MICROFLUIDICS FOR CANCER METASTASIS INVESTIGATION

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AGENDA

- ✓ Metastasis and different aspects of the problem
- ✓ Relationship between metastasis and Microfluidics
- ✓ Introduction to brain cancer
- ✓ MSC Device
- ✓ Results of the study
- ✓ conclusion

METASTASIS

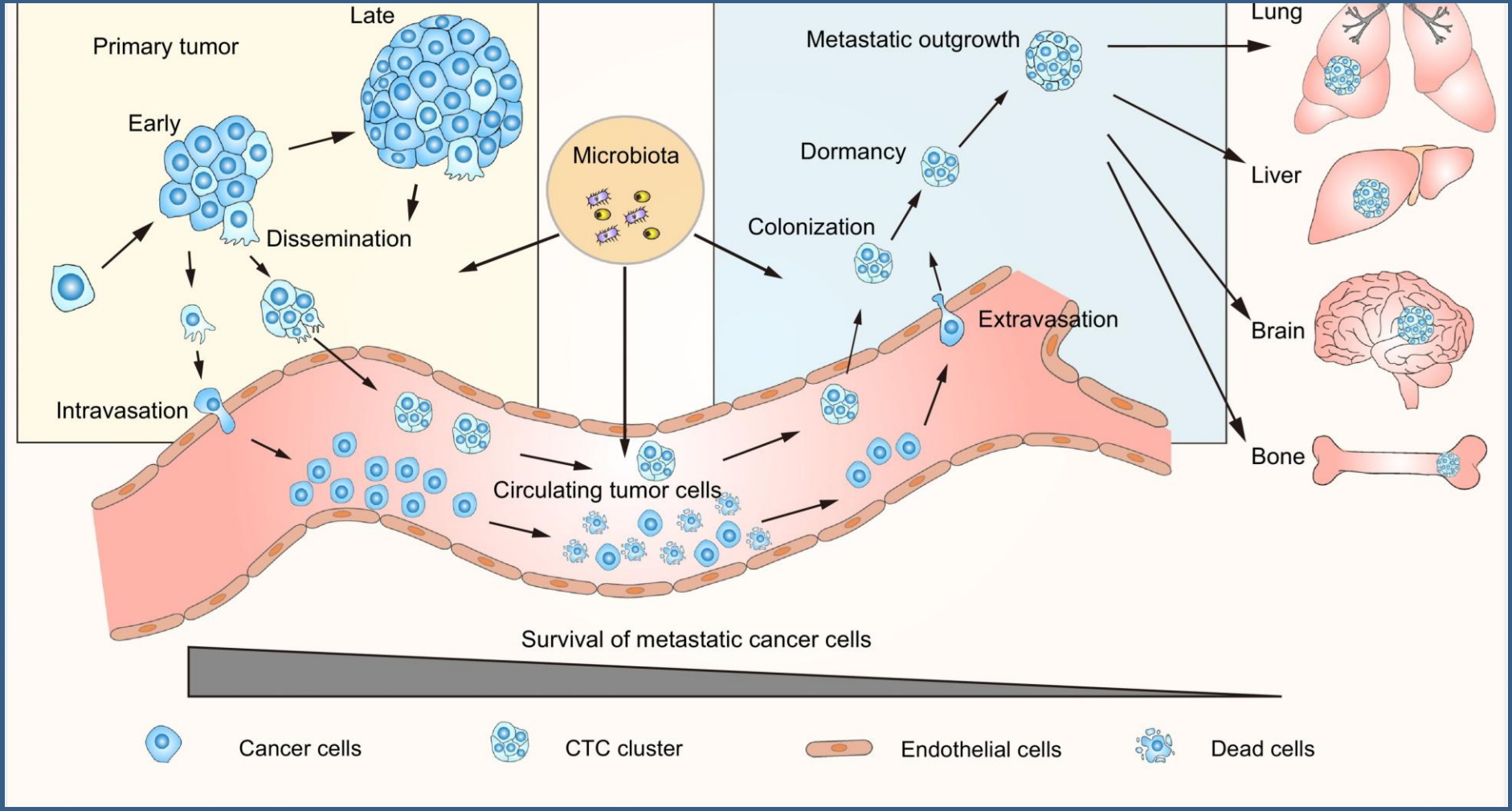
- Migration of cancer cells
- Breaking off from the primary tumor, traveling in the lymph or blood vessels, and eventually extravasating into a tissue
- Only 0.001–0.02% of cancer cells that enter circulation can form a metastatic tumor.
- But morbidity and mortality of patients increases significantly.
- Survival rate for metastases is under 30% (major cause of death from cancer reported by WHO)

WHY MICROFLUIDIC DEVICES

- Lack of a suitable model:
  1. Studying cancer metastasis in animal models.
  2. Use of human cancer cells in mice requires immunocompromised mice

- Need of a relevant model that enables the investigation of the molecular mechanisms underlying each step of metastasis.
- The solution: Microfluidic devices are used to replicate such microenvironments found in vivo
- Low numbers of cells and offer the potential for high-throughput screening.

