RECONSTITUTING FUNDAMENTALS OF BACTERIA MEDIATED CANCER THERAPY ON A CHIP

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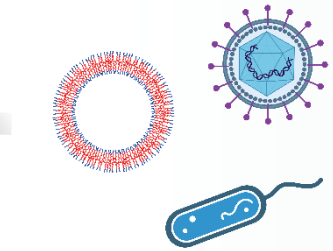
KIST Center for Healthcare Robotics

Bacteria as a versatile bio-sapper for cancer treatment

NANOVECTORS



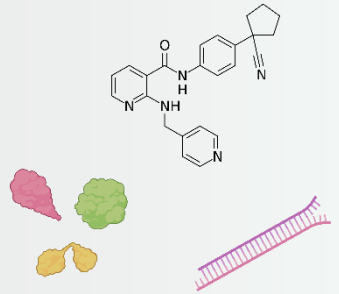
Bacteria

Nanovesicles

Oncolytic viruses

...

THERAPIES

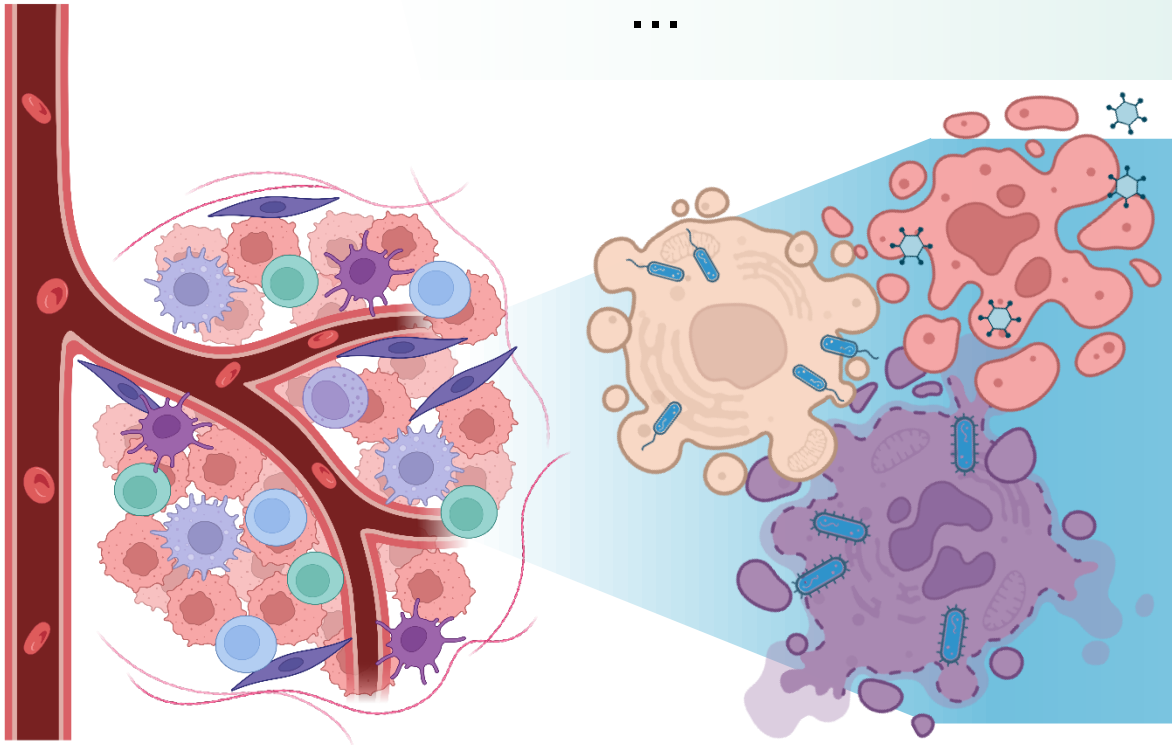


Chemical drugs

Proteins

DNA/RNA

...

