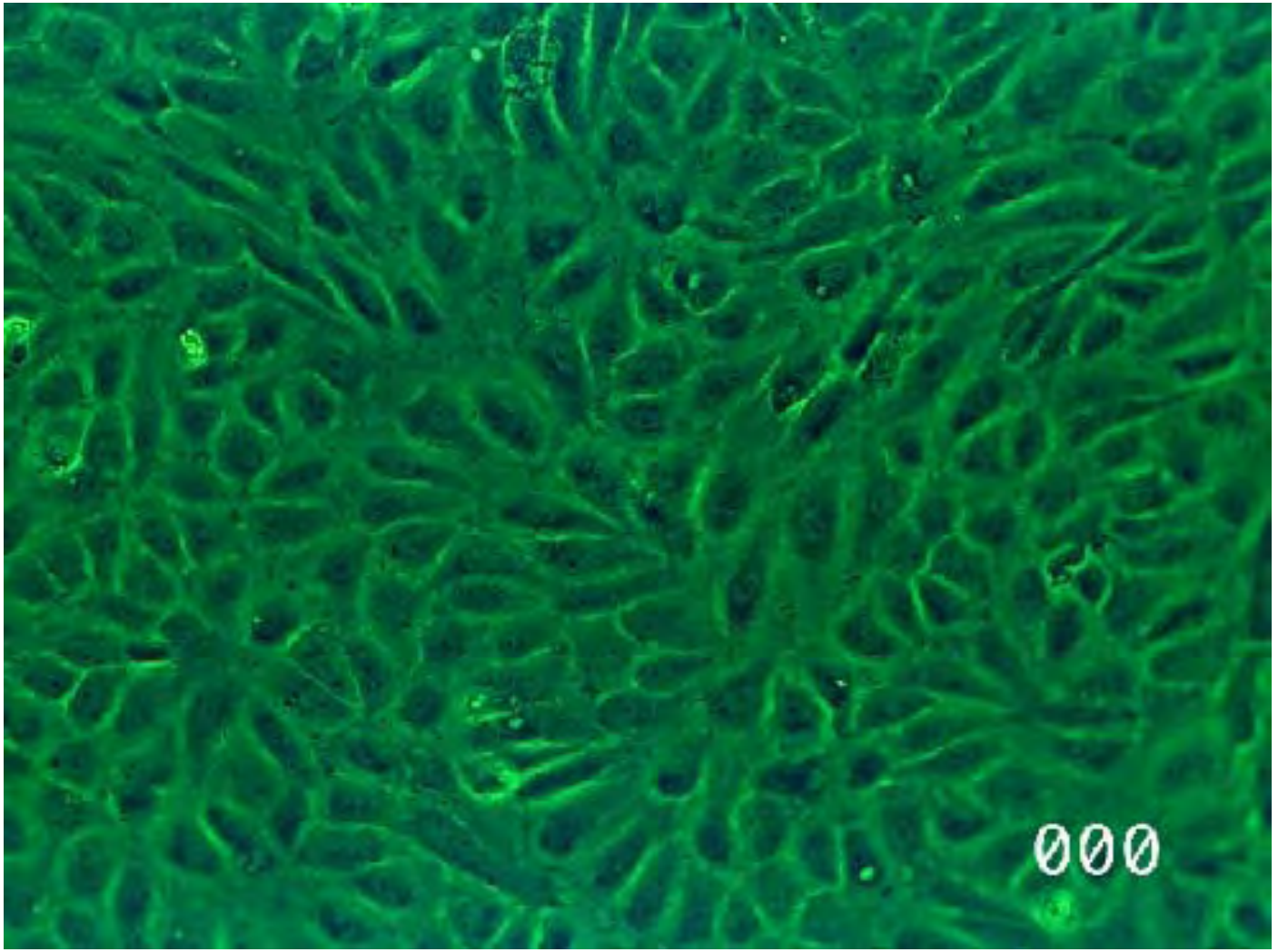
PDMS



Stretch System

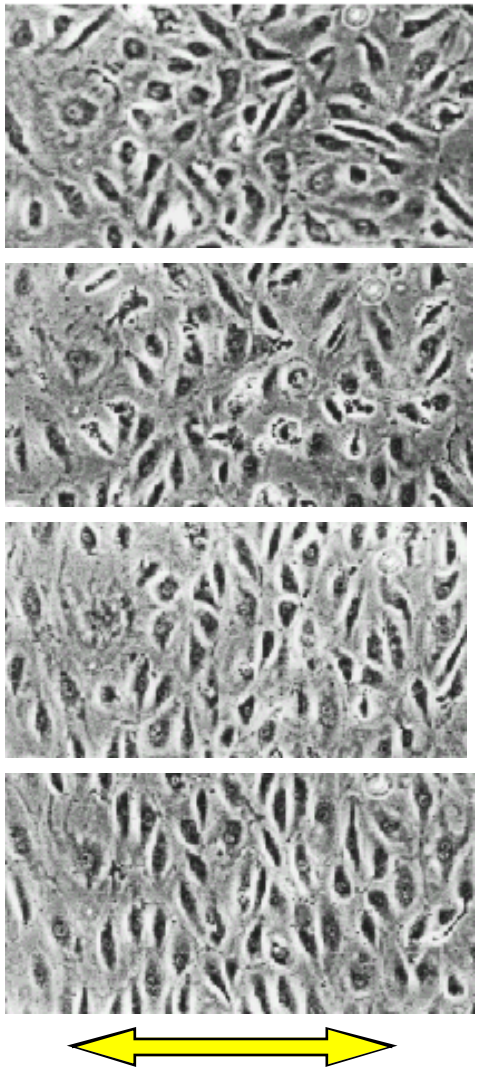


Stretch-induced Morphological change



1 Hz
20 %

Stretch-induced morphological change

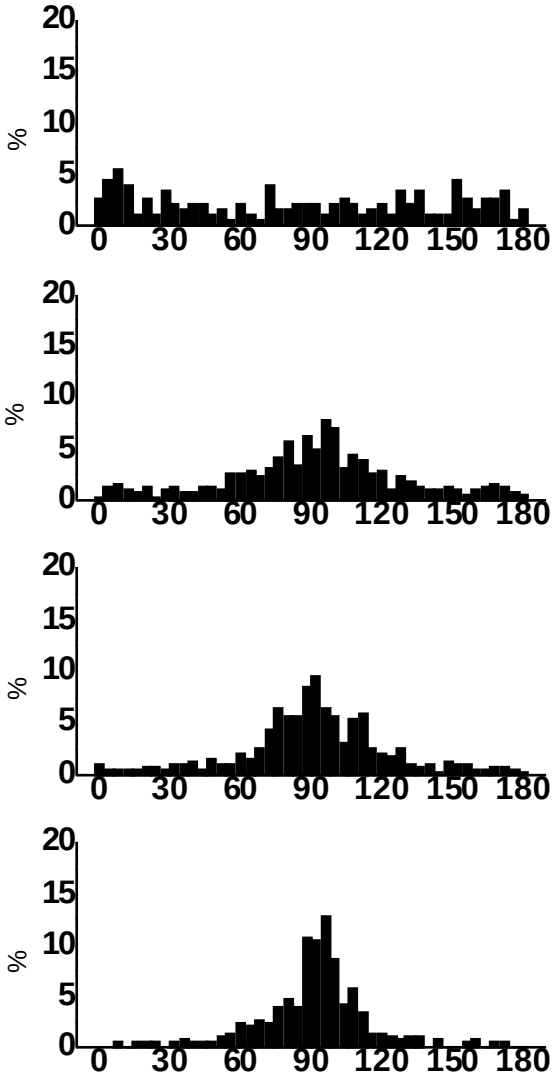


0 min

30 min

60 min

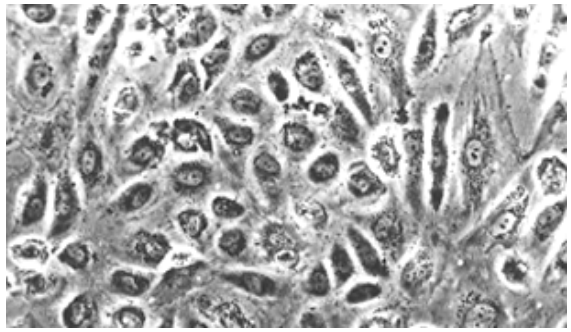
120 min



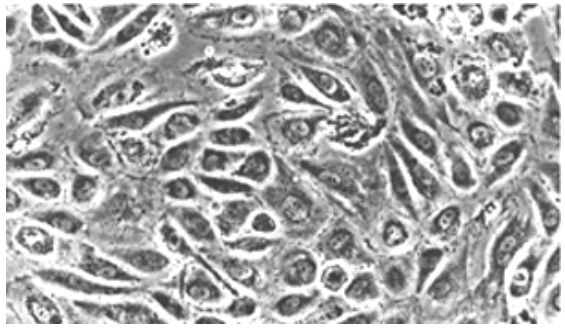
Stretch-induced morphological change

Stretch-activated channel dependent

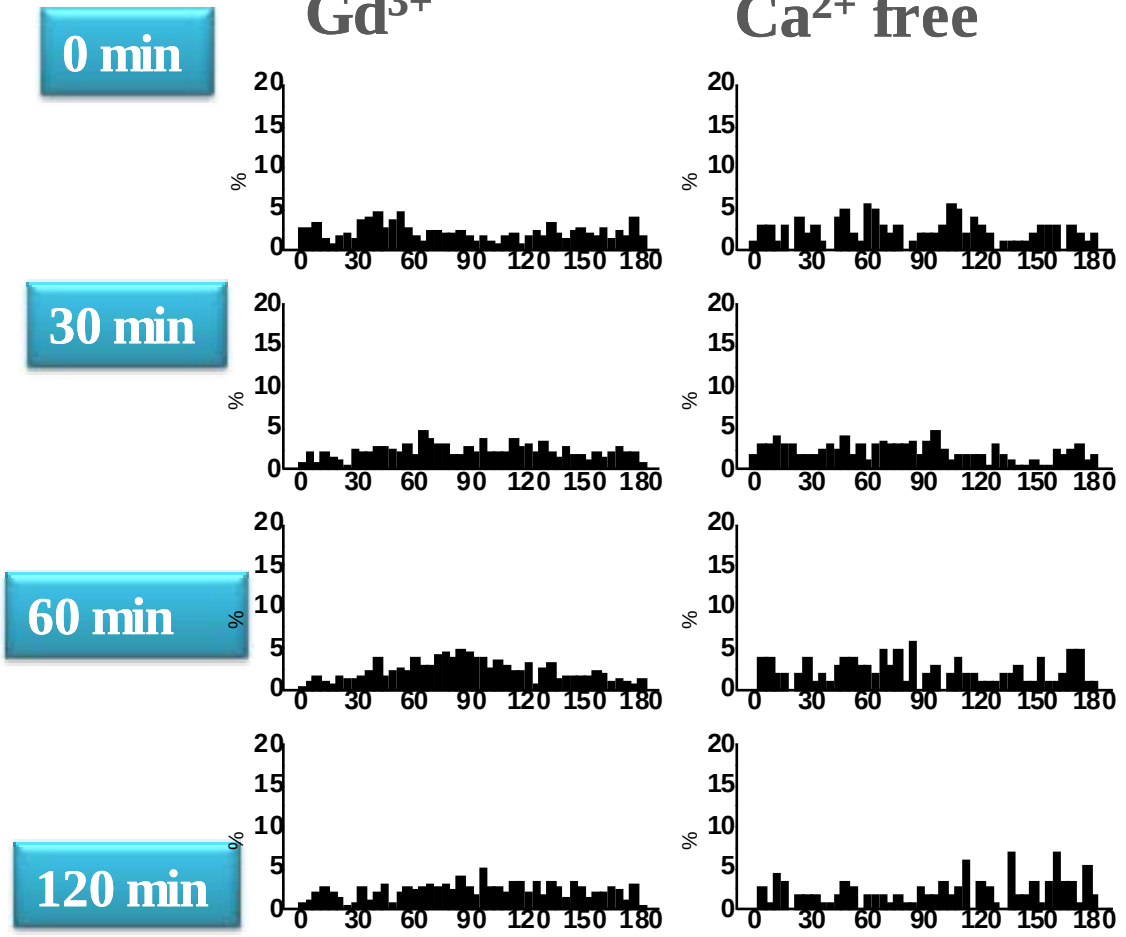
Gd^{3+}



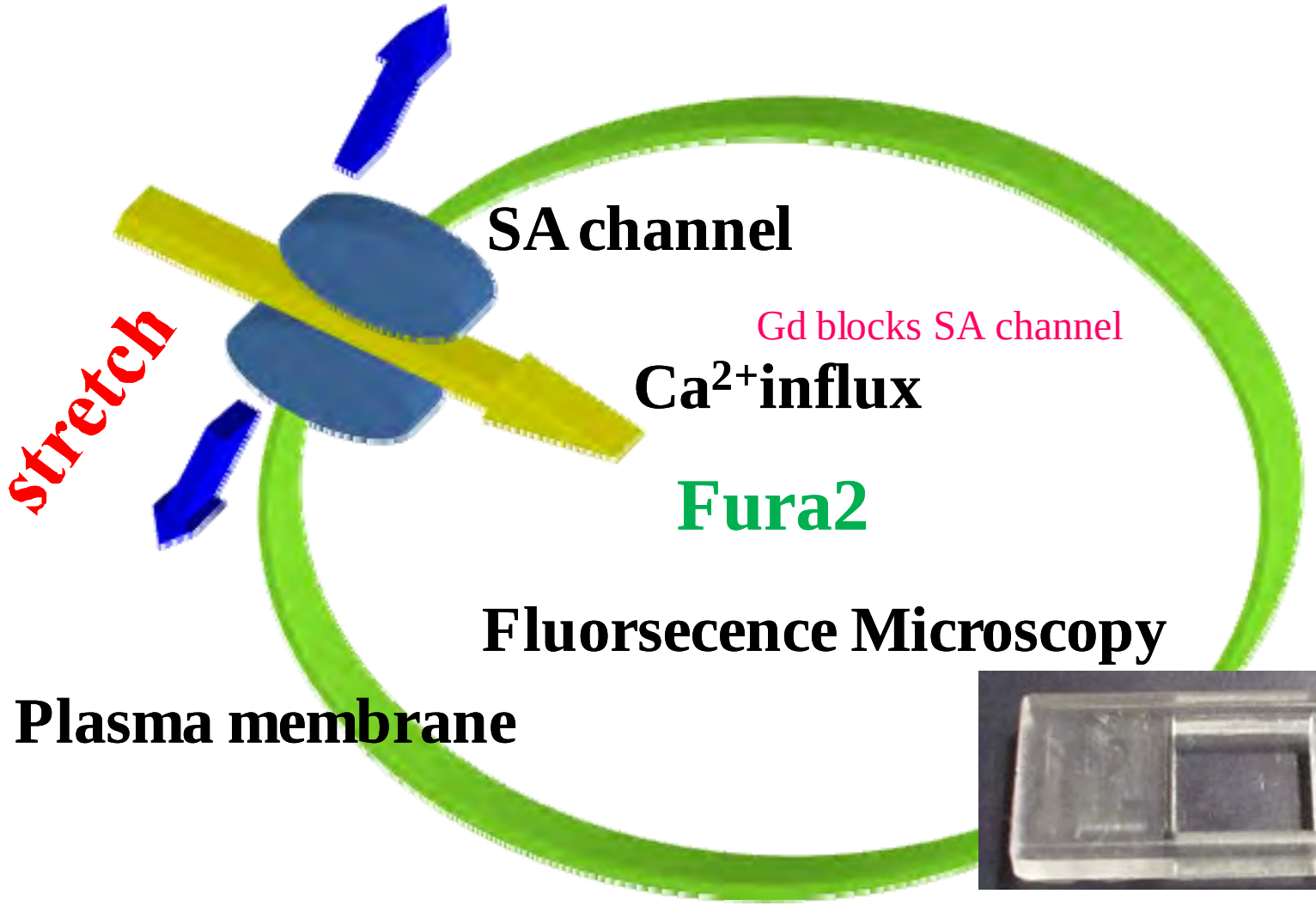
Ca^{2+} free



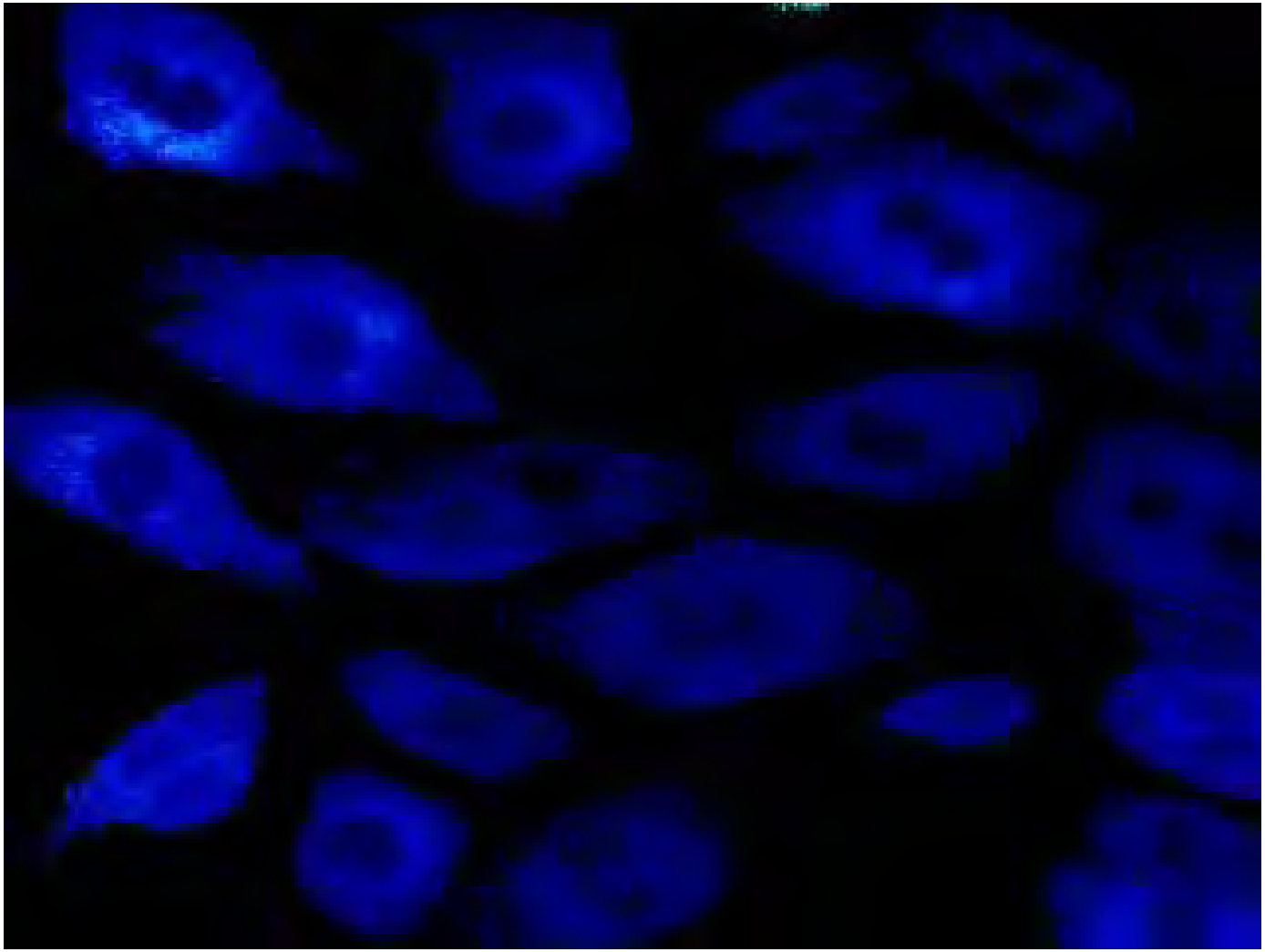
1 Hz, 20 % 60 min



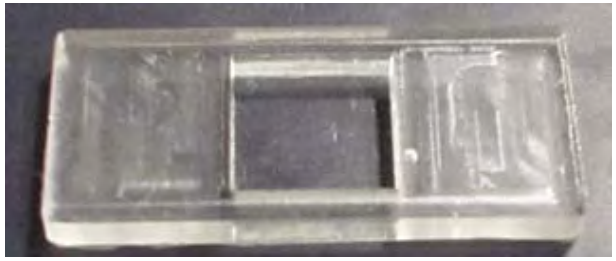
Stretch-induced Ca response