PROCESS OF METASTASIS



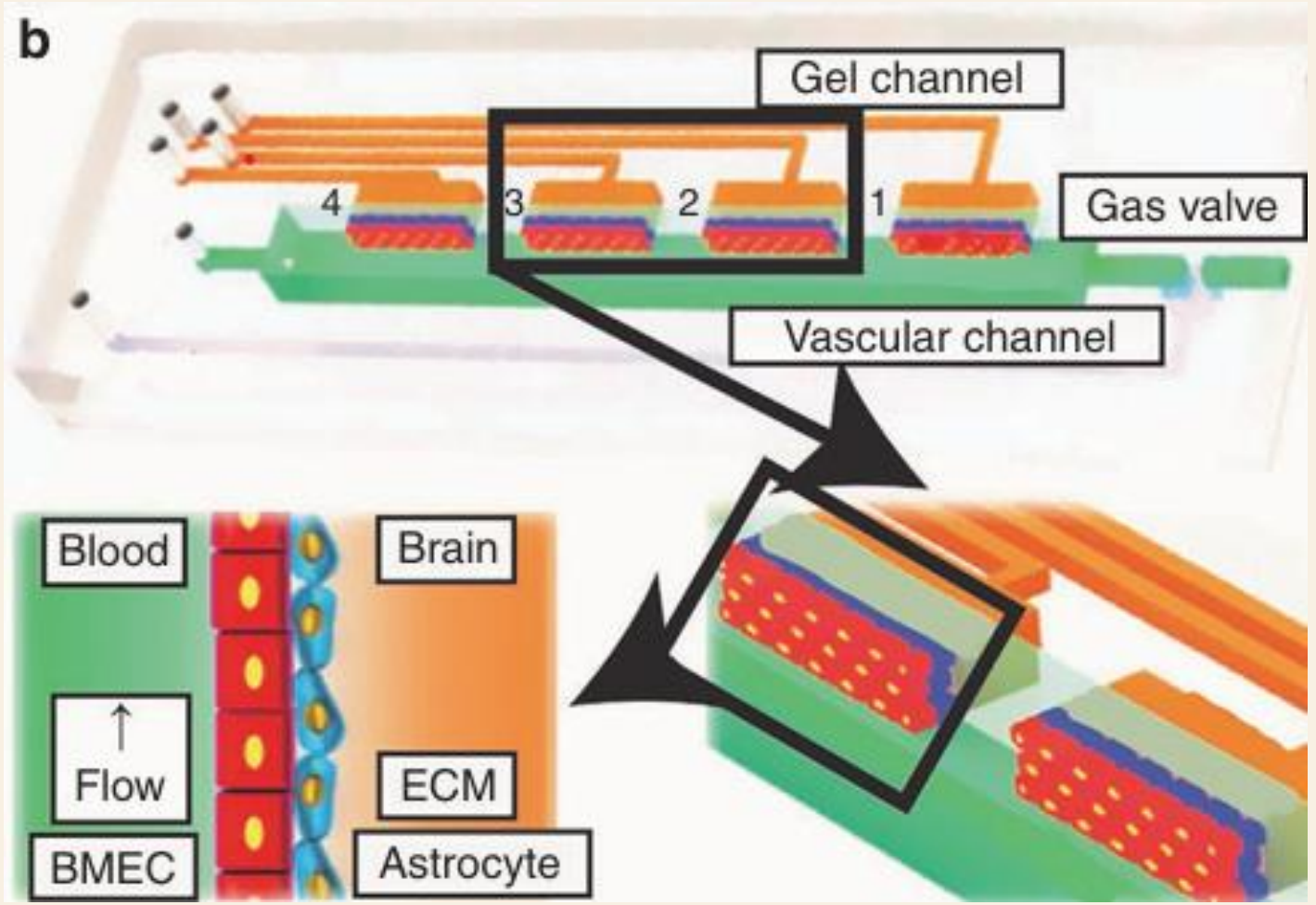
1. cancer cells invade the surrounding tissue (invasion)
2. Entering the lymphatic and/or blood circulatory system (intravasation)
3. Traveling through and survive the bloodstream (circulating)
4. Escaping from the blood vessel (extravasation)
5. Adapting and grow in a colonial environment (metastatic colony)