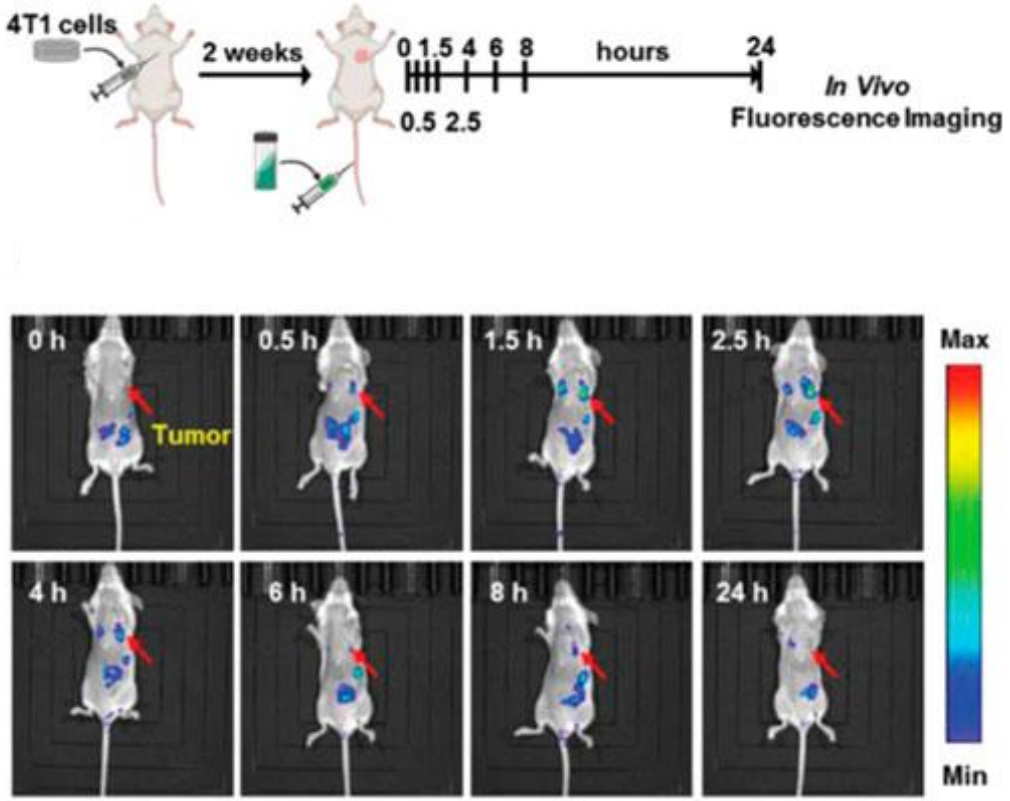


Bacteria-mediated cancer therapy (BMCT)

- Preferential colonization of tumor
- Increase therapeutic specificity
- Immunostimulation in TME
- Synergistic with other therapies

Traditional *in vivo* paradigm of BMCT research

- **Developing immuno-modulating micro-bio robot that can reach cancer after effectively avoiding human innate immunity**

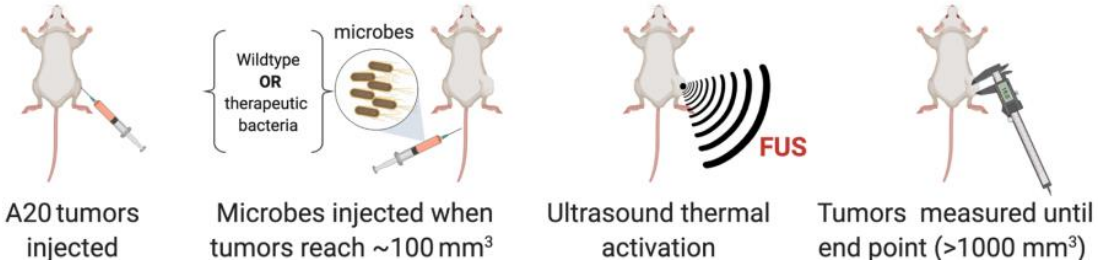


S. Liang, et al., *Frontiers in Bioengineering and Biotechnology* (2022).

REASEARCH PAIN POINTS

① **Restrictions in observing mechanism of action at cellular and tissue levels**