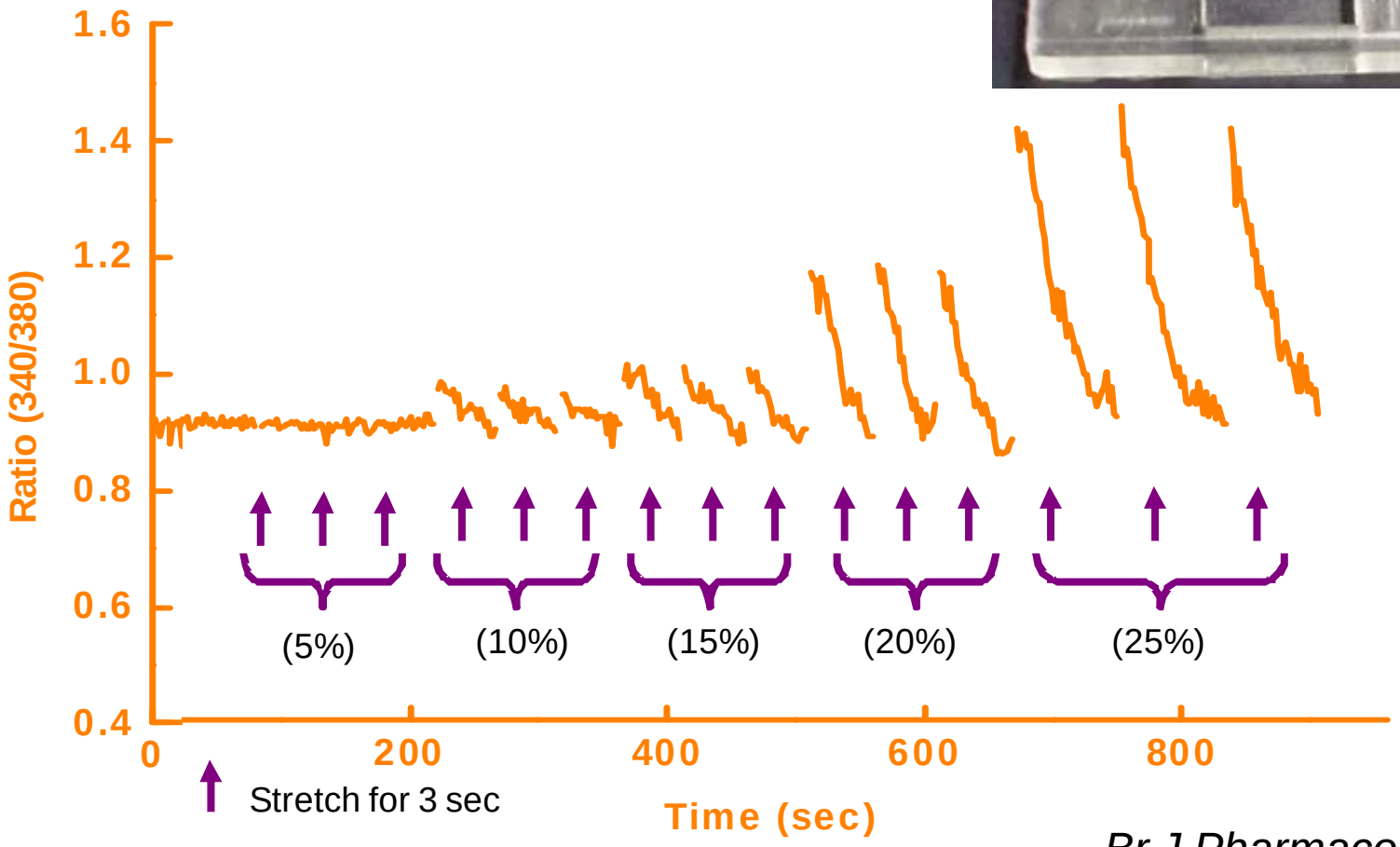


Stretch-induced Ca response



Stretch-induced Ca response

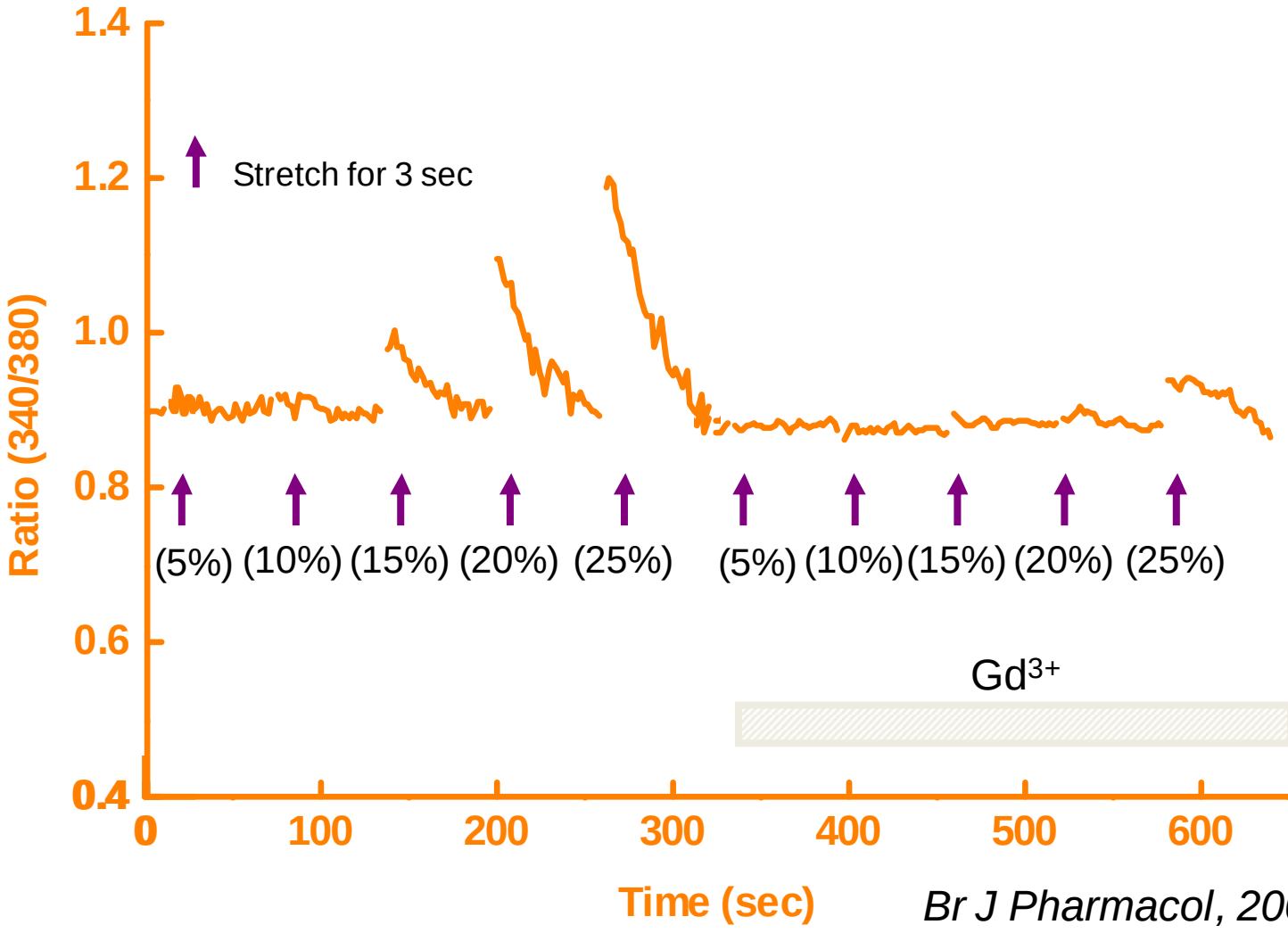




Br J Pharmacol, 2002

Stretch-induced Ca response

Stretch-activated channel dependent



Br J Pharmacol, 2002

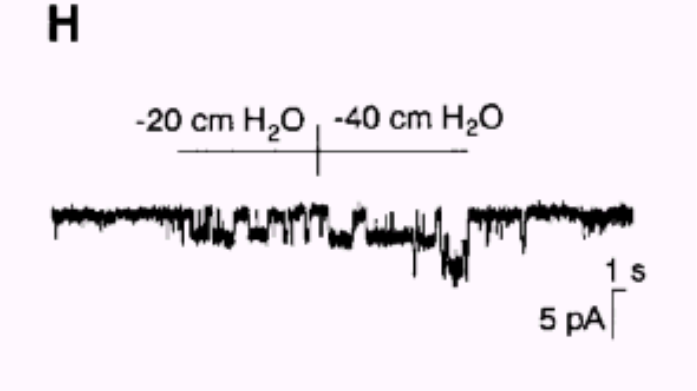
SA channel

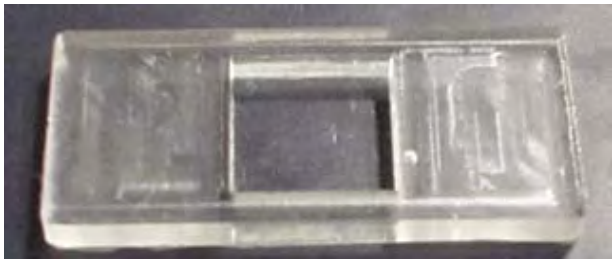
- MscL, MscS (Non-selective)
- **Mid-1 (Non-selective)**
- TREK/TRAAK (K^+ selective)
- **SAKCA (K^+ selective)**
- **TRPs (cation selective)**

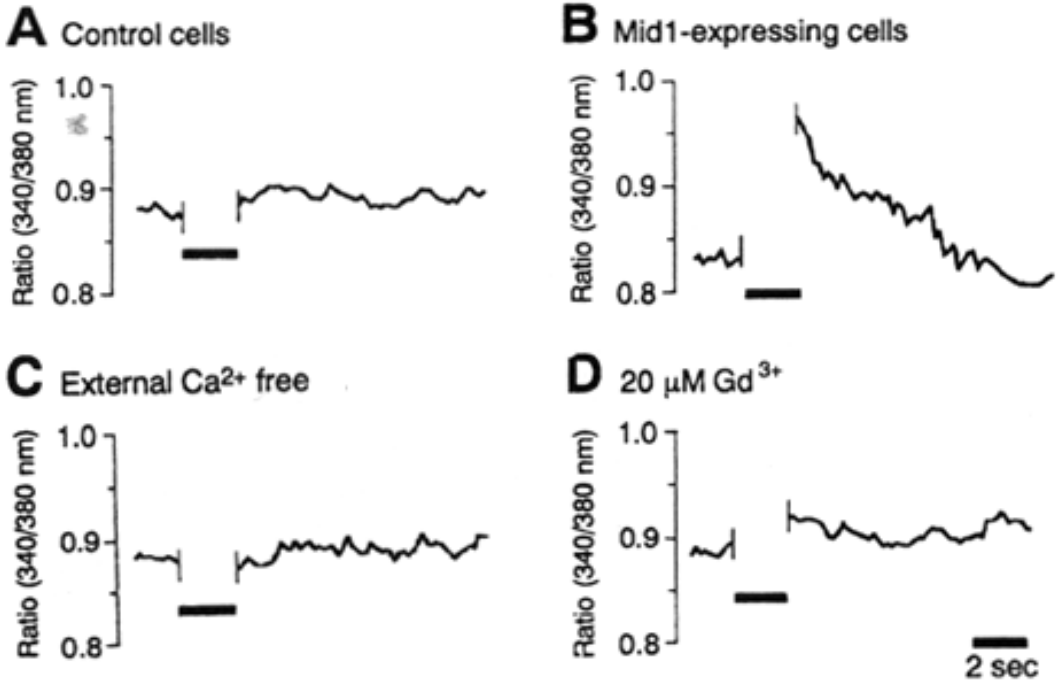
SA channel

Mid1

Molecular Identification of a Eukaryotic, Stretch-Activated Nonselective Cation Channel :Mid1