Emerging roles of intratumor microbiota in cancer metastasis. Fu, Aikun et al. Trends in Cell Biology, Volume 33, Issue 7, 583 - 593

DIFFERENT ASPECT OF STUDYING THE PROBLEM

1. Mechanical factors (focusing on both primary and secondary tumor sites)
   - Matrix stiffness
   - Matrix viscoelasticity
   - Tumor solid stress
   - Environmental confinement
   - Flow shear stress
   - Extracellular fluid viscosity
2. Biochemical factors (mostly focusing on exravasion)
3. Cells of the secondary site (Different types of cancer preferentially metastasize to different tissues. e.g. breast cancer mainly metastasizes to bone, lung, brain, and liver tissues, whereas prostate cancer primarily metastasizes to bone.)

Secondary site
Microfluidic device fabricated to study extravasion of cancer cells



Xu H, Li Z, Yu Y et al. A dynamic in vivo-like organotypic blood-brain barrier model to probe metastatic brain tumors. Scientific Reports 2016; 6: 36670.

A DROP FROM A SEA

Probing the mechanical properties of brain cancer cells using a microfluidic cell squeezer device

Z. S. Khan; S. A. Vanapalli

Aim of the study: investigating the mechanical properties of brain cancer cells in comparison of benign cells

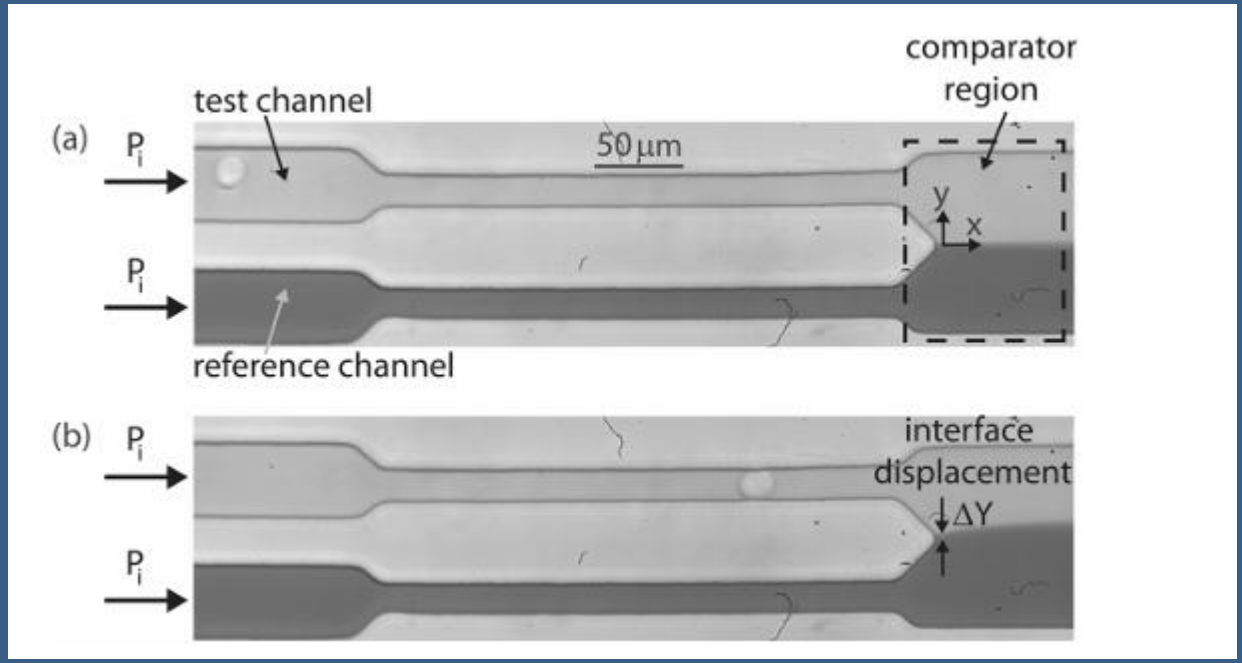
SOME FACTS ON BRAIN CANCER

- Occur at an incidence of approximately 0.006% per capita
- In children brain tumors account for approximately 23% of all cancers
- these tumors rarely metastasize outside of the central nervous system but frequently invade nearby tissues
- some rare cases: metastasize via the cerebro-spinal fluid pathways, and blood vessels.
- What are Benign cells?
- Stiffness of cancerous cells vs Benign cells