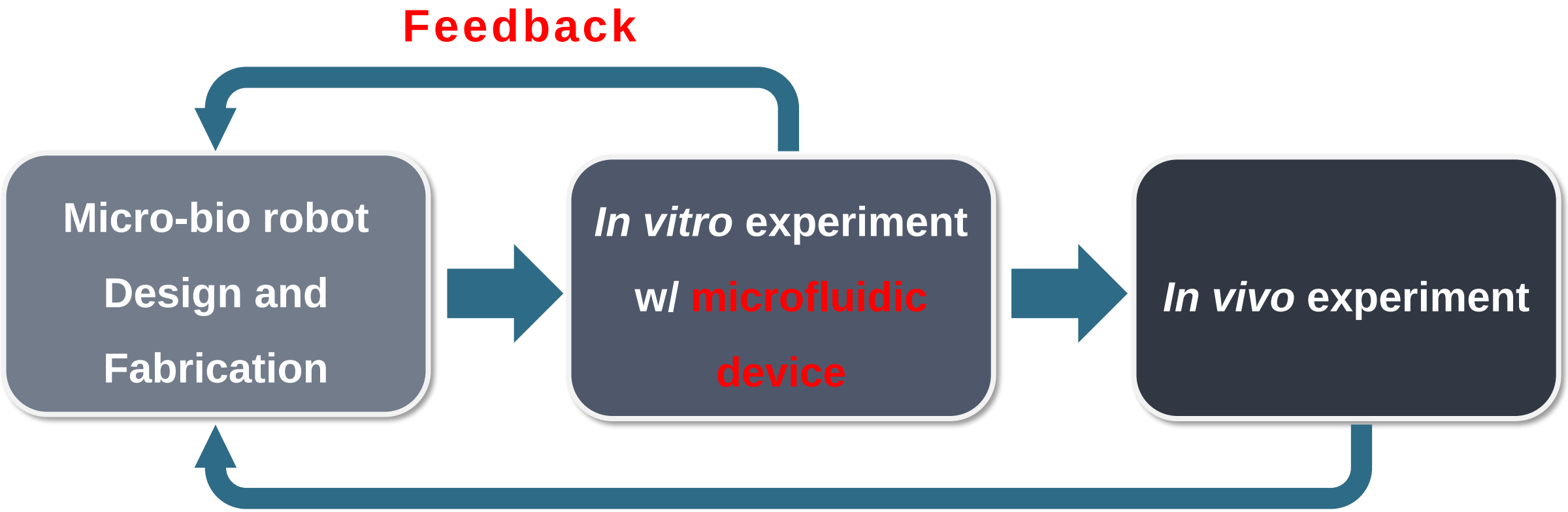
② **Disparities in fundamental physiology between humans and model organisms**



M. H. Abedi, et al., *Nature communications* (2022)

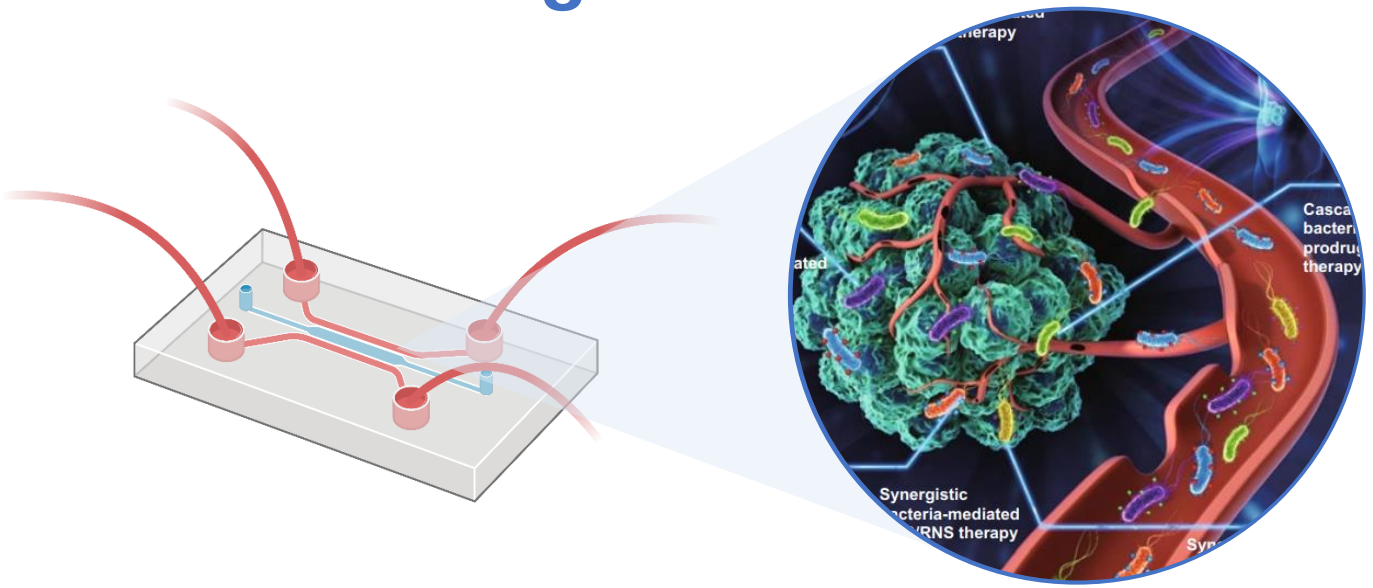
Building *in vitro* solution supporting the analysis of BMCT