Science, 285:882-885 1999

SA channel

SAKCA

```
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TVCCHCGVKNKEAQKINGGGDTQADGACKPTDEKEENVAAEVGWMTSVKD  100
WAGVMISAQTLTGRVLVVLVFALSIGALVIYFIDSSNPIESCQNFYKDFT  150
LQIDMAFNVFFLLYFGLRFIAANDKLWFWLEVNSVVDFFTVPPVFVSVYL  200
NRSWLGLRFLRALRLIQFSEILQFLNILKTSNSIKLVNLCSIFISTWLTA  250
AGFIHLVENSGDPWENFQNNQQLTYWECVYLLMVTMSTVGYGDVYAKTTL  300
GRLFMVFFILGGLAMFASYVPEIIELIGNRKKYGGSYSAVSGRKHIVVCG  350
HITLESVSNFLKDFLHKDRDDVNVEIVFLHNISPNPELEALFKRHFTQVE  400
FYQGSVLNPHDLARVKIESADACLILANKYCADPDAEDASNIMRVISIKN  450
YHPKIRIITQMLQYHNKAHLLNIPSWNWKEGDDAICLAELKLGFIAQSCL  500
APGLSTMLANLFSMRSFIKIEEDTWQKYYLEGVANEMYTEYLSSAFVGLS  550
FPAVCELVFAKLKLLMIAIEYKSEKRESSILINPGNHVKIQEGTLGFFIA  600
SDAKEVKRAFFYCKACHDDITDPKRIKKCGCKRPKMSIYKRMKLACCFDC  650
GRSERDCSCMSGSVHSNMDTLERAFPLSSVSVNDCSTSLRAFEDEQPSTL  700
SPKKKQRNGGMRNSPNSSPKLMRHDPLLIPGNEQIDNMDANVKKYDSTGM  750
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RASNFHYHELKHIVFVGSLEYLRREWETLHNFPKVSILPGTPLSRADLRA  850
VNINLCDMCVILSANQNNIDDASLQDKECILASLNIKSMQFDDSIGVLQA  900
NSQGFTPPGMDRSSPDNSPVHGLLRQPSITTGANIPIITELVNDSNVQFL  950
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TGGATPELEALIAEENALRGGYSTPQTLANRDRCRVAQLALYDGPFADLG 1050
DGGCYGDLFCKALKTYNMFCFGIYRLRDAHLSTPSQCTKRYVITNPPYEF 1100
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RPNRTKTRDSREKQKYVQEDRL                               1172
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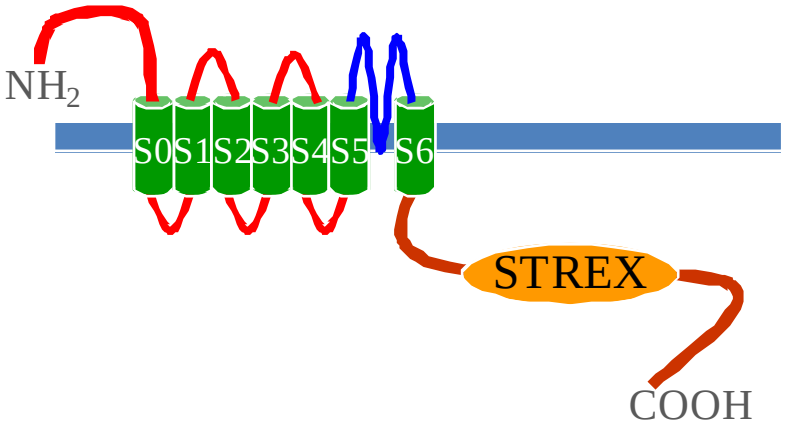
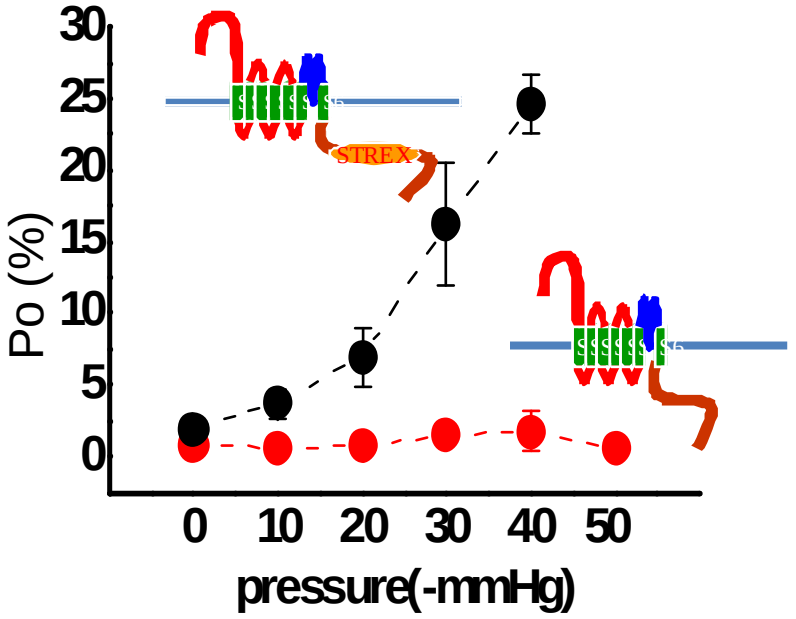


