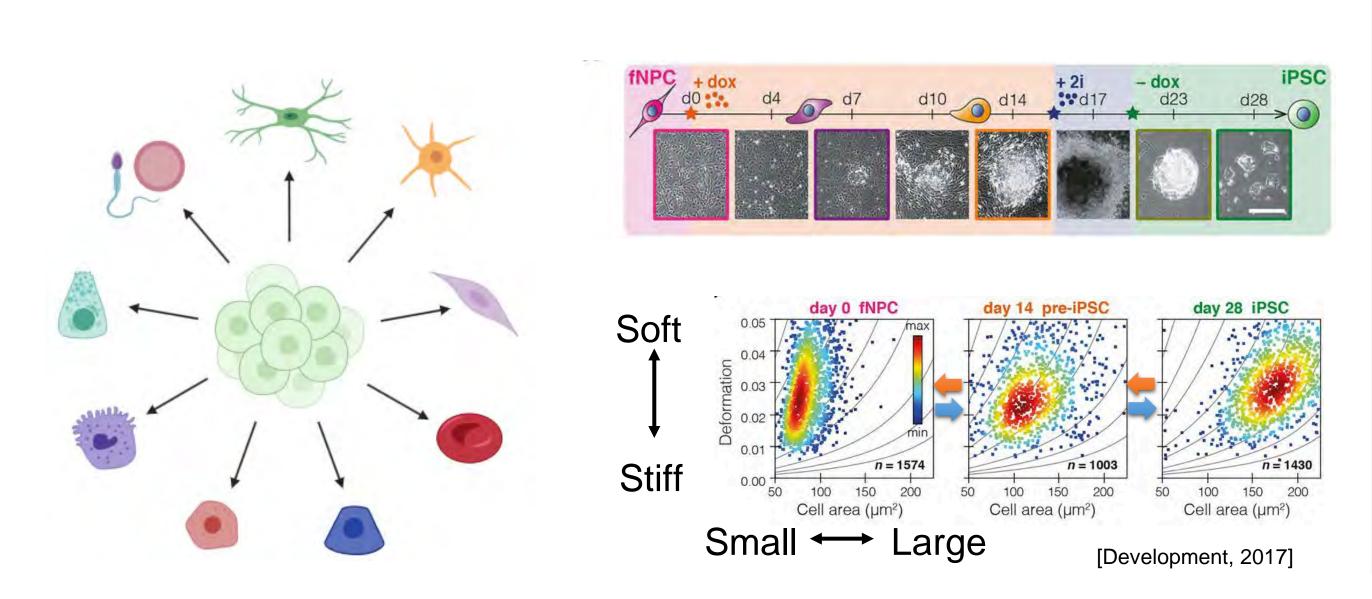


Development of non-labeled, non-destructive single stem cell analysis technology for cell therapy

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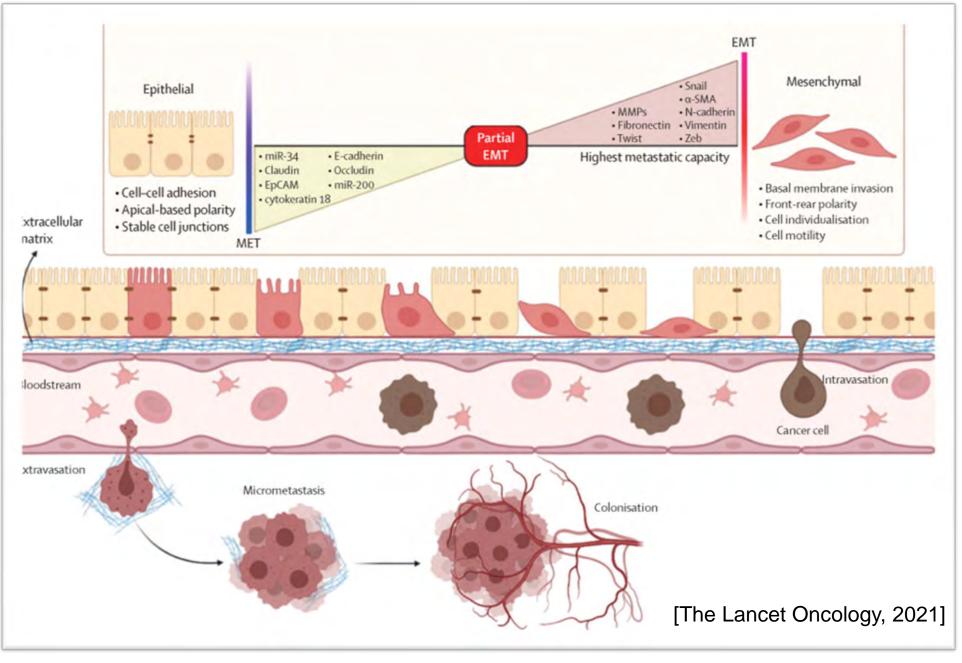
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Research Background