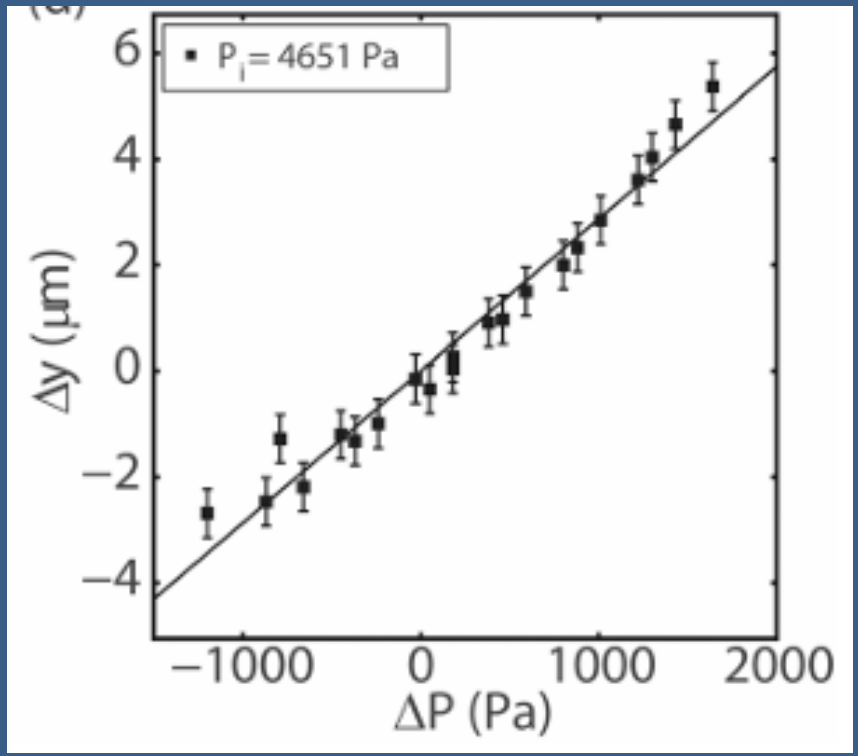
WHAT DID THEY USE?

Microfluidic Cell Squeezer (MCS) device:

- **two parallel microchannels**: a test channel (for cell analysis) and a reference channel.
- measures the **excess pressure drop (ΔP)** as cells pass through a narrow constriction.
- The system records **cell speed, elongation, and entry time** into the constriction.