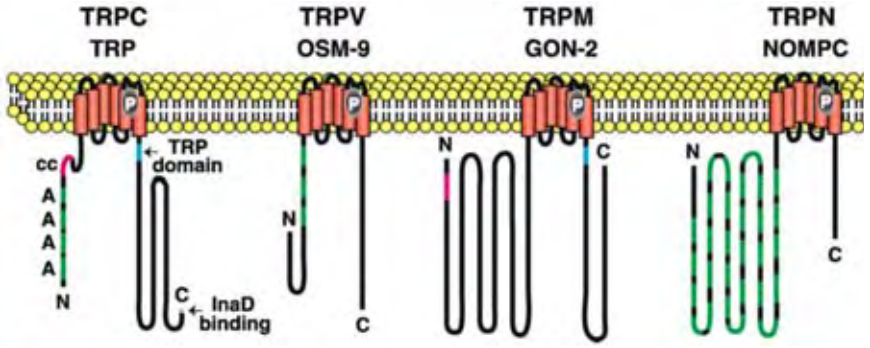


STREX: Stress Axis Regurated EXon

SA channel

Transient Receptor Potential channels

- Drosophila Retina
- non-selective cation channel
- 6 putative transmembrane helices
- ankyrin repeats (N-terminal)
- variety of sensory system
- TRPV1→Thermosensor
- Mechanosensor?
- TRPV2→SA channel?



Current Opinion in Neurobiology 2004, **14**:362–369

JCB Article

A novel mechanism of myocyte degeneration involving the Ca^{2+}-permeable growth factor–regulated channel

Yuko Iwata,[1] Yuki Katanosaka,[1] Yuji Arai,[2] Kazuo Komamura,[3] Kunio Miyatake,[4] and Munekazu Shigekawa[1]

[1]Department of Molecular Physiology, [2]Department of Bioscience, and [3]Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, and [4]Division of Cardiology, National Cardiovascular Center Hospital, Suita, Osaka 565-8565, Japan

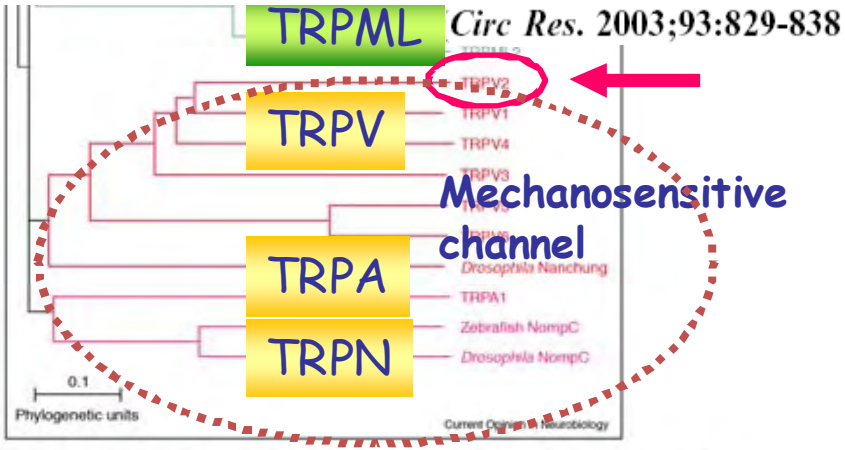
The Journal of Cell Biology, Volume 161, Number 5, June 9, 2003 957–967

Cellular Biology

TRPV2 Is a Component of Osmotically Sensitive Cation Channels in Murine Aortic Myocytes

Katsuhiko Muraki, Yuko Iwata, Yuki Katanosaka, Tomohiro Ito, Susumu Ohya, Munekazu Shigekawa, Yuji Imaizumi

Circ Res. 2003;93:829-838



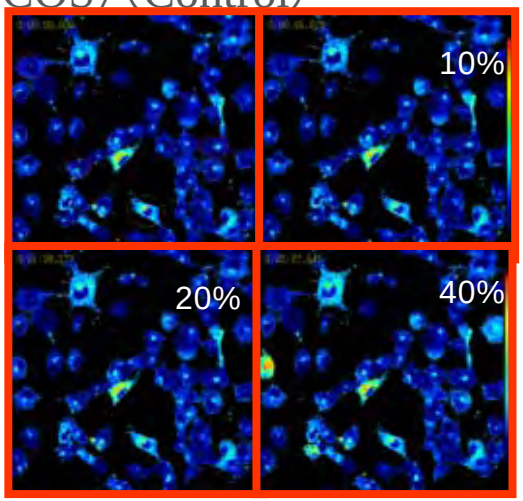
A phylogeny tree to show how the human TRP channels are related. As TRPC2 is a pseudogene in humans, the mouse is represented.

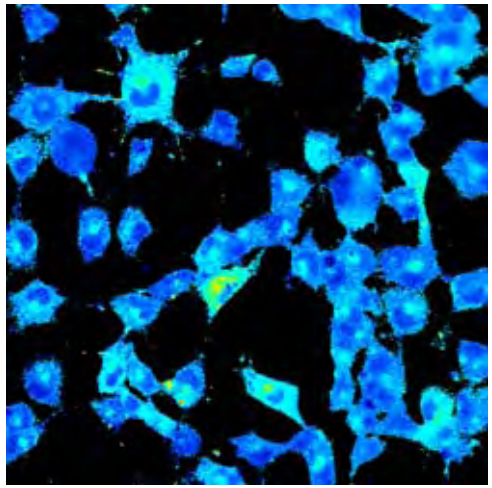
SA channel

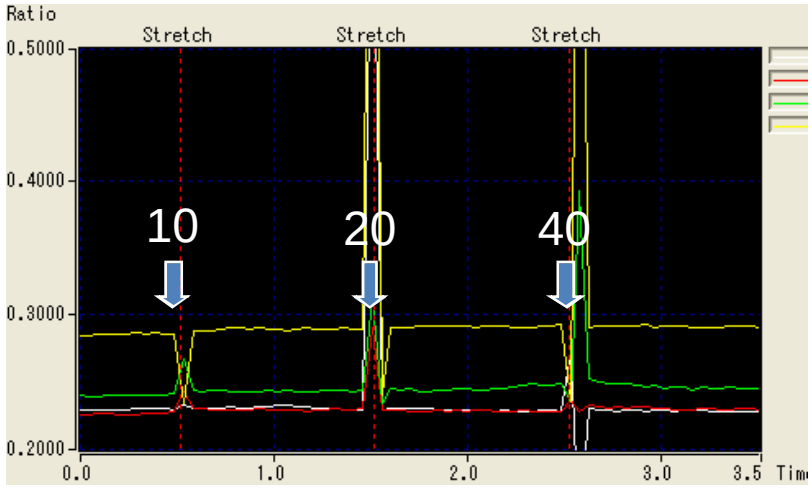
TRPV2

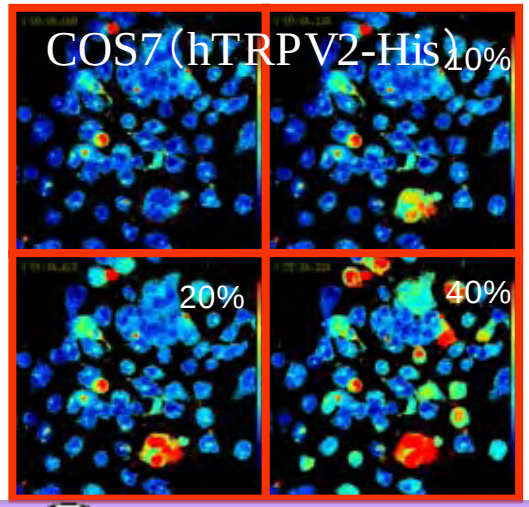
Transient Rceptor Potential channels