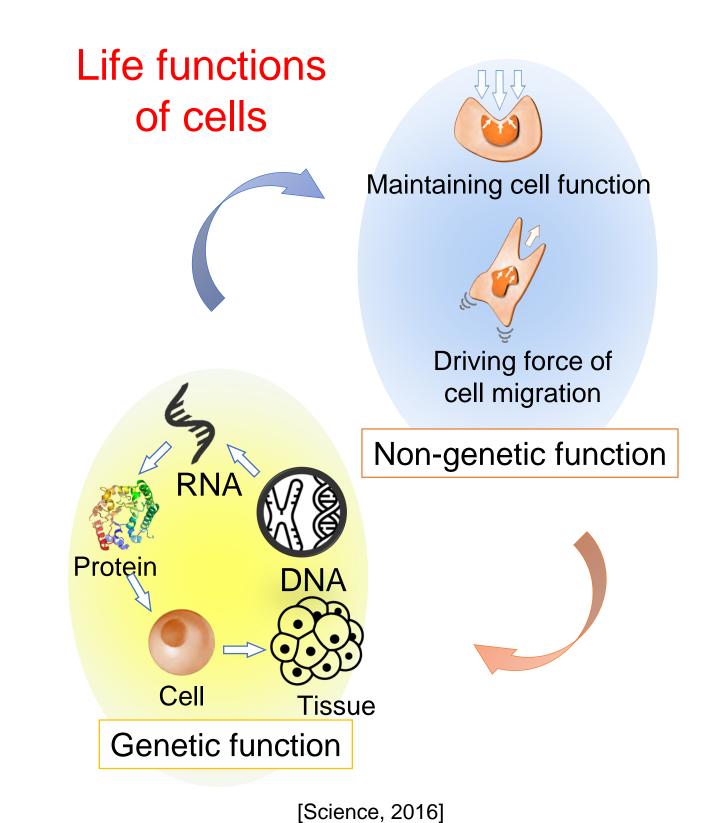
Pluripotency, Multipotency

The ability of a cell to differentiate into any cell type or a limited range of cell types



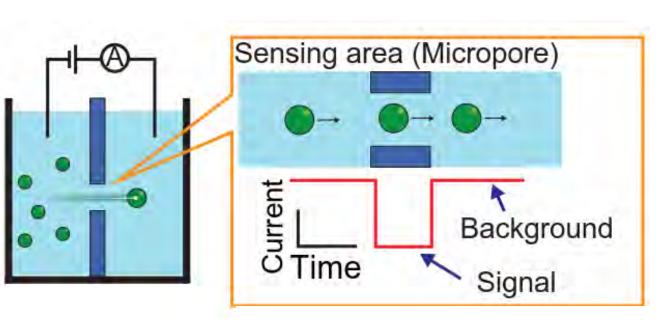
EMT (Epithelial-Mesenchymal Transition)

A biologic process that allows a polarized epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity and invasiveness.