- **Supplementing tranditional in vivo paradigm for the scrutinization of BMCT**



Reconstituting fundamentals of BMCT on a chip

- **Research goal**



X. Lou, et al., *Nano-Micro Letters* (2021)

Concepts to be emulated

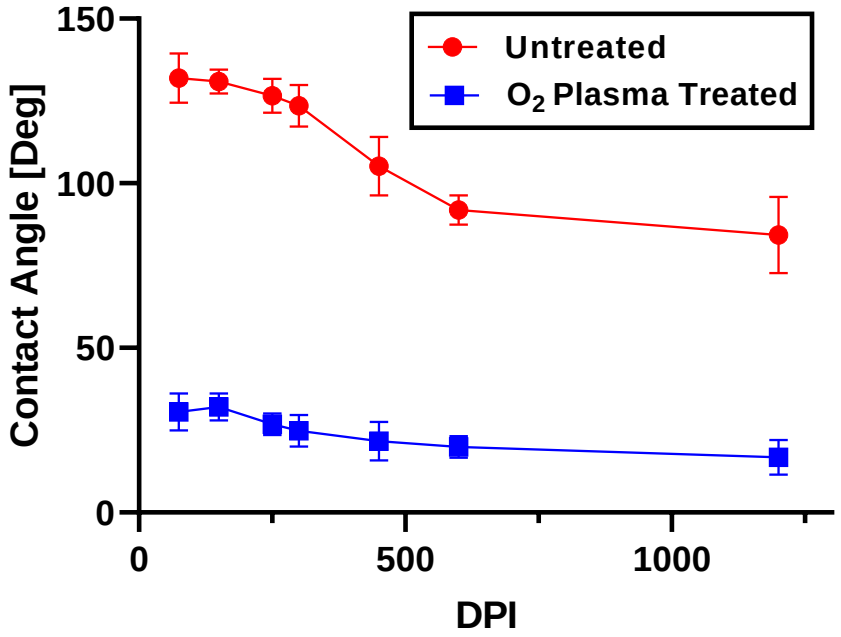
- Basics of bacteria-colonized tumor microenvironment (TME)
- Immunostimulation by bacterial components in TME

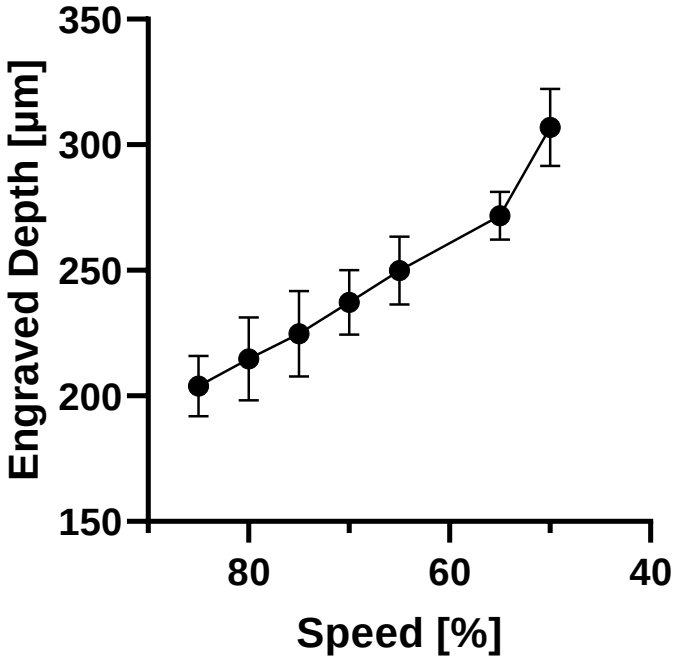
Designing the microfluidic device → **Modeling bacteria infection** → **On-chip TME generation**