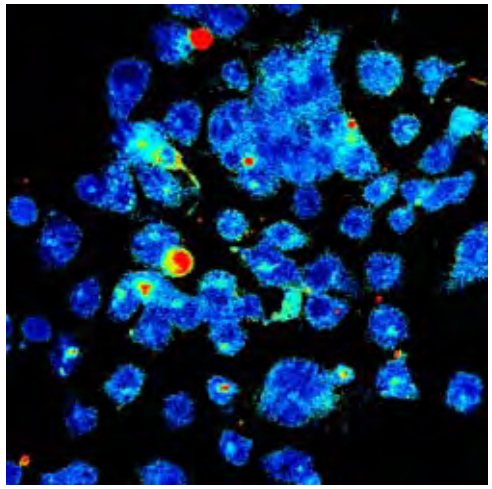
COS7（Control）

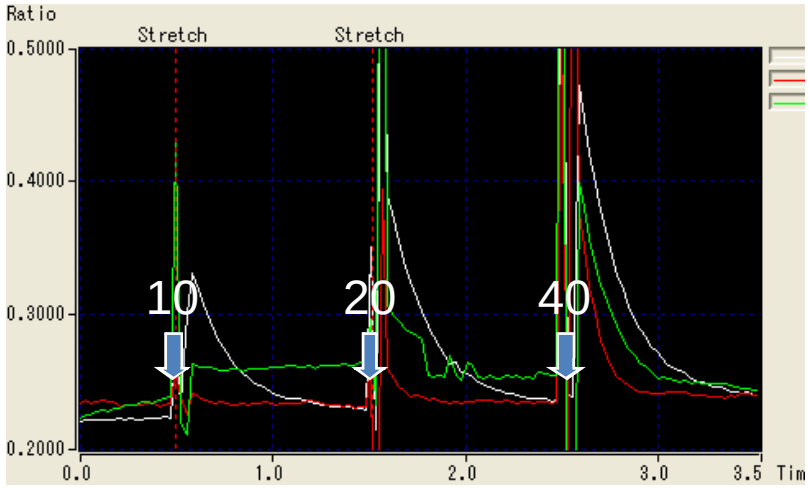








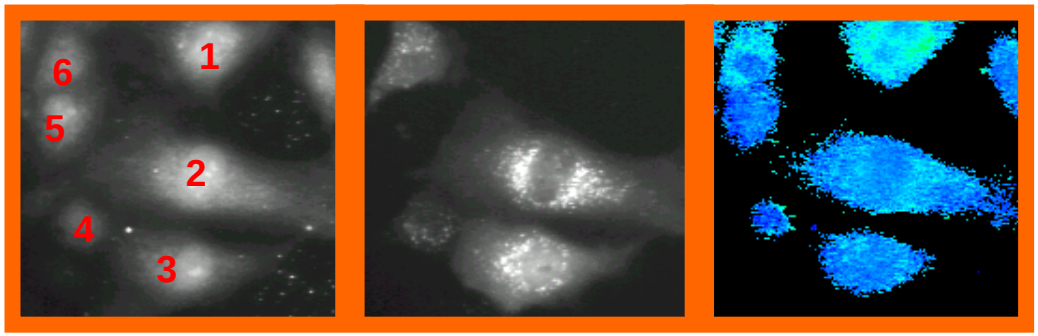




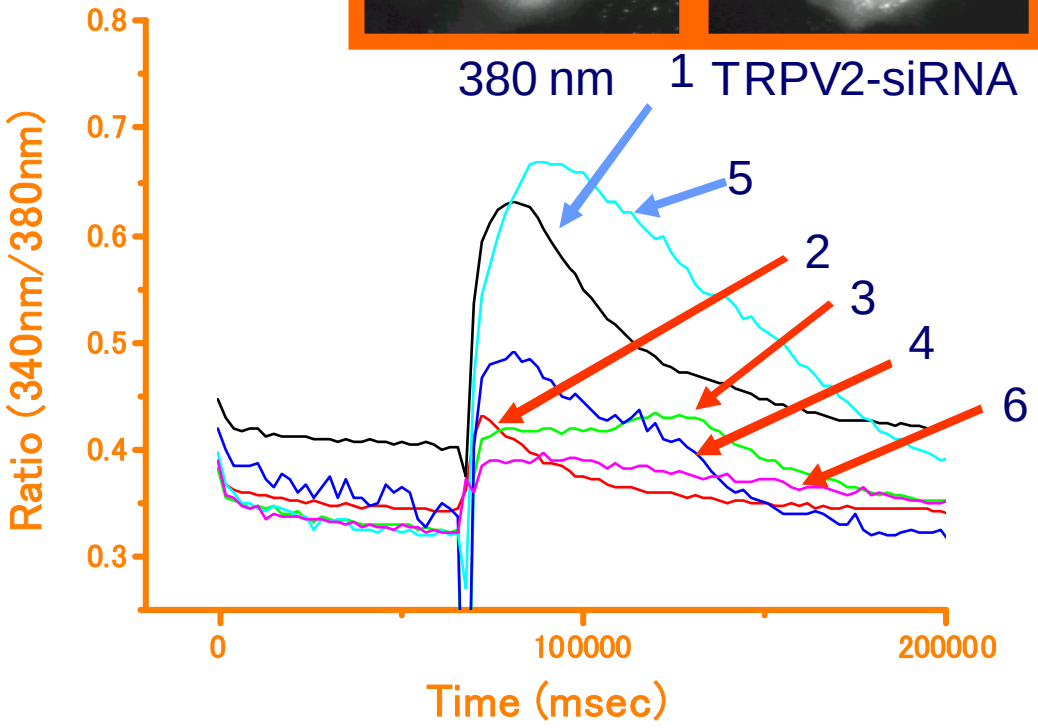
SA channel

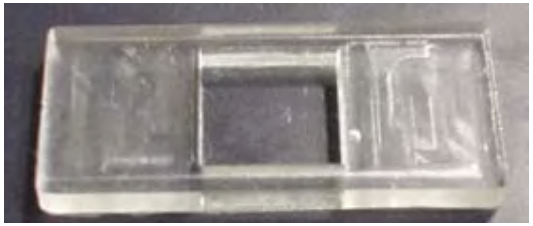
TRPV2

Effect of TRPV2-siRNA on stretch-induced $[Ca^{2+}]_i$ response



HUVEC

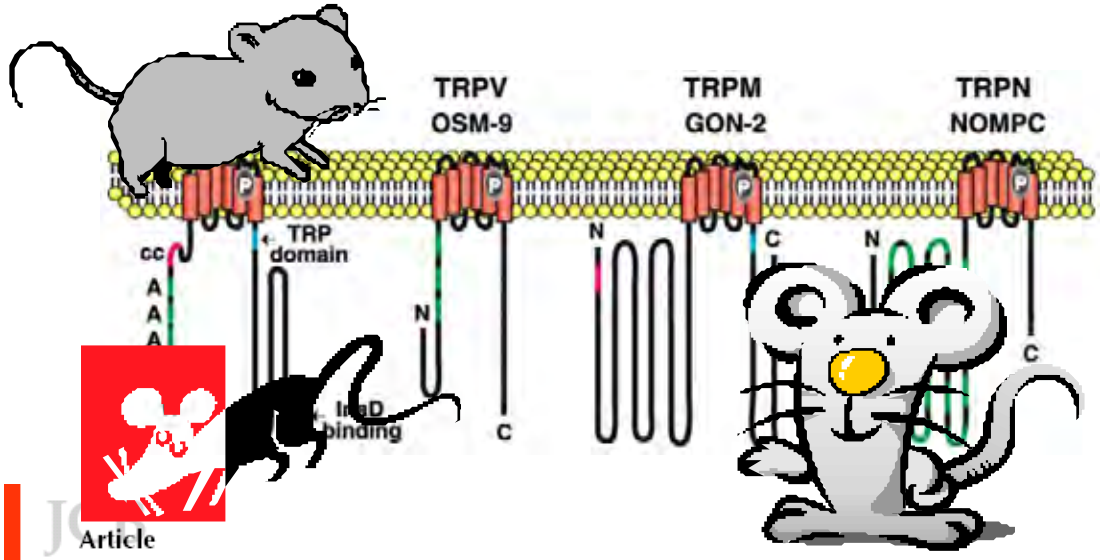




SA channel

TRPV2 TG, KO, KI mouse

Inducible, Tissue specific, Human KI
Double KO



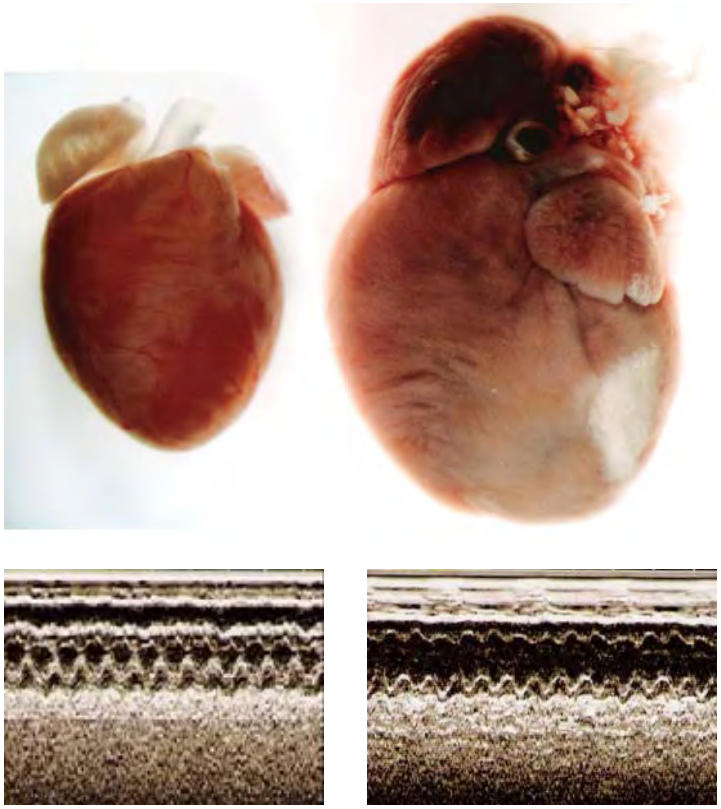
Article

A novel mechanism of myocyte degeneration involving the Ca^{2+}-permeable growth factor–regulated channel

Yuko Iwata,[1] Yuki Katanosaka,[1] Yuji Arai,[2] Kazuo Komamura,[3] Kunio Miyatake,[4] and Munekazu Shigekawa[1]

[1]Department of Molecular Physiology, [2]Department of Bioscience, and [3]Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, and [4]Division of Cardiology, National Cardiovascular Center Hospital, Suita, Osaka 565-8565, Japan

Y. Iwata and Y. Katanosaka contributed equally to this work.

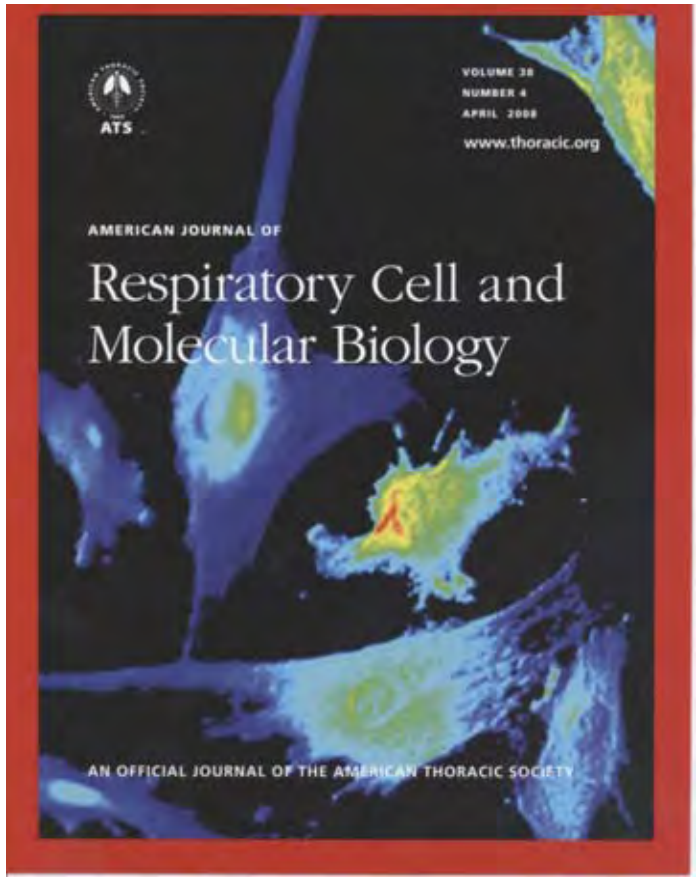


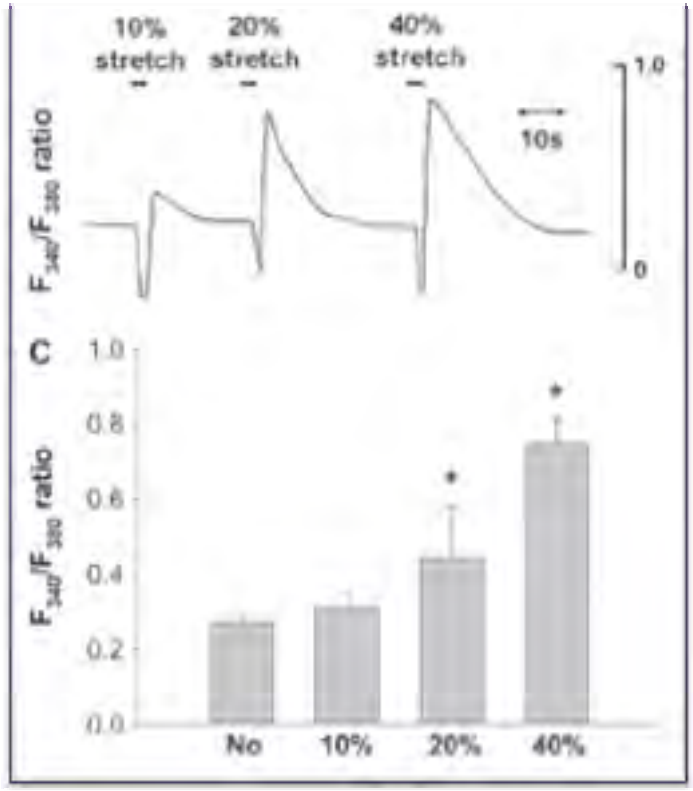
Wild V2 TG

SA channel

TRPV2

A Novel Ca^{2+} Influx Pathway Activated by Mechanical Stretch in Human Airway Smooth Muscle Cells



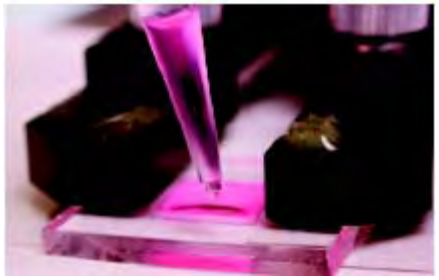


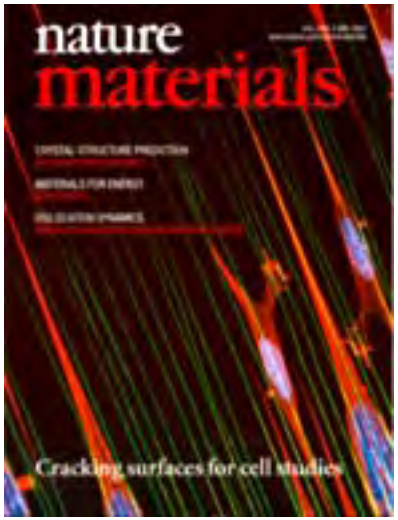
Ito et al., ARCMB, 2008

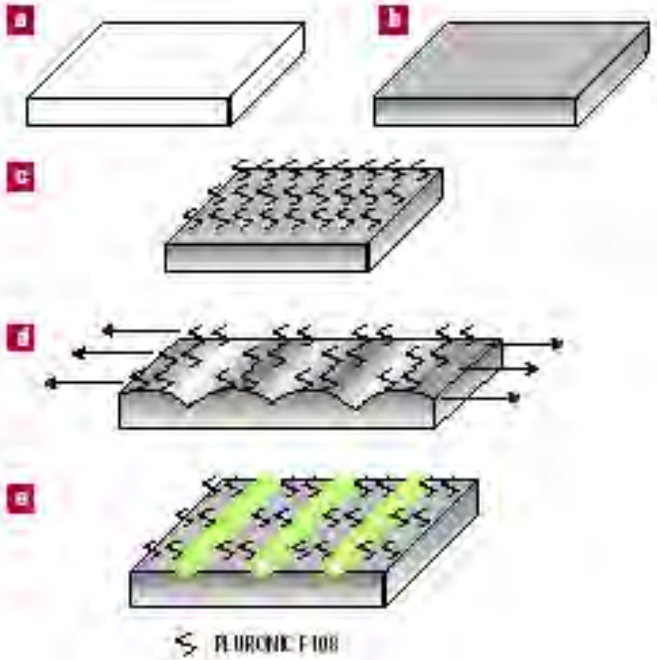
MECHANOTRANSDUCTION

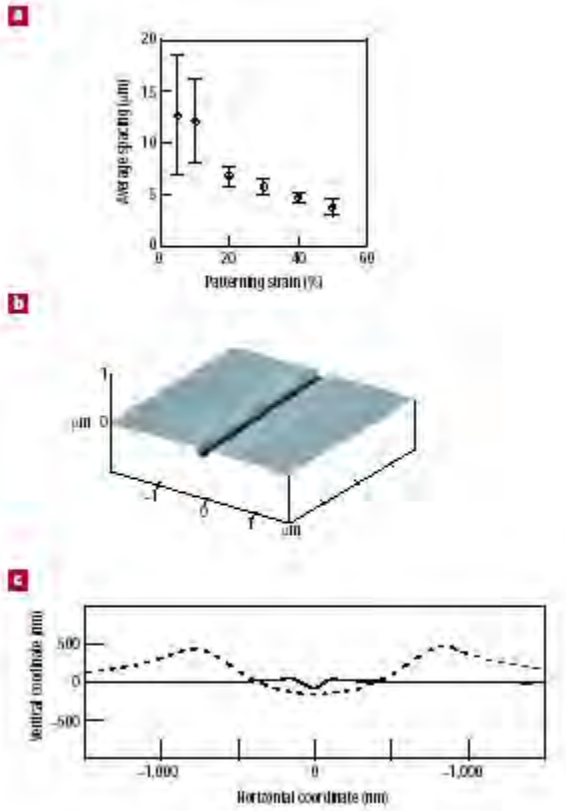
Fabrication of reconfigurable protein matrices by cracking