lonic sensing for cell deformability

Principle: Coulter counter



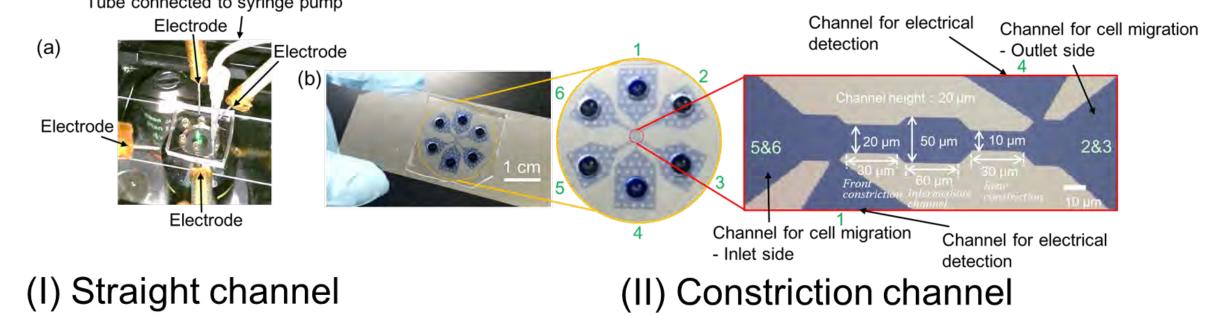
Particle Signal amplitude volume

Detectable information

- Size of molecules and cells Numeber of molecules and cells
- in sample solution

lonic sensing on a chip for cell diagnosis Tube connected to syringe pump

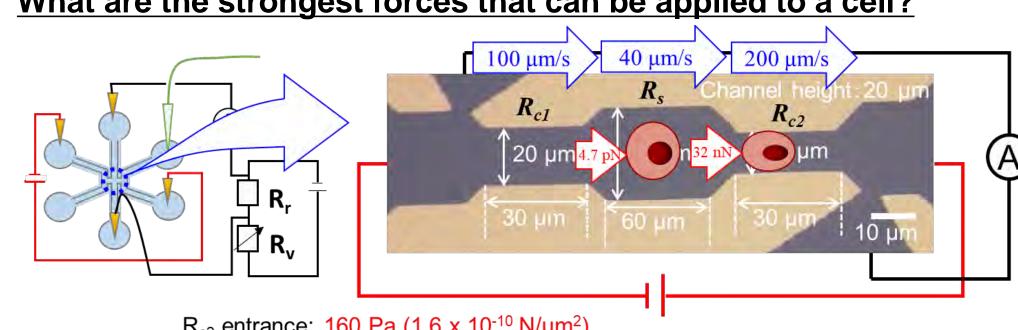
Time [s]



Duration time (Cell deformability) Signal intensity Signal intensity (Sample size)

Time [s] [Sano et al., Anal. Chem., 2019]

What are the strongest forces that can be applied to a cell?



 R_{c2} entrance: 160 Pa (1.6 x 10⁻¹⁰ N/ μ m²) \rightarrow 32 nN (32 x 10⁻⁹ N) at the cross section of R_{c2} ,10 μ m x 20 μ m. Assumption: A HeLa cell, Φ=14 μm, in free solution In R_s at 40 μ m/s fluid, the cell experiences 4.7 pN (4.7 x 10⁻¹² N) In R_{c2} at 200 μ m/s fluid, the cell experiences 23 pN (23 x 10⁻¹² N)

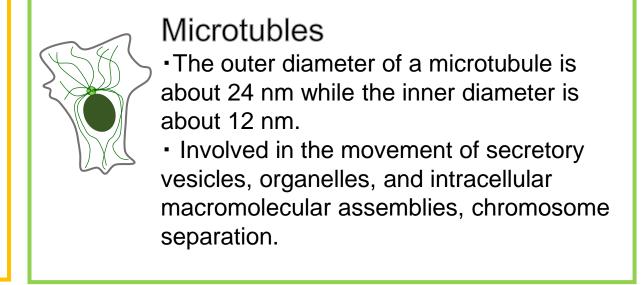
	T T G G G G G G G G G G G G G G G G G G	1110410	1 Minute
Fibroblasts	1.2-1.7x10 ⁻⁹ N/µm ² 3-4x10 ⁻¹⁰ N/µm ²	Traction Compression	Journal of Cell Science 110: 2109-2116. (1997)
Neutrophils Endothelial cells	~10 ⁻¹⁰ N/µm ²	Aspiration into micropipettes	Biophysical Journal 56: 151-160. (1989) J Biomech Eng-T Asme 109: 27-34. (1987)
Epithelial cells	1-5x10 ⁻⁹ N/μm ²	Cell poking	P Natl Acad Sci-Biol 79: 5327-5331. (1982), Journal of Cell Science 107: 1105-1114. (1994)
Fibroblast protrusions Neurites	1.5-3x10 ⁻¹⁰ N 10 ⁻¹⁰ - 10 ⁻⁹ N	microneedles	J Cell Biol 111: 2513-2526. (1990) J Cell Biol 109: 3073-3083. (1989)