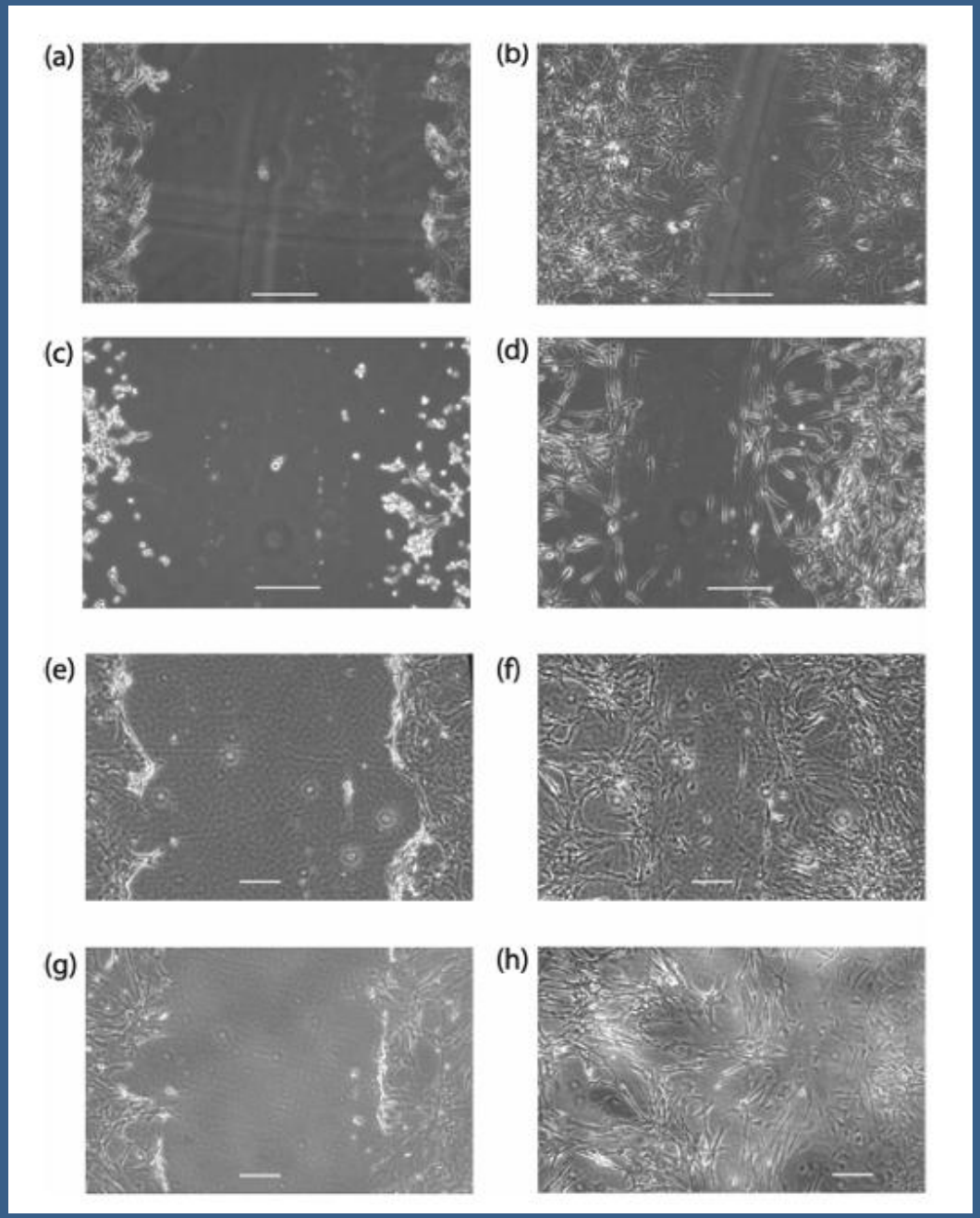


CALIBRATION CURVE



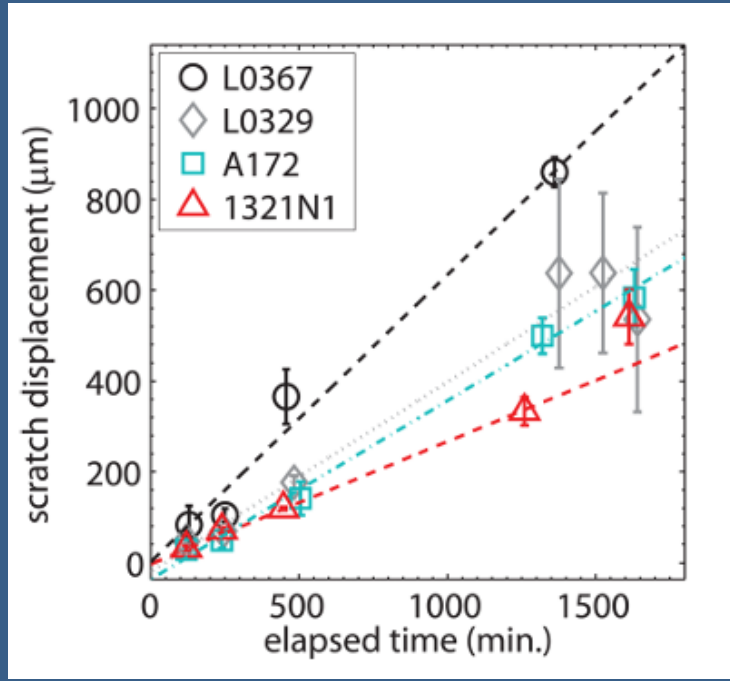
EXPERIMENTAL TECHNIQUES

- **Cell Types**: Cancerous (A172, 1321N1) and benign (L0329, L0367) glial cells
- **Wound Healing Assay**: Used to compare migration rates of cancerous vs. benign glial cells.
- **Data Collection**: high speed imaging and MATLAB analysis
    1. Pressure drop
    2. Cell elongation: taking the ratio of the major axis of the cells inside the constriction to the major axis prior to entering the constriction
    3. Velocity
    4. Entry time The time elapsed between the point of first contact of the cells with the constriction and the point of full containment within the constriction.