Nature Materials 4 (5) : 403-406, 2005





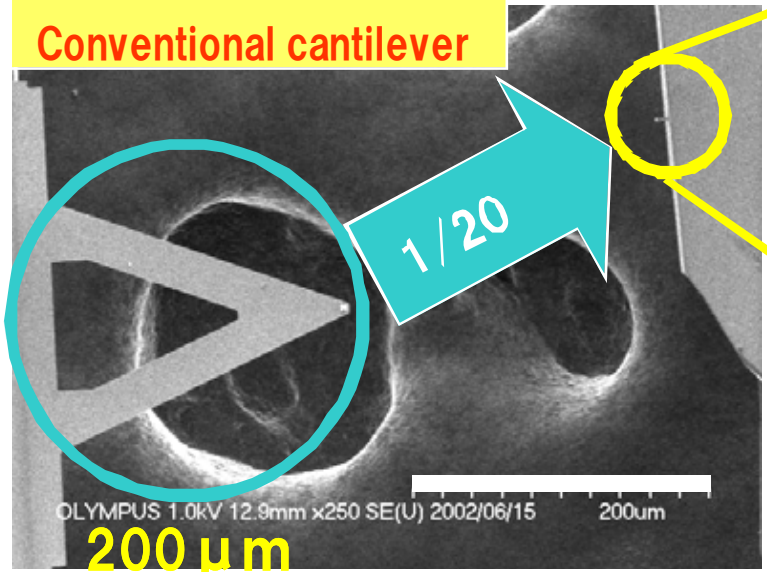




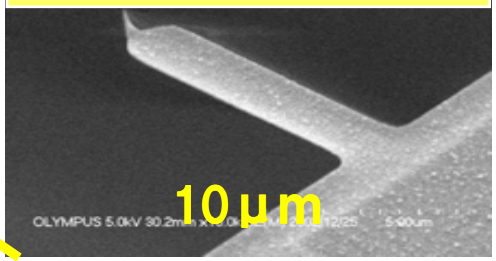
"Nano-crack"

Fast Scanning Atomic Force Microscopy

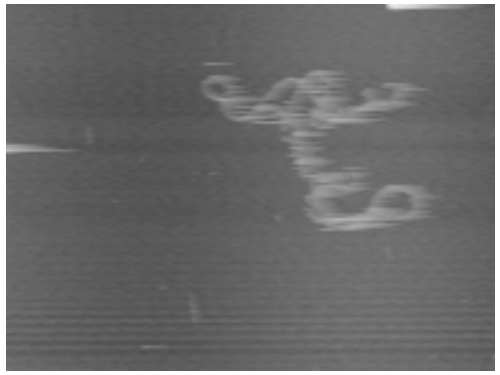
Conventional cantilever



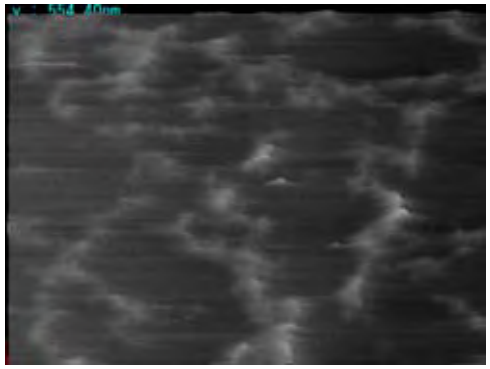
Fast Scanning cantilever



Olympus



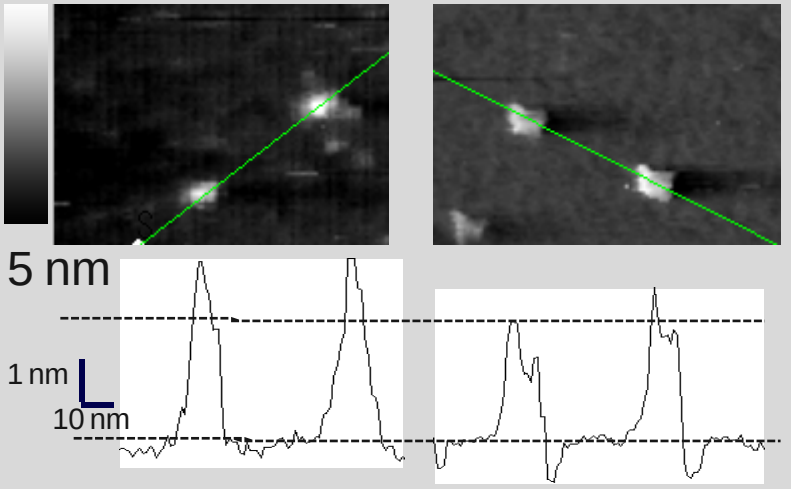
plasmid
(800 x 600 nm)



Fibronectin
(740 x 560 nm)

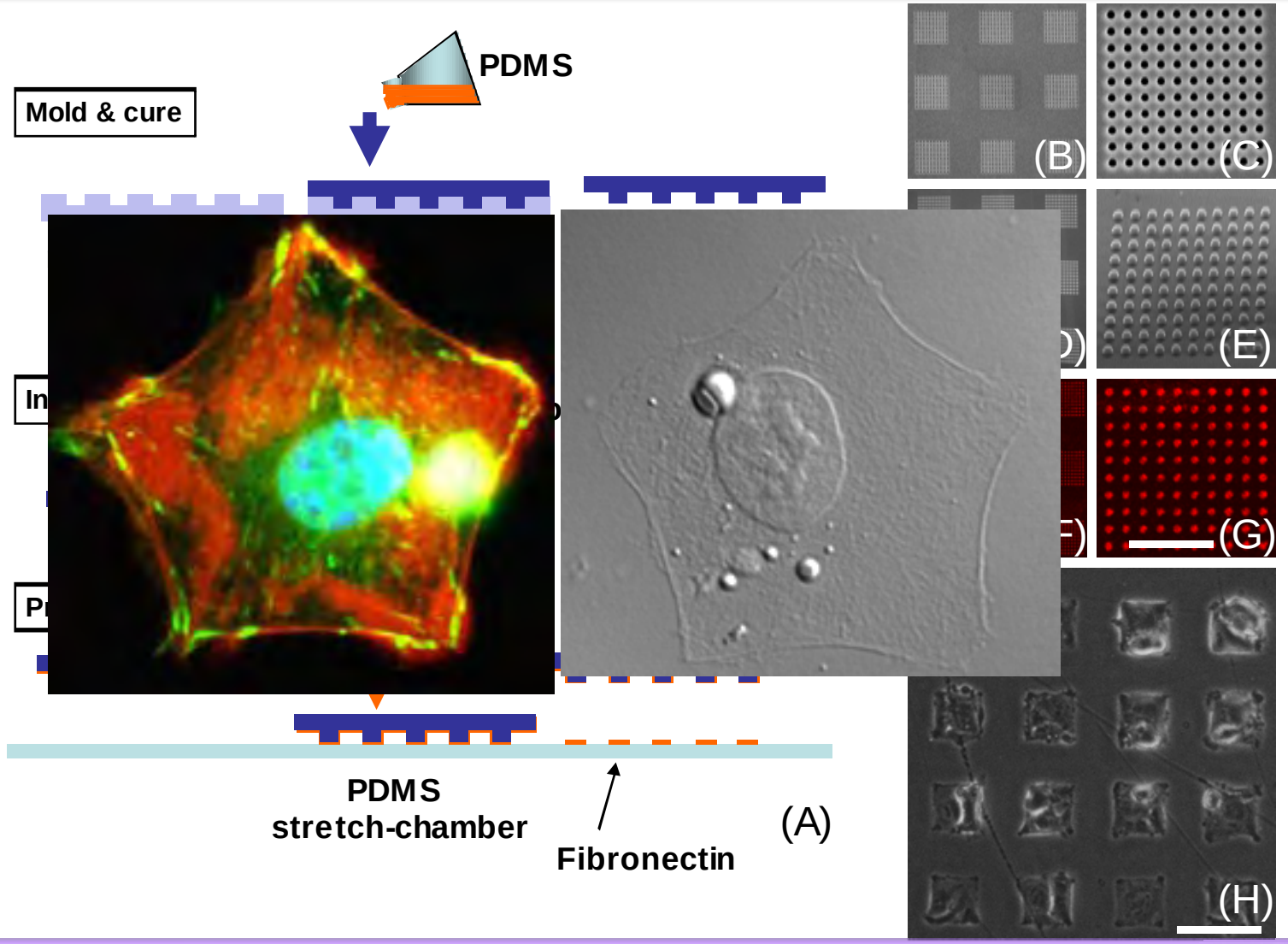
MscL / WT

MscL / G22N
Open mutant



Stretch-induced Responses in Patterned HUVEC

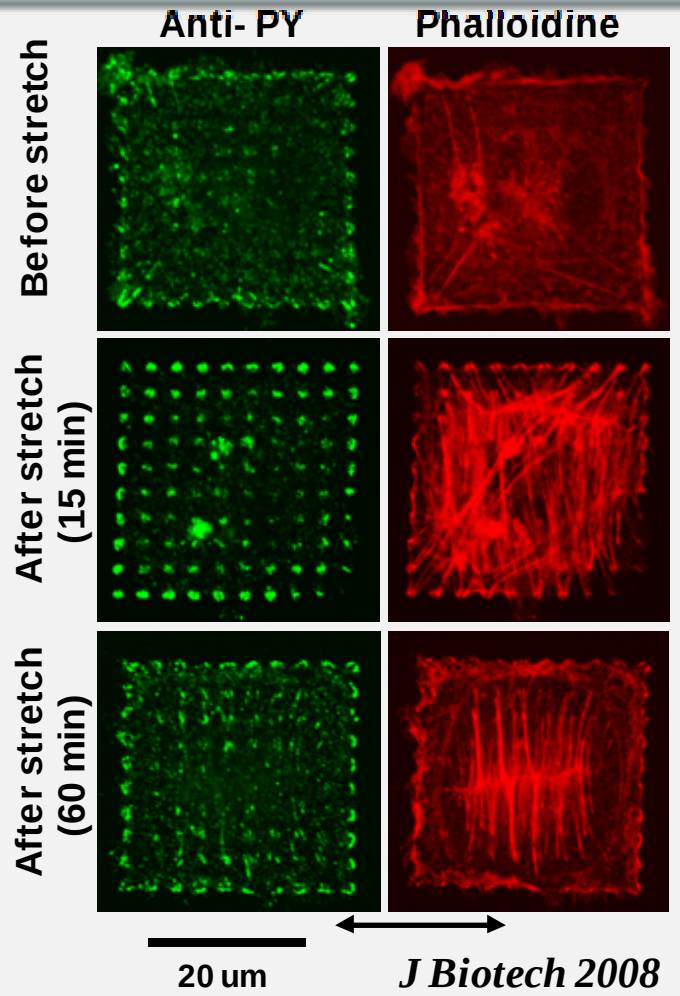
Microcontact Printing



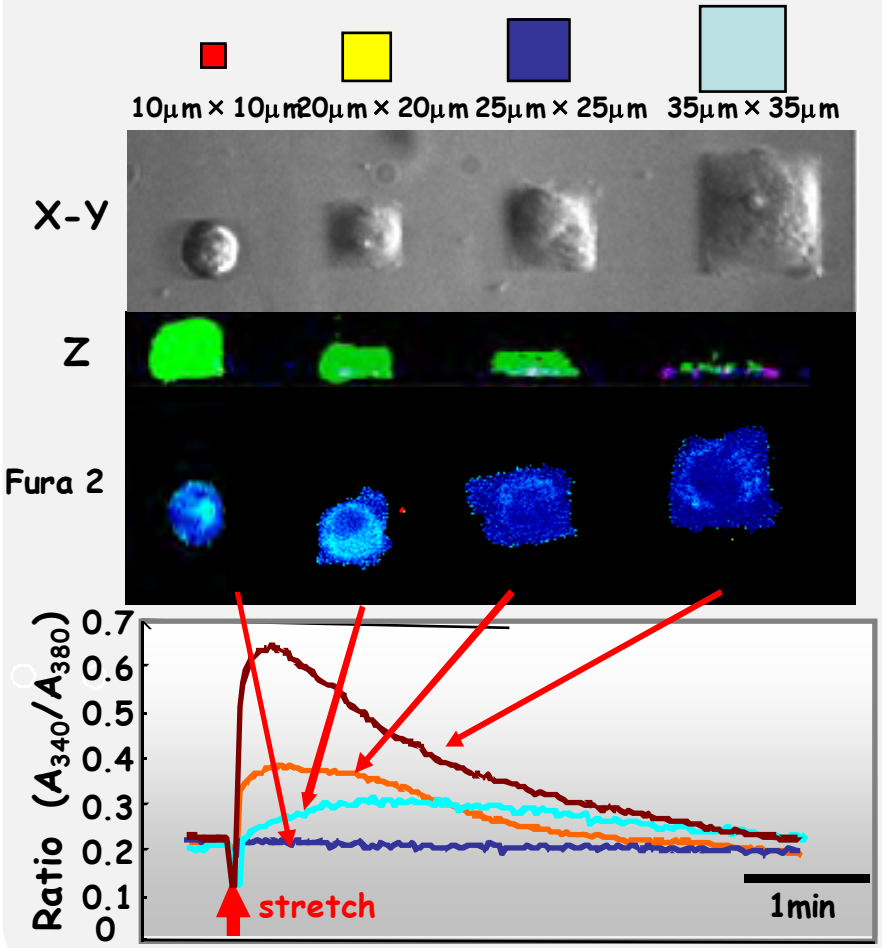
Stretch-induced Responses in Patterned HUVEC

Microcontact Printing on Stretch chamber

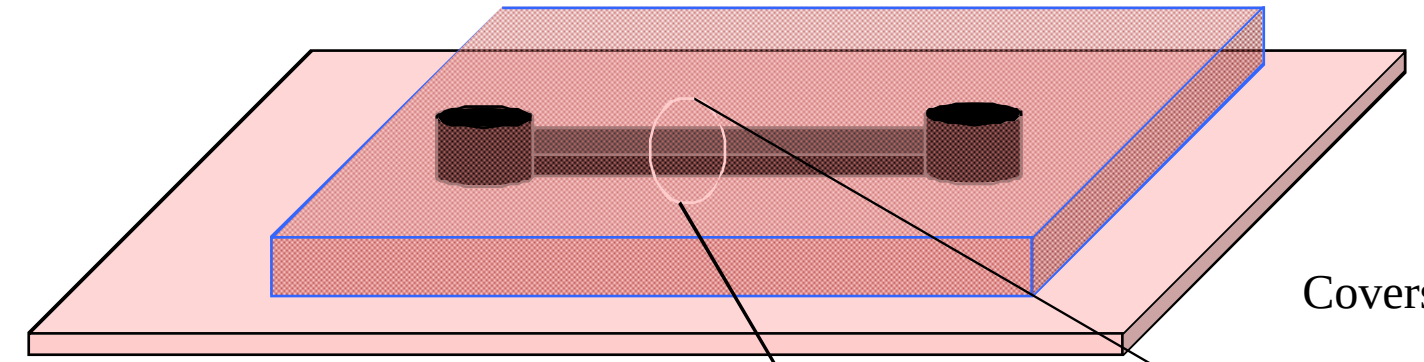
Tyrosine phosphorylation

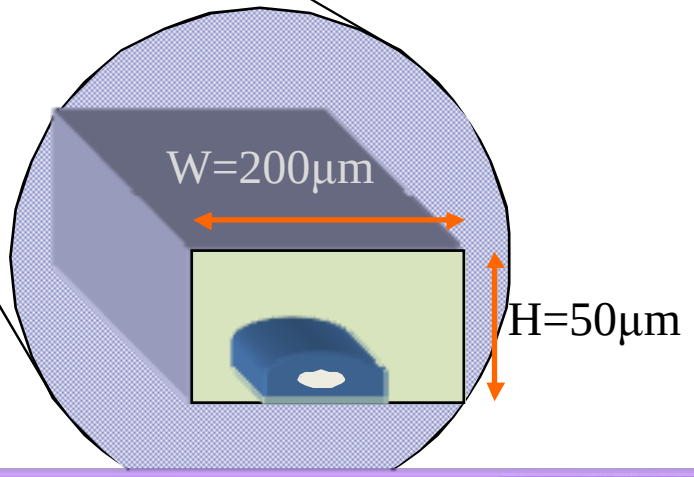


Stretch-induced $[Ca^{2+}]_i$



Cells in microchannel



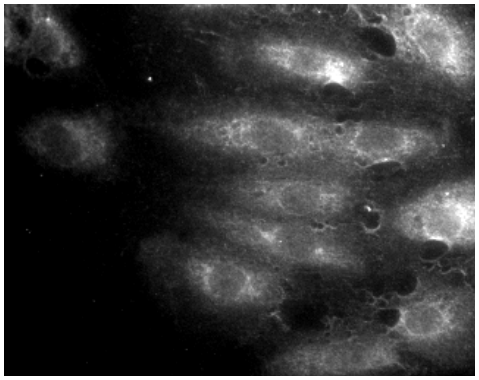


①High shear
②Laminar flow

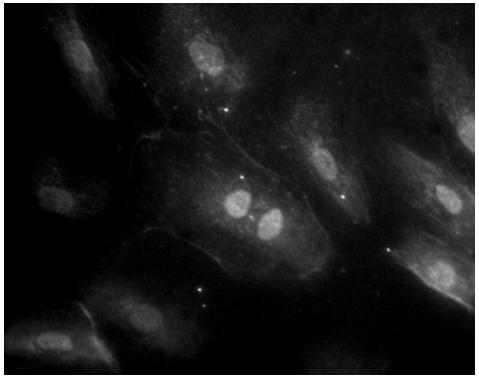
MECHANOTRANSDUCTION

Cell response in microchannel

Nuclear Factor kB

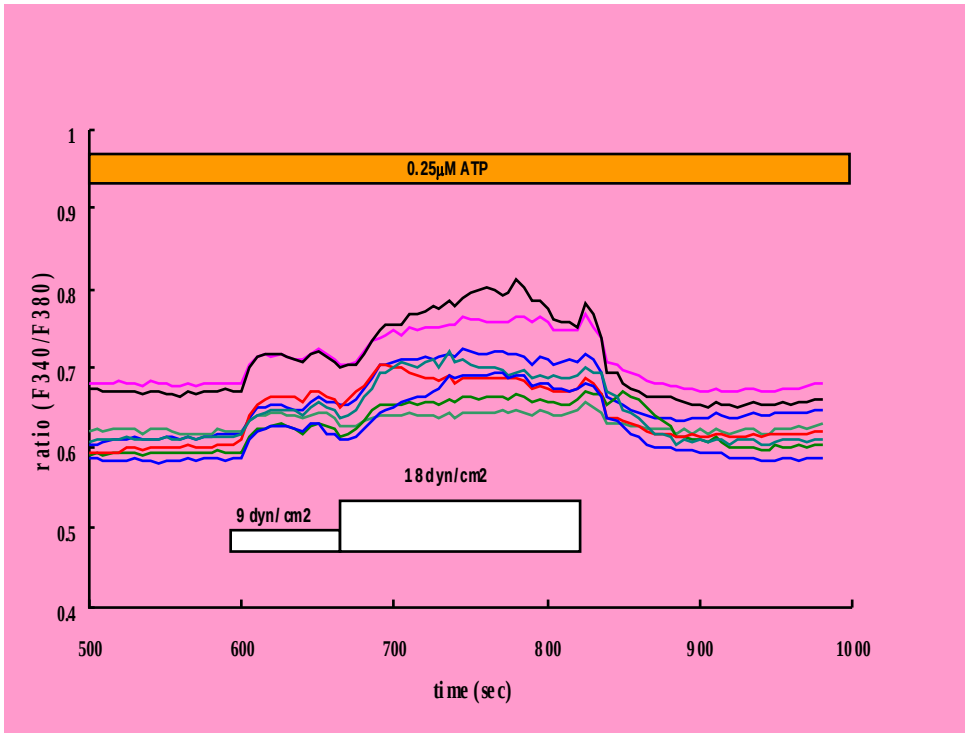


CONTROL

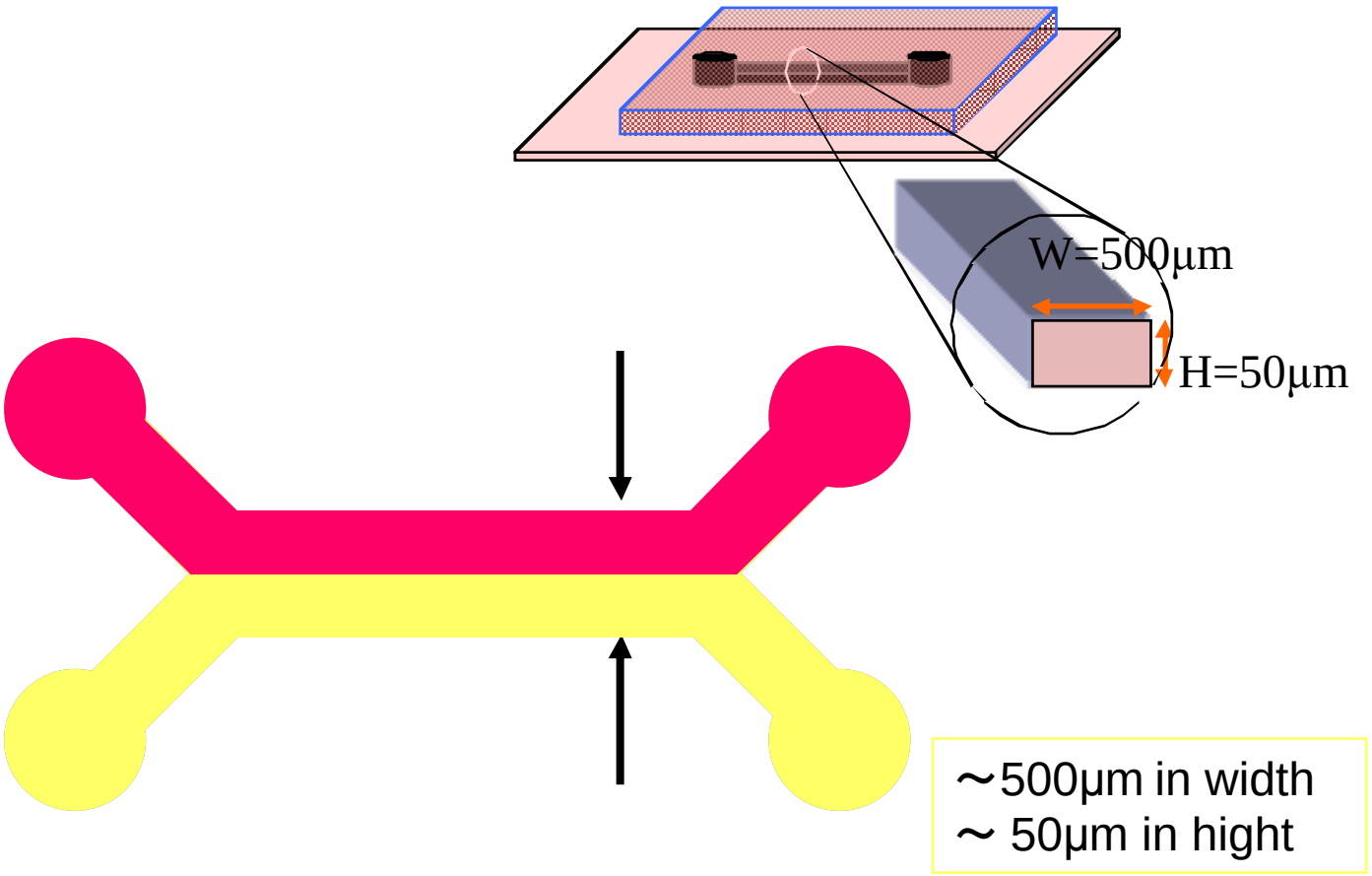


SHEAR-STRESS

$[Ca^{2+}]_i$ in response to shear stress

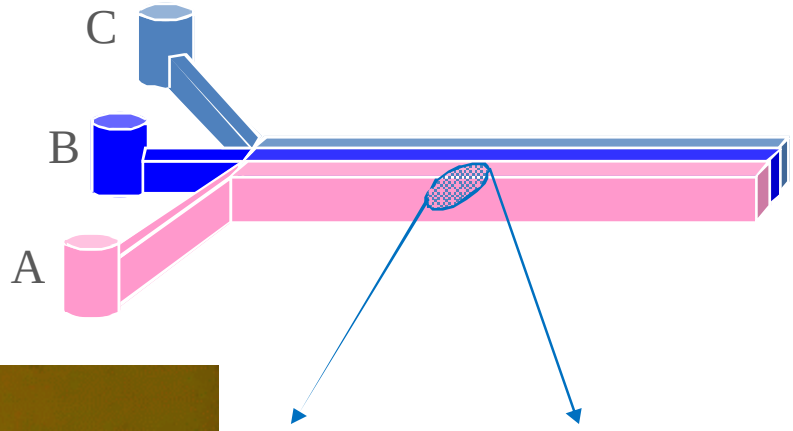


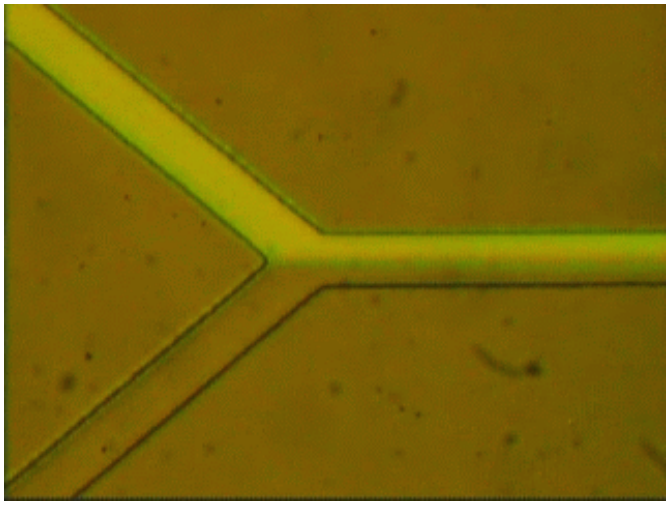
Principle of laminar flow

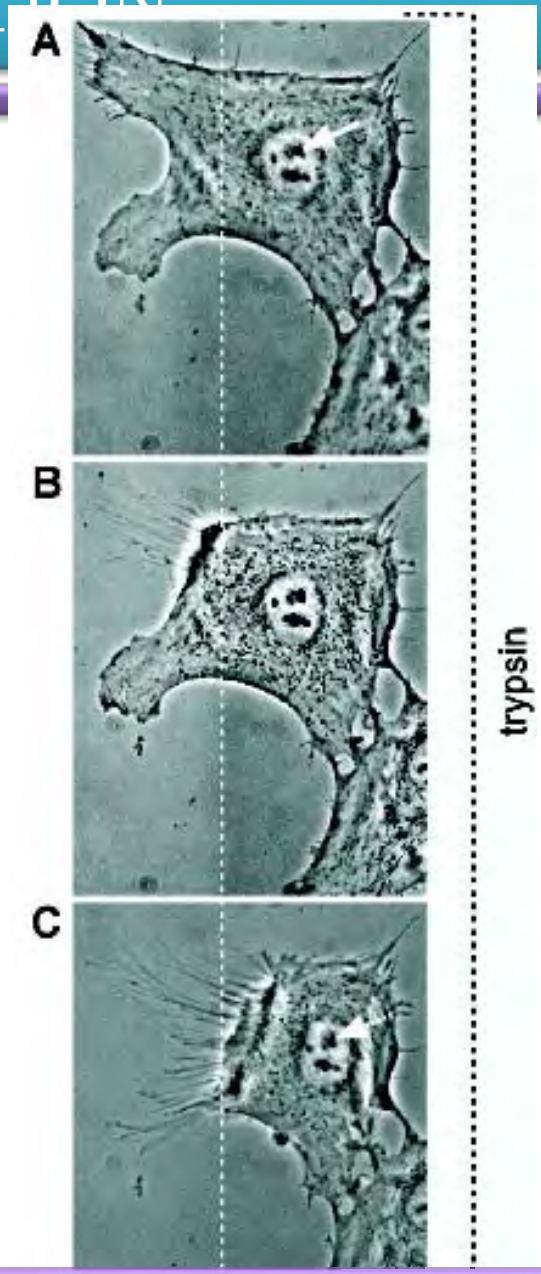


MECHANOTRANSDUCTION

Principle of laminar flow







"Subcellular positioning of small molecules"

Nature, 2001

PARTCEL (pattented)