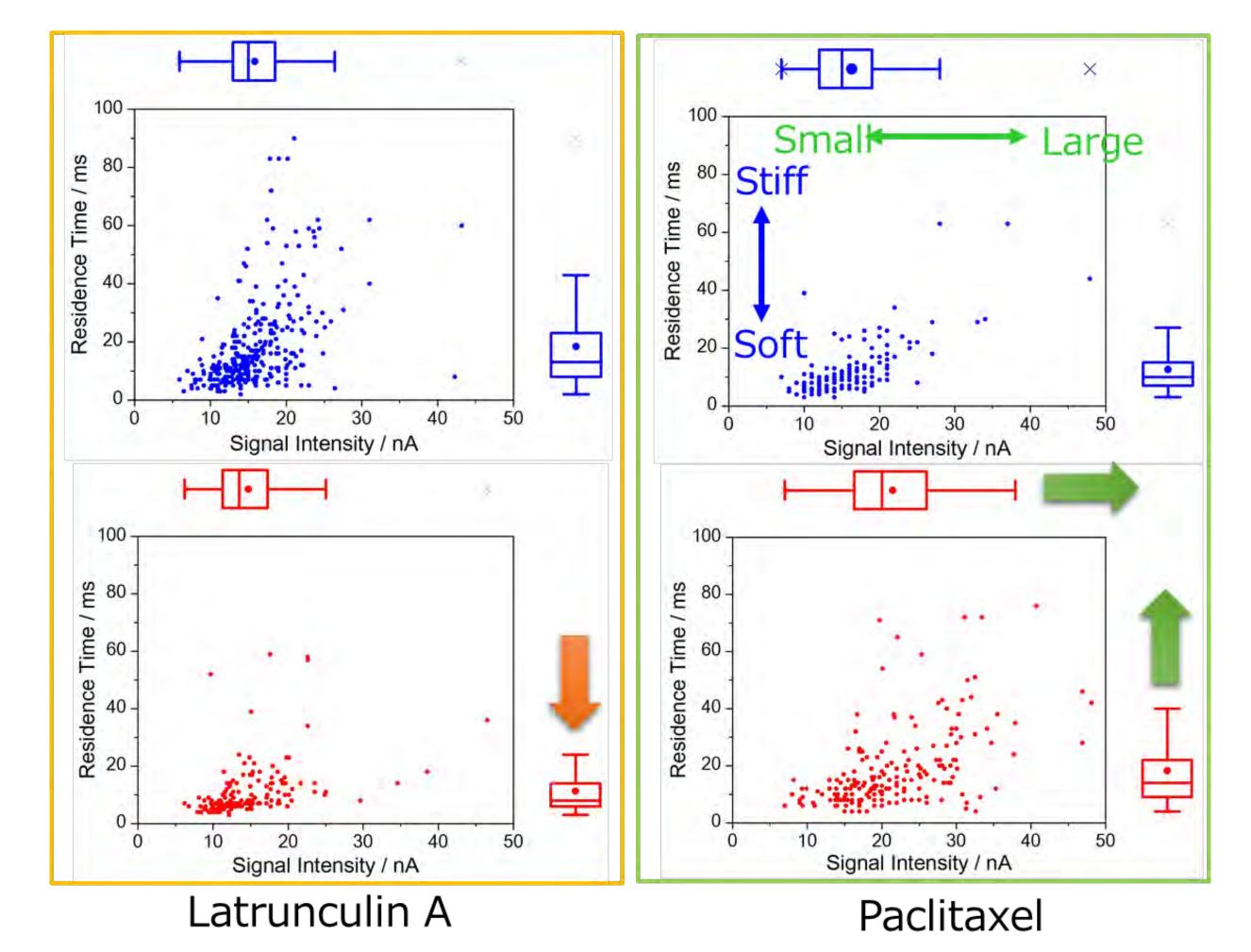
Cell diagnosis

Demonstration of a model experiment

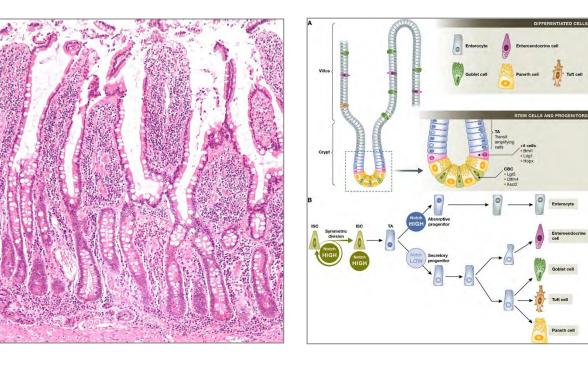
Actin filaments (Microfilaments) •The mass is roughly 42-kDa and it is the

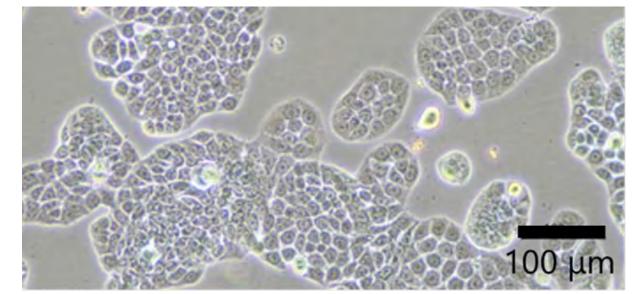
monomeric subunit of two types of filaments in cells, G-actin and F-actin. Involved in muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, and the establishment and maintenance of cell junctions and cell shape.