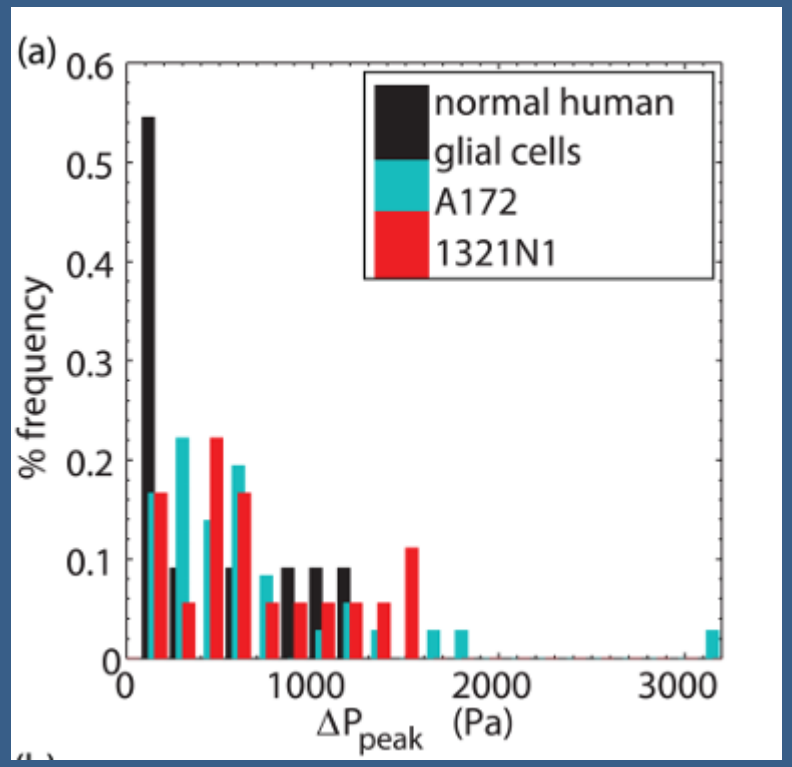
WOUND HEALING ASSAY

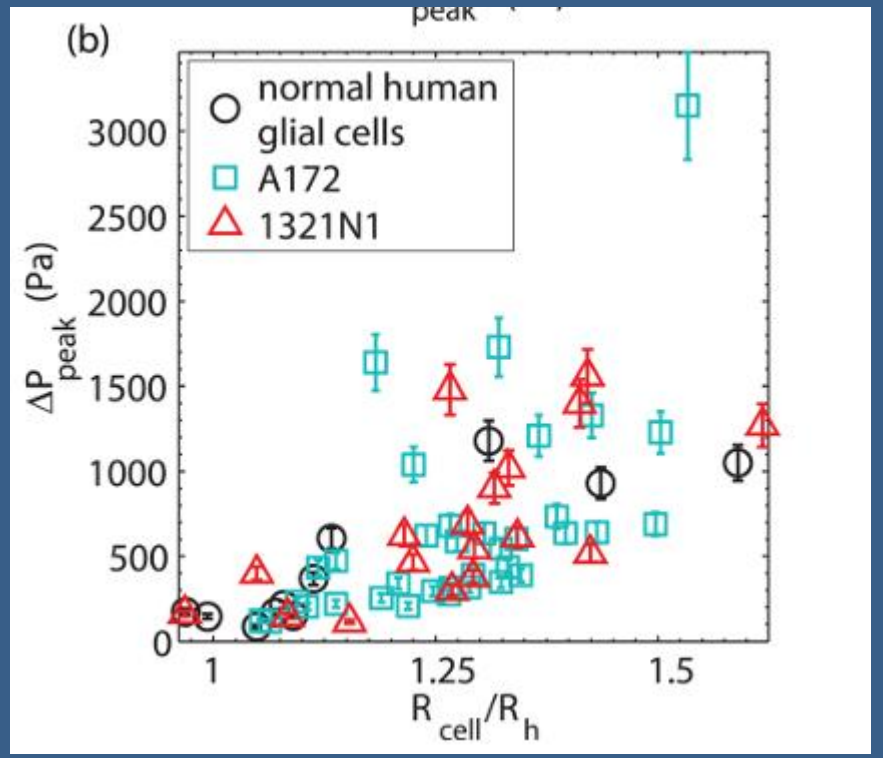
The wound closing velocity was determined by manually finding the average distance between the edges of each scratch at known time intervals using IMAGEJ software, plotting these distances against time, and fitting to determine the slope