Mechanobiology in Reproductive Medicine

INFERTILITY

Human
Assisted
Reproductive
Therapy

Mechanobiology in Reproductive Medicine

INFERTILITY in JAPAN

- Decreasing in Population
  - Death(1,077,000)>Birth(1,067,000)
- Decreasing in Birthrate
- Total fertility rate : ~1.3
- Various cause

- Infertility
  - One in 5-10 couples
    - ➢Male factor=Female factor

- 2-3 % : IVF

出生率1.25 少子化が加速

政府、「推計人口」引き下げ

昨年

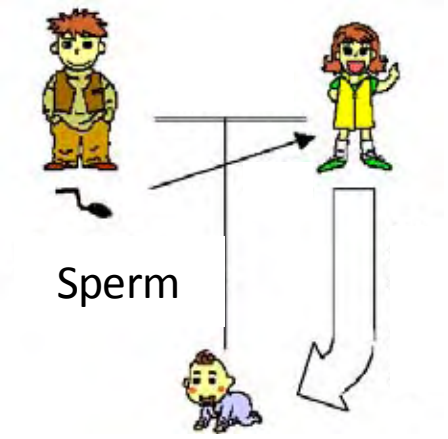
厚生労働省が一日発表した二〇〇五年の人口動態統計（概数）によると、一人の女性が生涯に産むと推定される子どもの数を表す合計特殊出生率は一・二五となった。〇四年の一・二九を大幅に下回り、五年連続で過去最低を更新した。政府は年金制度を維持するために一・三九への回復を前提

平成18年6月2日日経新聞

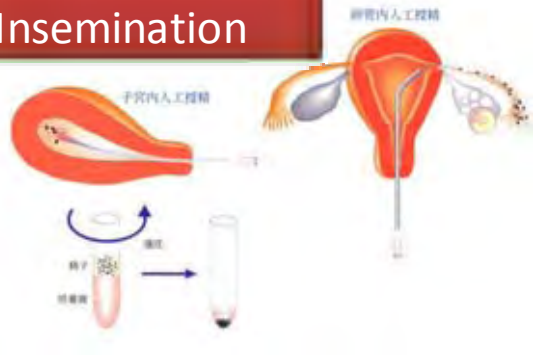
INFERTILIGY TREATMENT

- Timing treatment (Ovulation)
- AIH (Artificial Insemination by Husband)
- IVF (In Vitro Fertilization)
  - IVF-conventional
  - IVF-ICSI（Intracytoplasmic sperm injection）

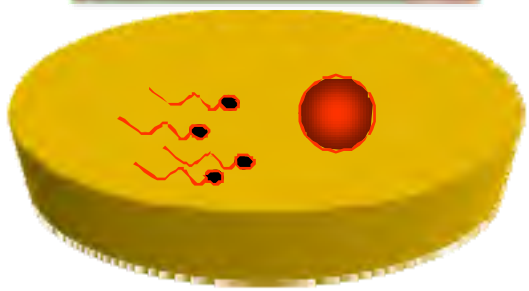
ICSI



Artificial Insemination

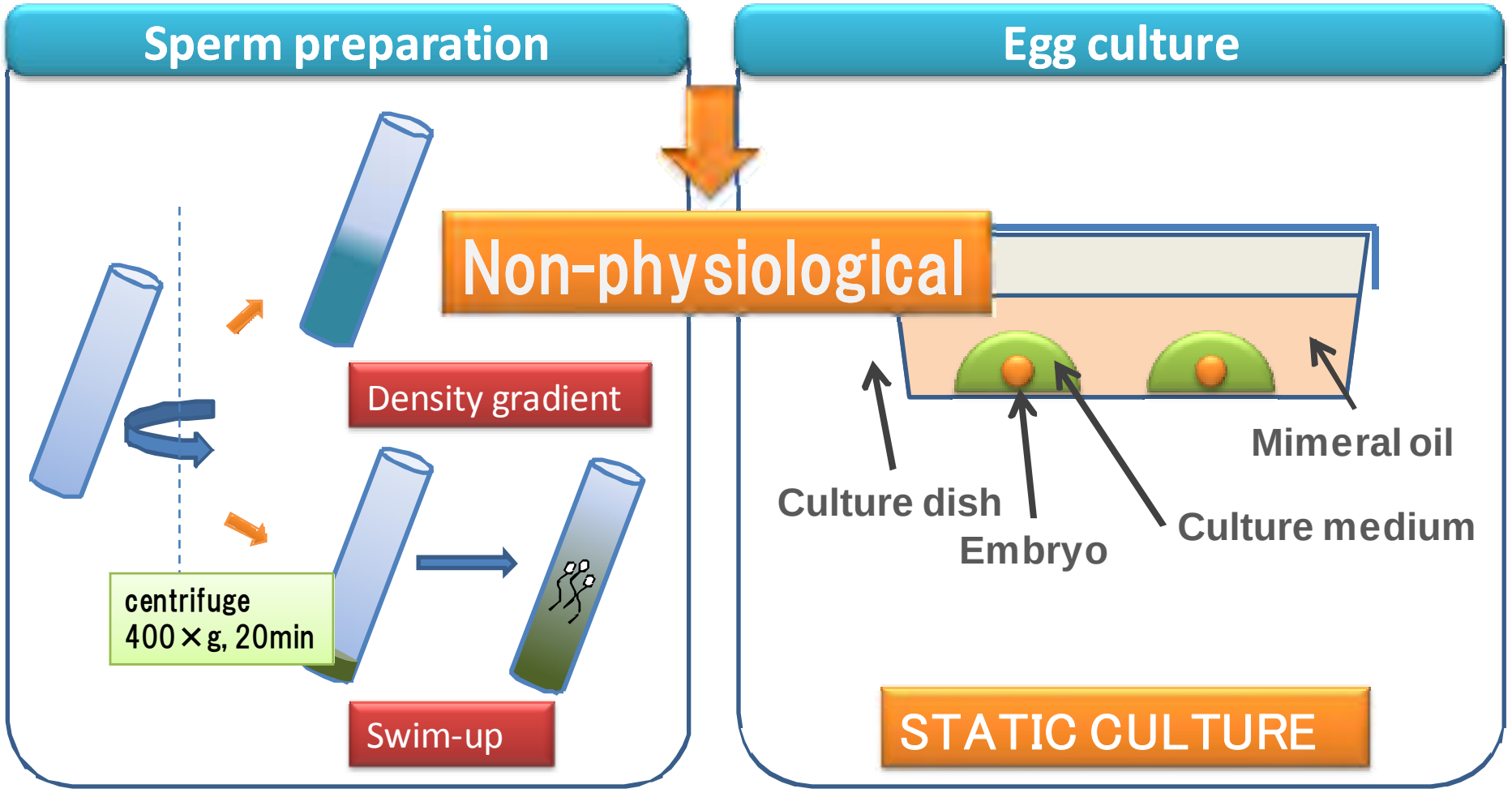


Conventional IVF



REPRODUCTIVE MEDICINE

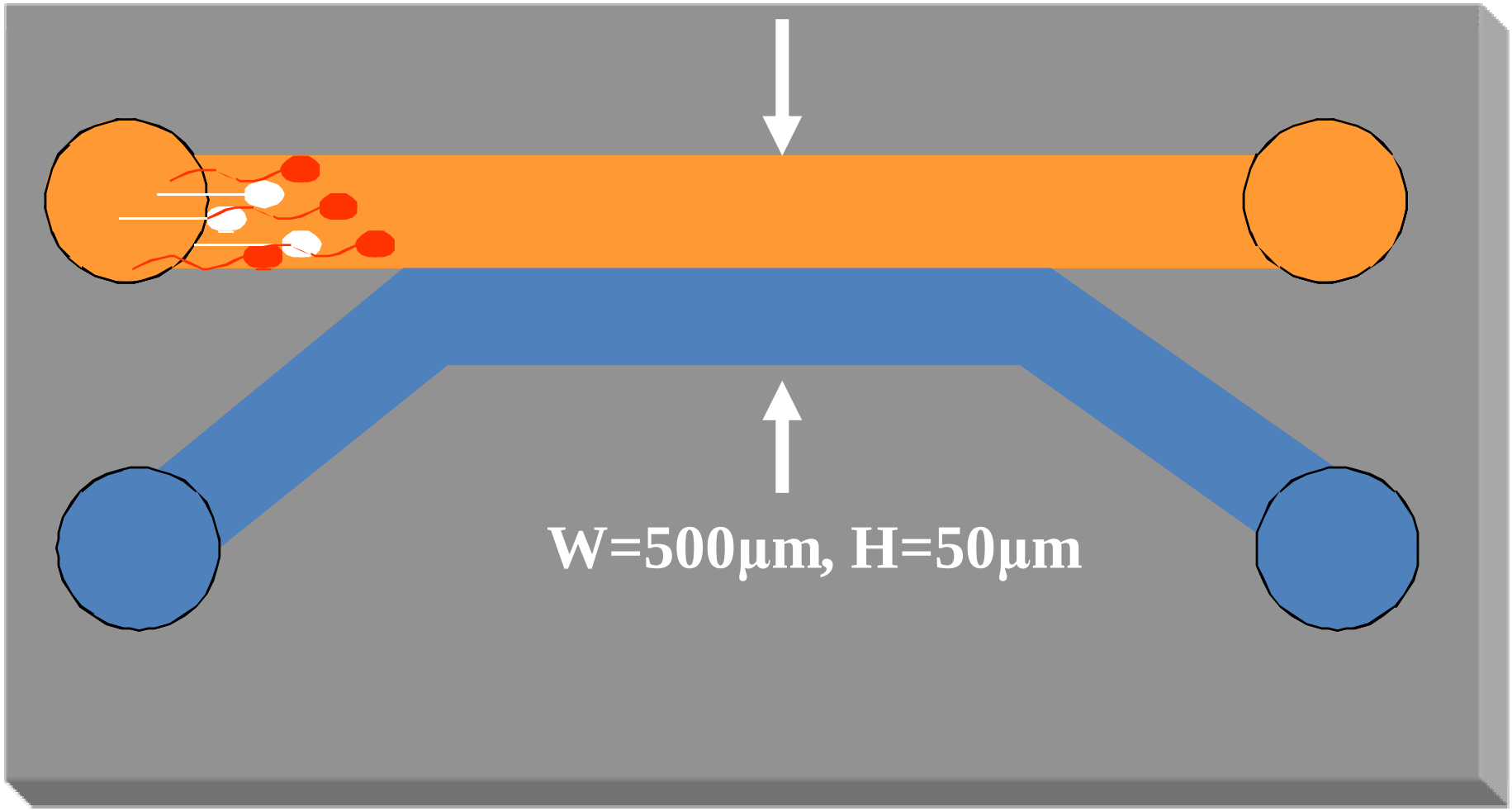
Conventional Human Assisted Reproductive Treatment (HART)



REPRODUCTIVE MEDICINE

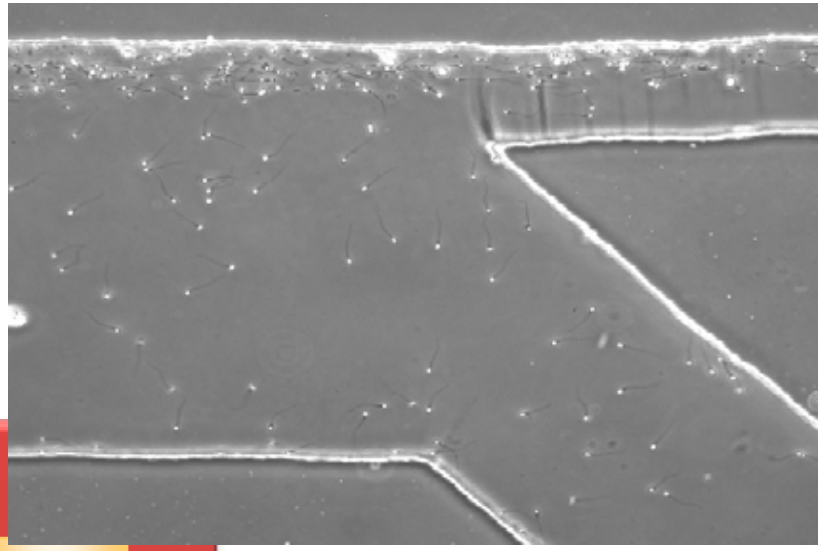
1. Microfluidic Sperm Sorter System
2. Mechanically Active Embryo Culture System

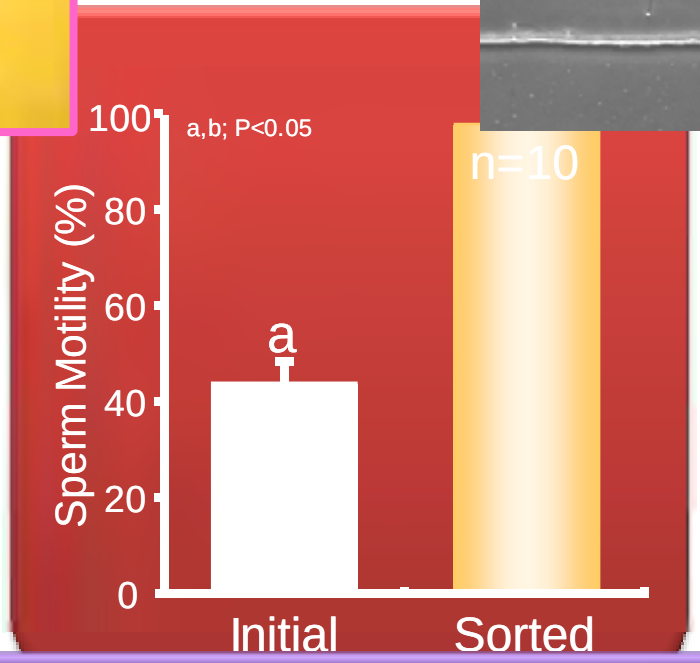
Sperm Sorter /microfluidics



REPRODUCTIVE MEDICINE

Sperm Sorter /microfluidics

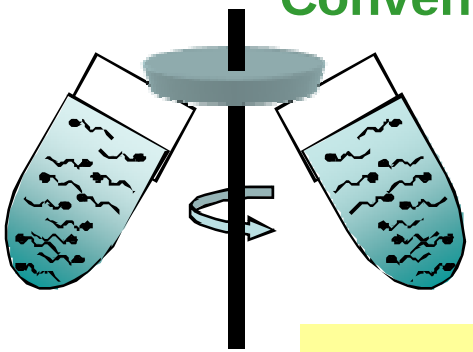




REPRODUCTIVE MEDICINE

No Centrifugation

Conventional protocol



400 x *g*

Motile sperm

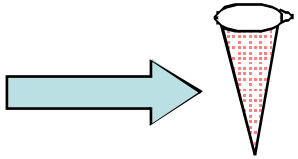