Control over manufacturing for desired dimensions

- Channel height control through speed adjustment

EPILOG LASER mini



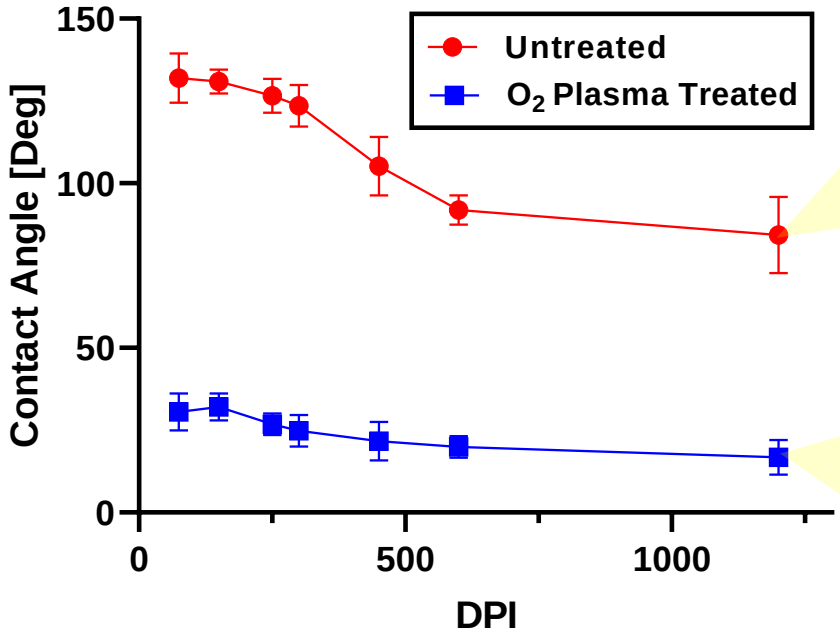


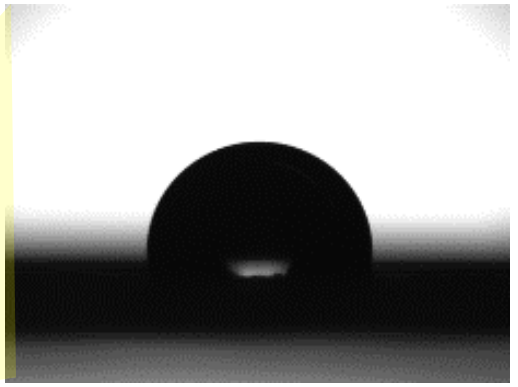
Speed 100% corresponds to the actual speed of 85 mm/s

Surface property of the laser engraved PMMA body

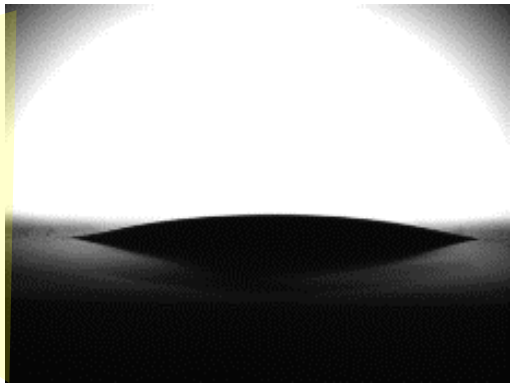
- Contact angle corresponding to engraving DPI