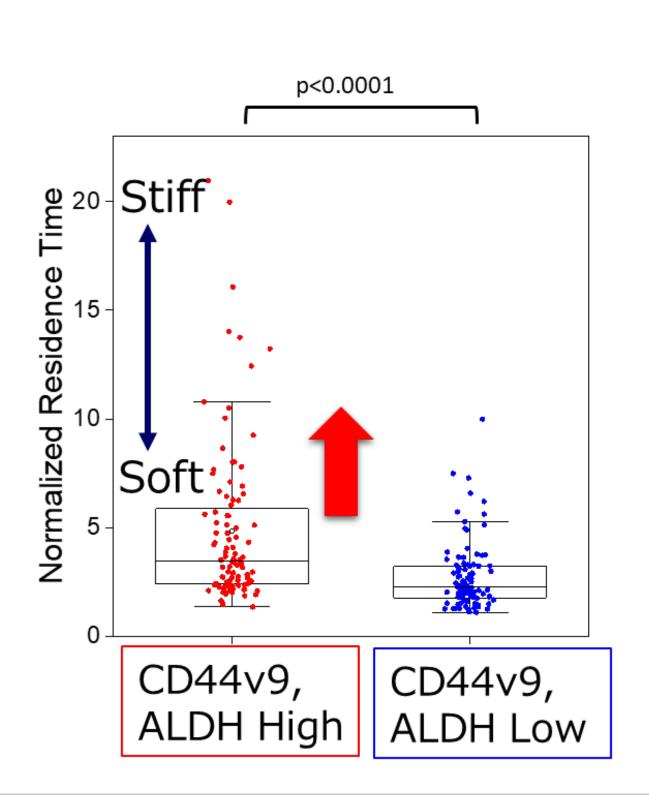




Cancer stem-like cell measurements







HT-29 cell as a model of a highly pluripotent cell

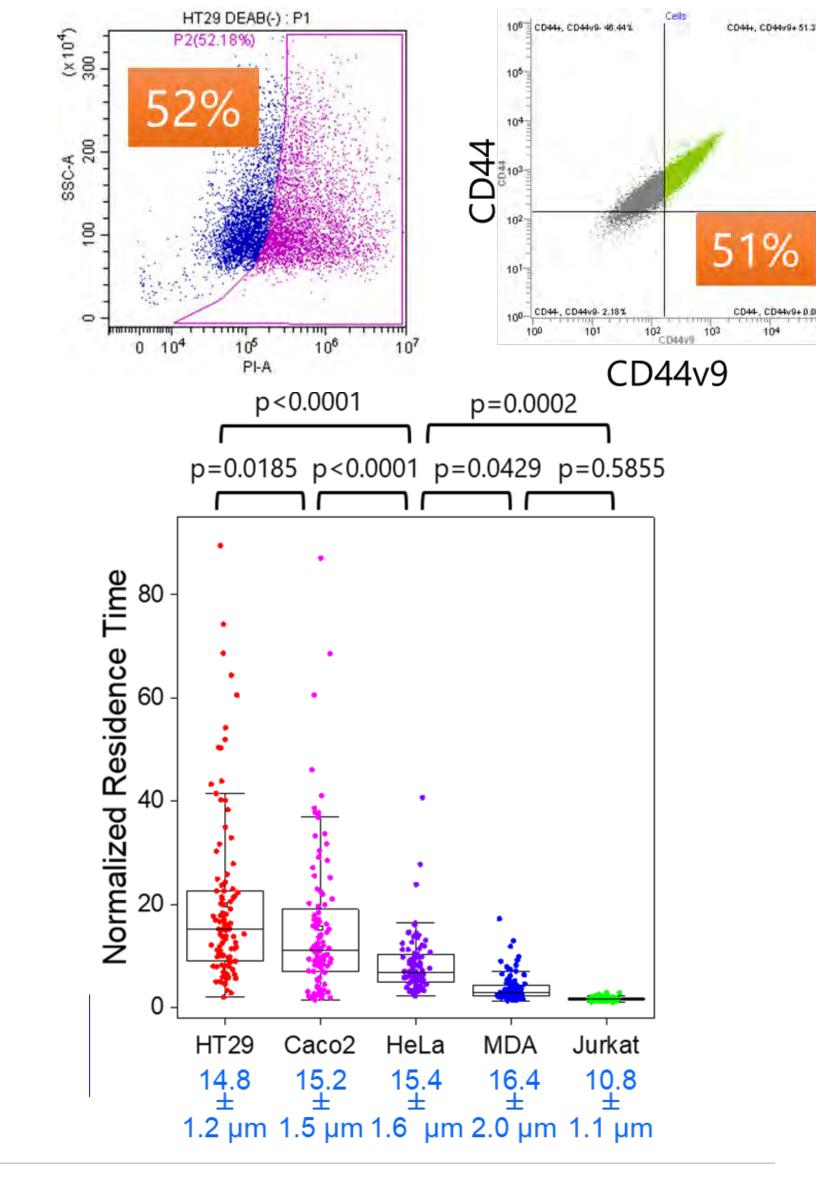
High heterogeneity cell population

High pluripotency

Multipotency of the stem-like cells are evaluated by

Aldehyde dehydrogenase (ALDH) activity

Membrane protein marker (CD44, CD44v9) expression (Fluorescent labeling is necessary, Expensive, Cannot use the re-collected cells after the assay, and so on.)



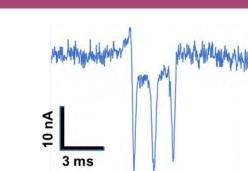
Summary

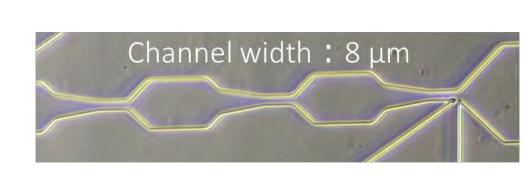
□An analytical technique that could detect the mechanical properties of cells at the single-cell level using electrical currents was developed. This technique enabled labelfree and non-destructive detection within tens of milliseconds at a single cell level.

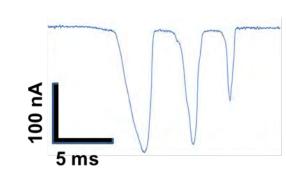
□A correlation between the biochemical information and mechanical properties of stem cells was demonstrated using model cells.

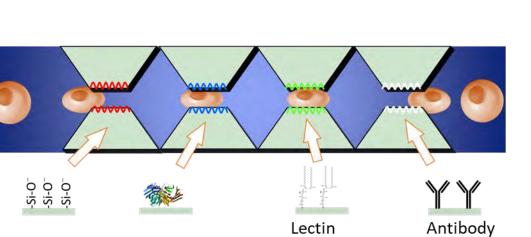
Future perspective











modification

Deformability of a cell Multiplex immunoassay Non-labeling, Non-destructive, Re-collectable assay

A single cell immunoassay