PRESSURE DROP (ΔP) ANALYSIS

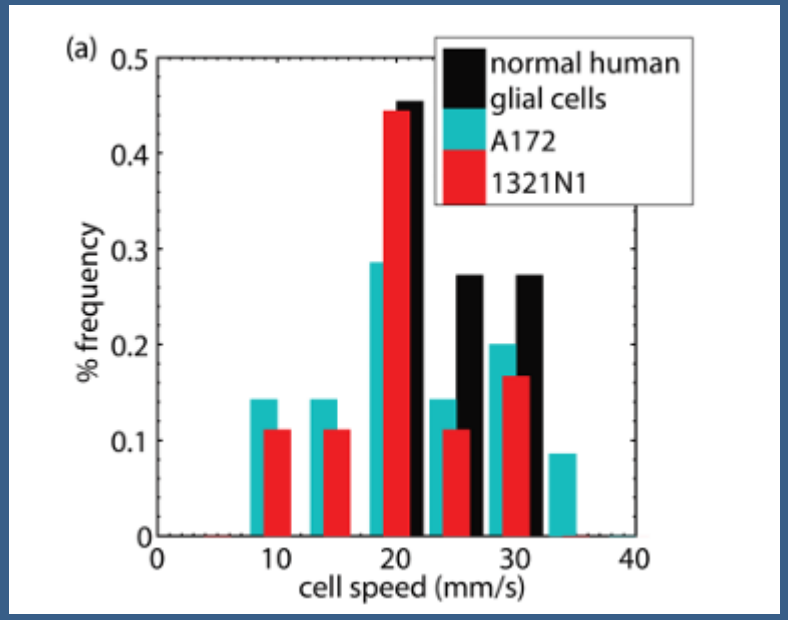
- No statistically significant difference in peak pressure drop (ΔP) between benign and cancerous brain cells.
- Pressure drop increased significantly with cell size, suggesting that cell confinement plays a role in mechanical resistance.

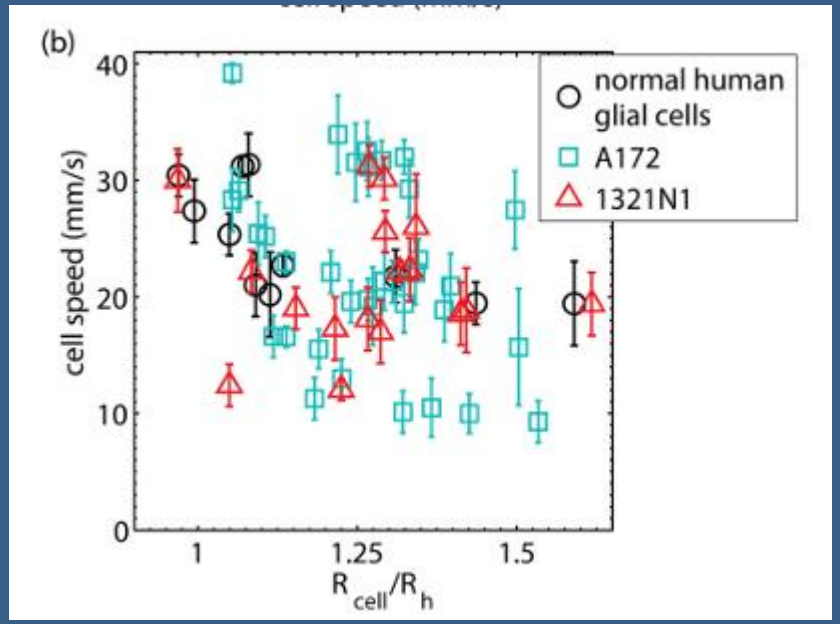




CELL ENTRY TIME INTO THE CONSTRICTION

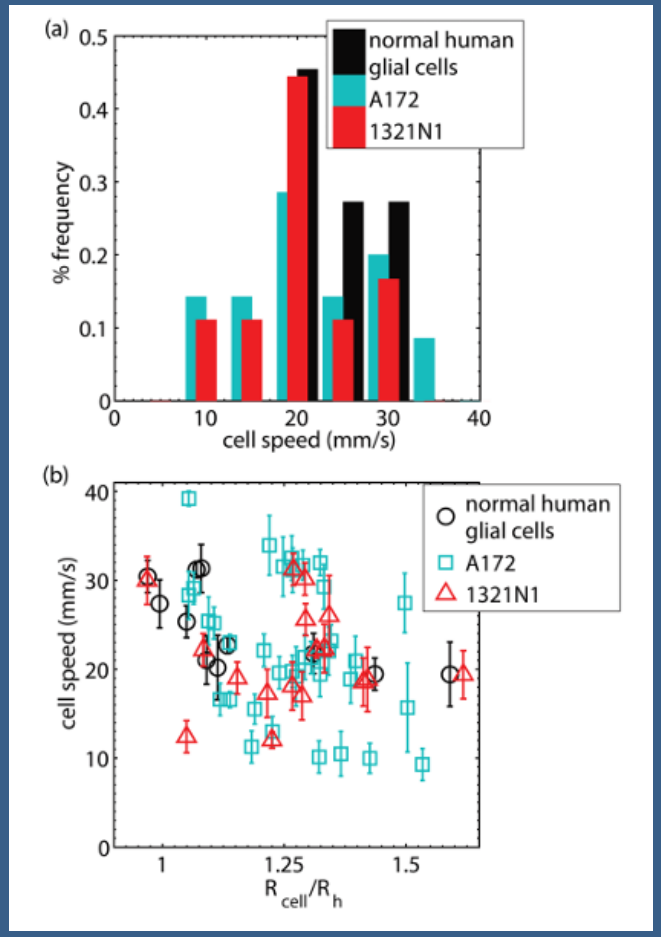
- **Benign glial cells entered the constriction faster** than cancerous cells.
- This is **opposite** to extraneural cancers (breast, lung), where malignant cells enter faster due to their softer nature.
- Entry time is a **more sensitive marker of malignancy** than pressure drop.