SWIM UP !!

MFSS & Dilution

semen

+

medium



Dilution

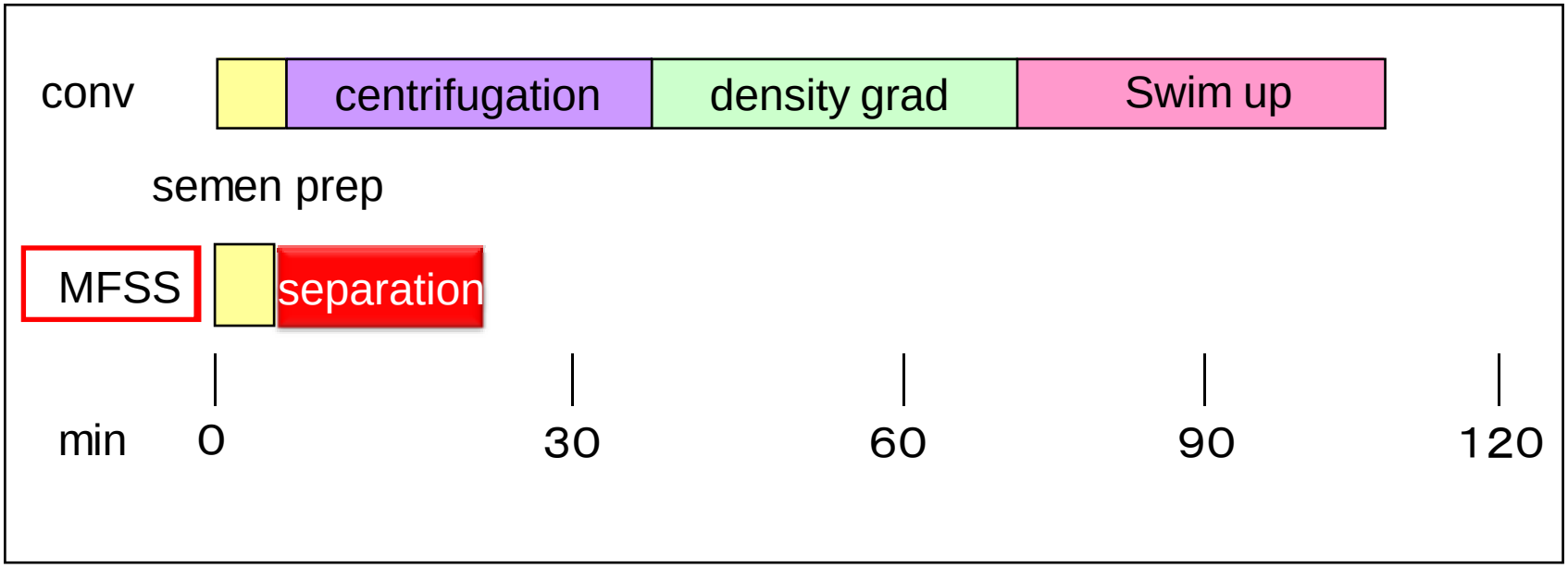
MFSS

No centrifugation

> X 3

REPRODUCTIVE MEDICINE

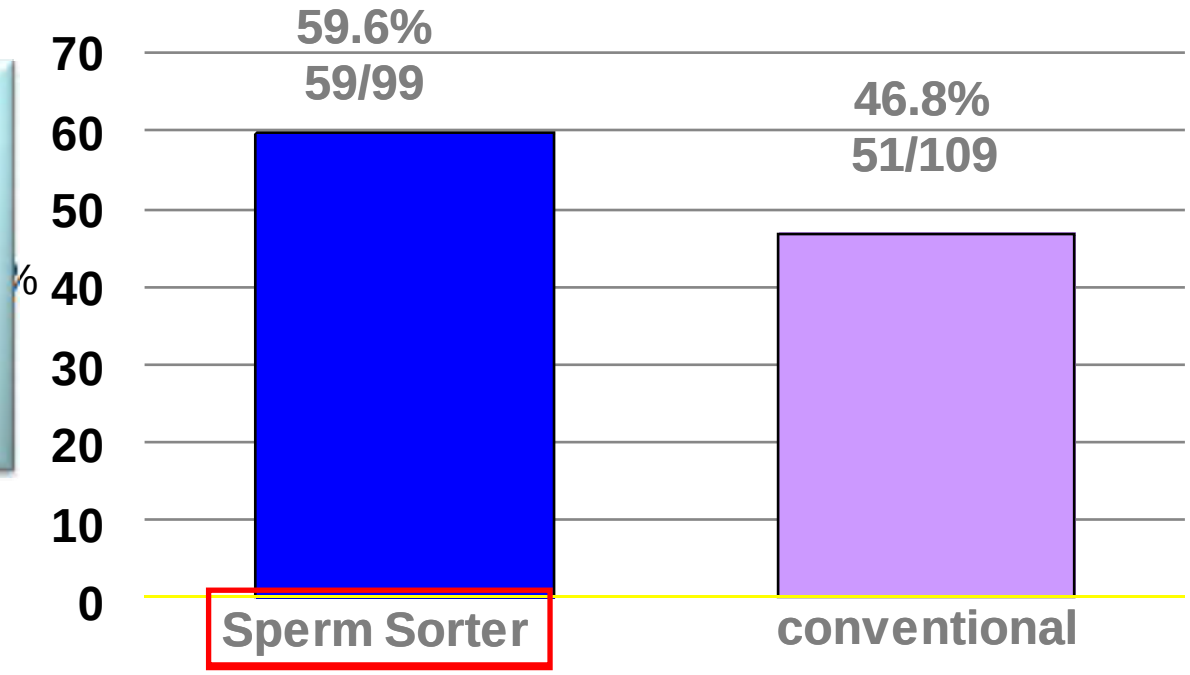
Short processing time



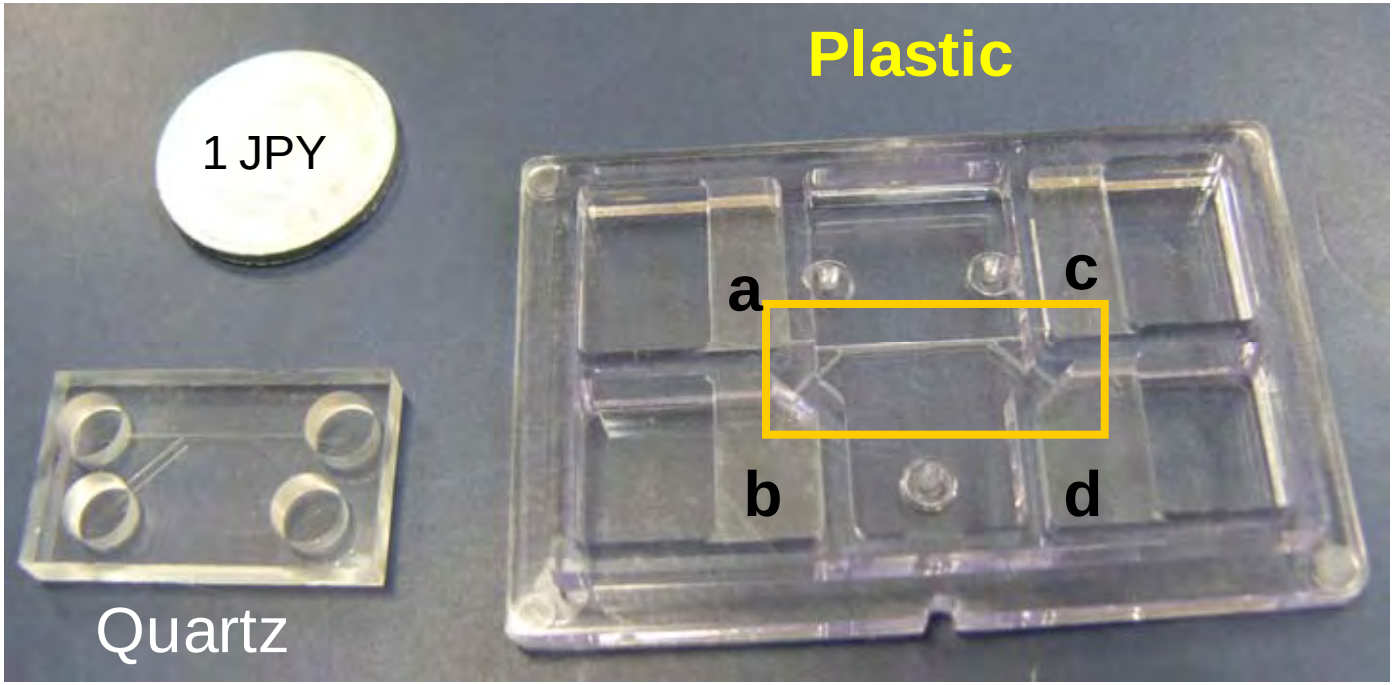
REPRODUCTIVE MEDICINE

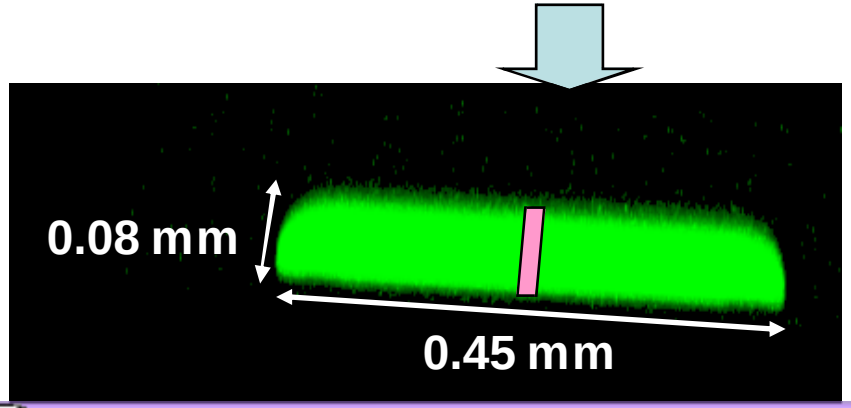
Fertilization rate ↑

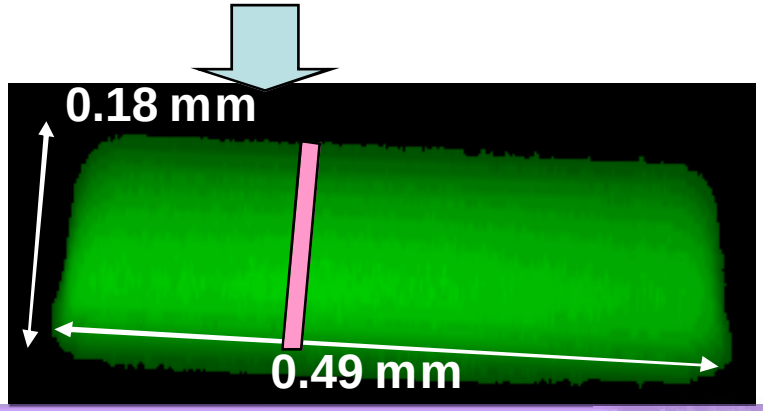
- IRB: Passed 13/5/2005
- 40 couples with informed consent
- > 4 eggs ⇒ ICSI
- Embryo transplantation



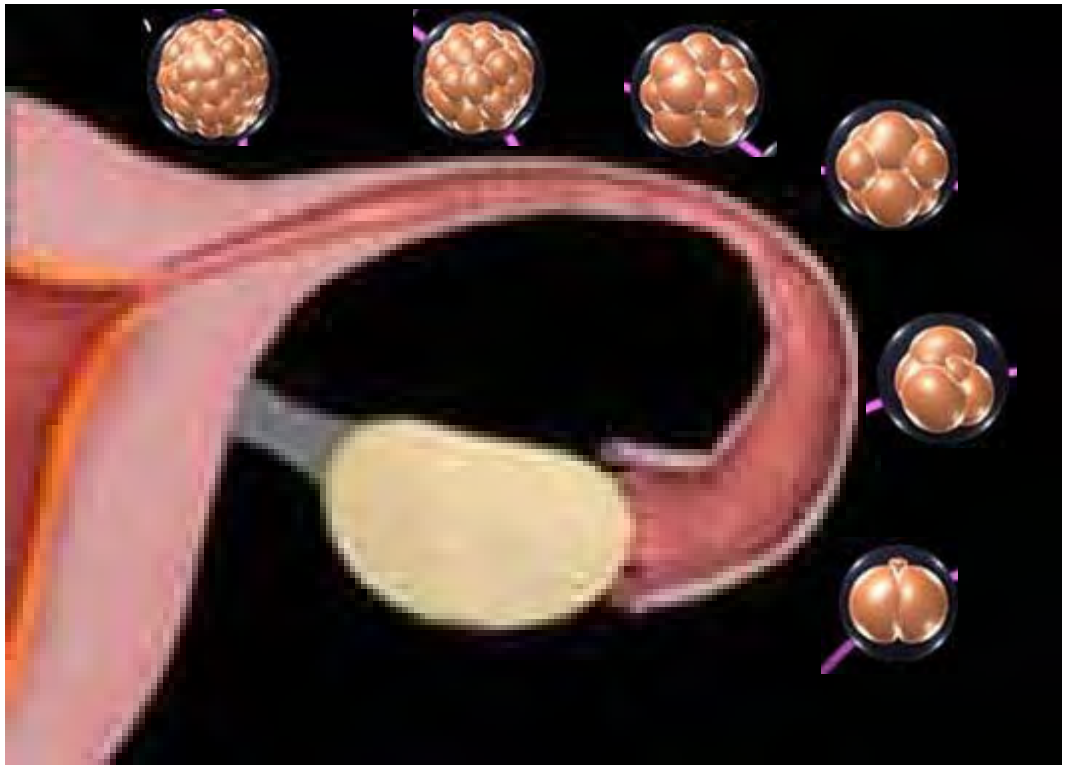
REPRODUCTIVE MEDICINE

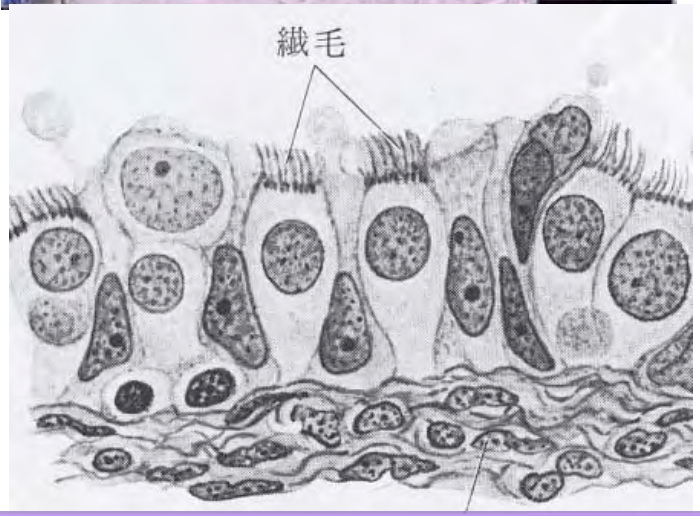






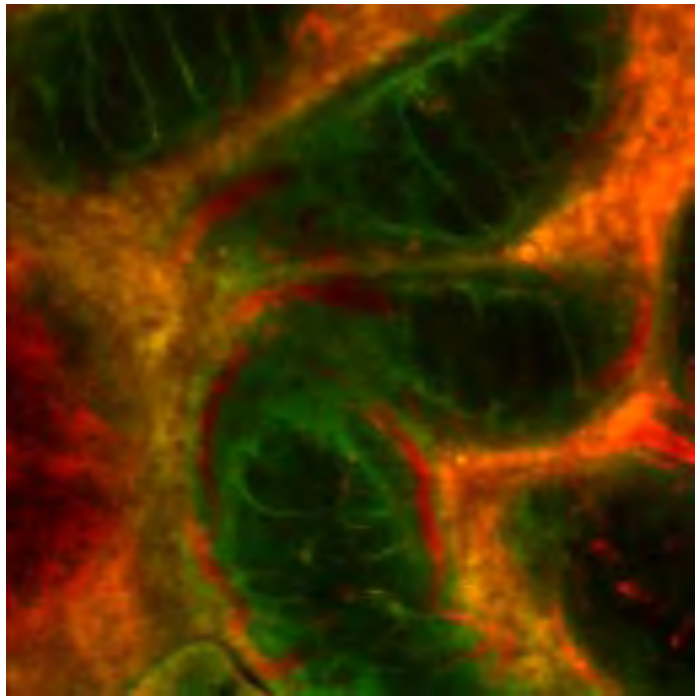
REPRODUCTIVE MEDICINE



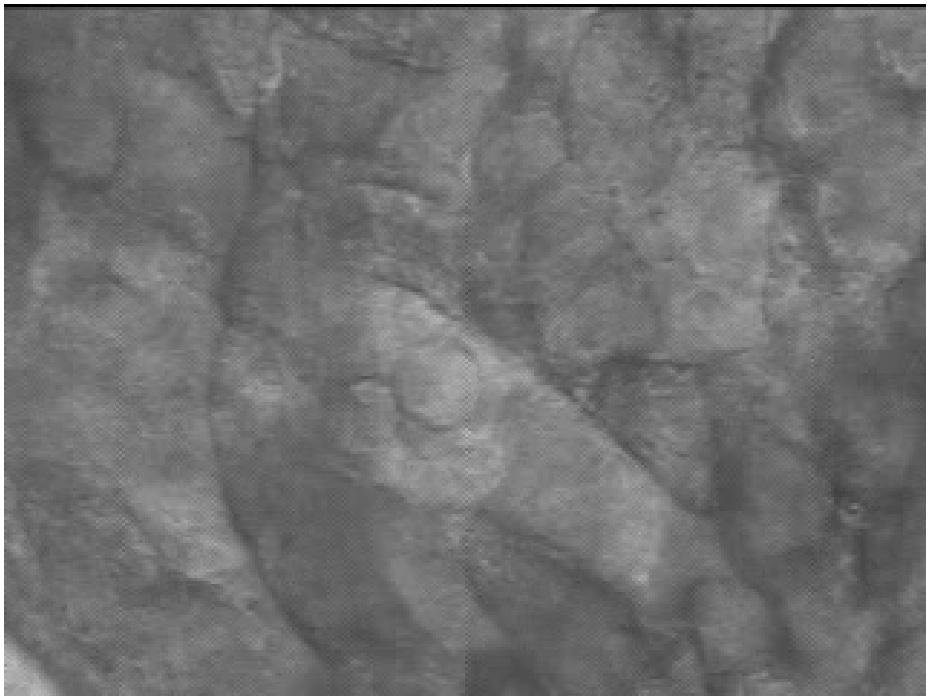


REPRODUCTIVE MEDICINE

RAT FALLOPIAN TUBE

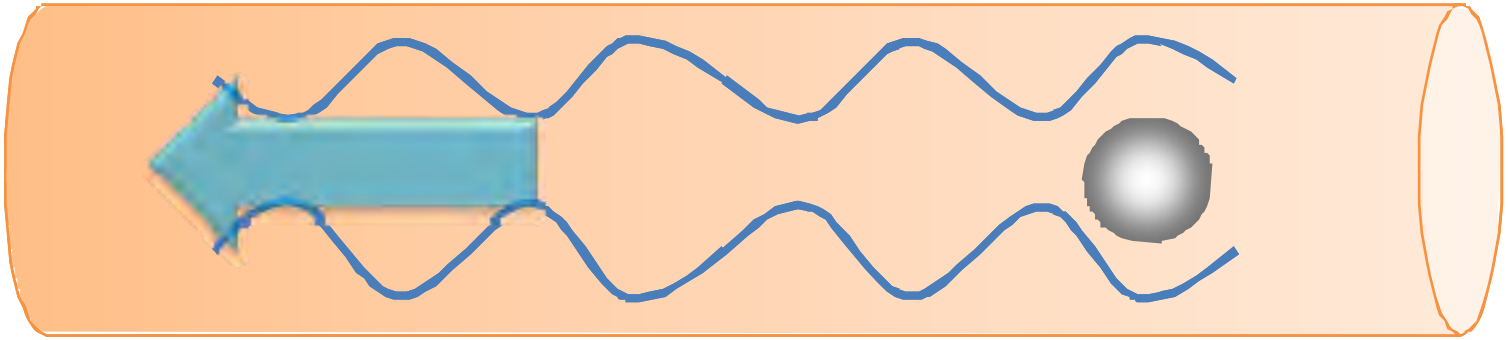


Mouse eggs in fallopian tube

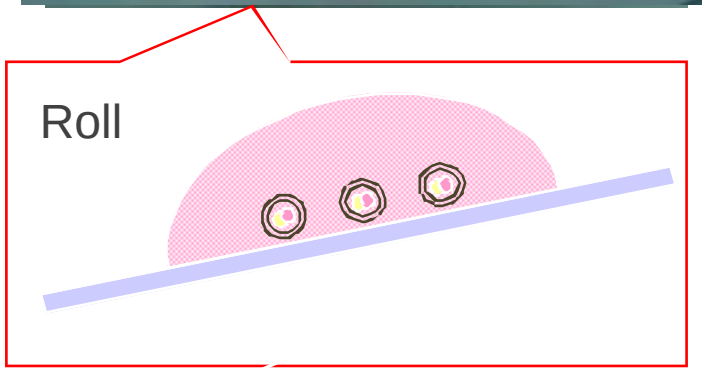


Mechanical stresses applied to embryo in oviduct

- Shear Stress
- Compression
- Stretch
- Turbulence
- Friction



Tilting Embryo Culture System (TECS)



Velocity (mm/sec) 0.003 Shear stress (dynes/cm^2) 0.0018

20deg tilting for 10 min, 4-6 mouse eggs/drop

	Blastocysts/total	%
Control	63/136	46
TECS	77/130	59

*p=0.035

STREX Inc.

Acknowledgement

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  - X. Tang
  - F. Lopez

BIO Manipulation

System Cell Engineering by Multi-scale Manipulation

ICORP 国際共同研究 International Cooperative Research Project

CREST 戦略的創造研究推進事業 Core Research for Evolutional Science and Technology

STREX ストレックス株式会社

NEDO