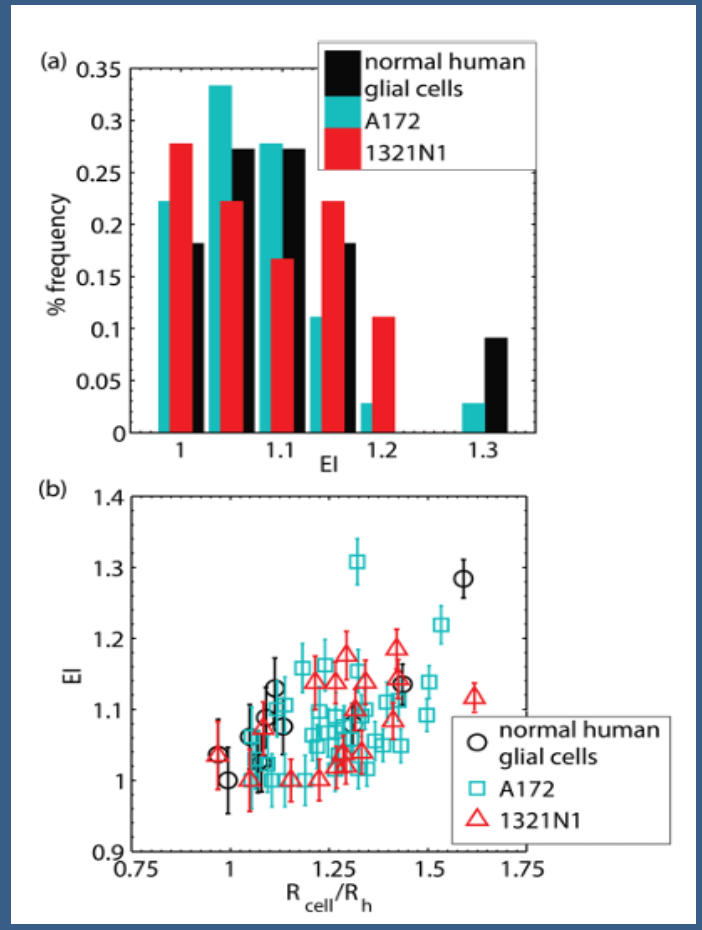




CELL ELONGATION AND SPEED

- No significant difference in elongation index (EI) between cancerous and benign cells.
- No significant difference in cell speed inside the constriction.





CONCLUSION AND FUTURE RESEARCH

- Brain cancer cells appear mechanically stiffer than benign glial cells.
- Entry time into microchannels is the best marker for distinguishing malignant from benign cells.
- These results contradict prior research on extraneural cancers, which found malignant cells to be softer.
- The study suggests that brain tumors follow a different metastatic pattern than extraneural cancers, possibly due to the unique CNS environment.
- The **Microfluidic Cell Squeezer (MCS)** is an effective tool for studying mechanics of cells.

- Future studies should be directed towards probing the detailed micromechanics of tumor cells, including the role of the nucleus.
- Microfluidic devices can be engineered with multiple squeezing channels to enable high throughput mechanical characterization of tumor cells.

REFERENCES

[1] Khan ZS, Vanapalli SA. Probing the mechanical properties of brain cancer cells using a microfluidic cell squeezer device. Biomicrofluidics 2013; 7: 11806.

[2] Emerging roles of intratumor microbiota in cancer metastasis. Fu, Aikun et al. Trends in Cell Biology, Volume 33, Issue 7, 583 – 593

[3] Ma, Yu-Heng Vivian et al. "A Review of Microfluidic Approaches for Investigating Cancer Extravasation during Metastasis." *Microsystems & nanoengineering* 4.1 (2018): 17104. Print.

[4] Liang, Lanfeng et al. "Insights into the Mechanobiology of Cancer Metastasis via Microfluidic Technologies." *APL Bioengineering* 8.2 (2024): 021506. Print.

[5] Xu H, Li Z, Yu Y et al. A dynamic in vivo-like organotypic blood-brain barrier model to probe metastatic brain tumors. Scientific Reports 2016; 6: 36670.

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