EPILOG LASER mini





Untreated, $\theta = 91.0°$

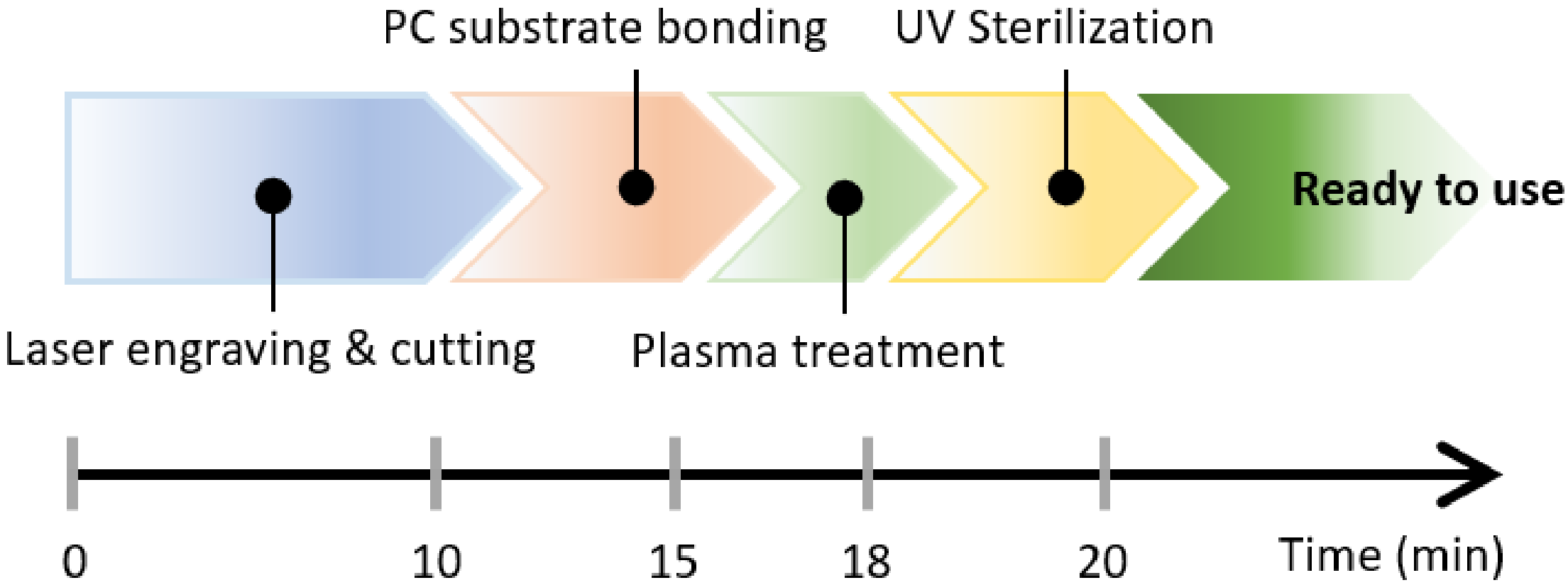


Plasma Treated
2 min, $\theta = 14.2°$

Enables spontaneous capillary flow under hydrophilic condition

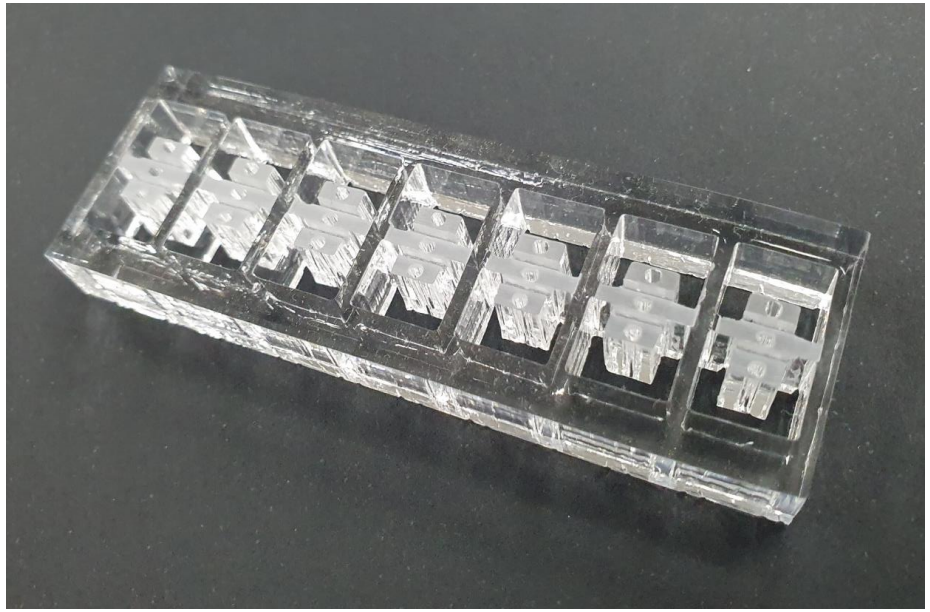
Rapid prototyping for the microfluidic device fabrication

- **Fabrication step for the final chip preparation**

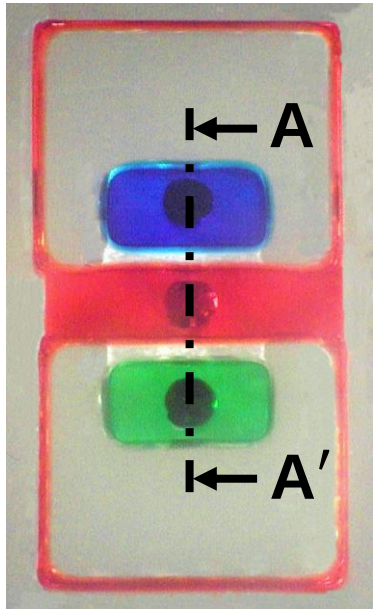


User-friendly platform with straightforward design

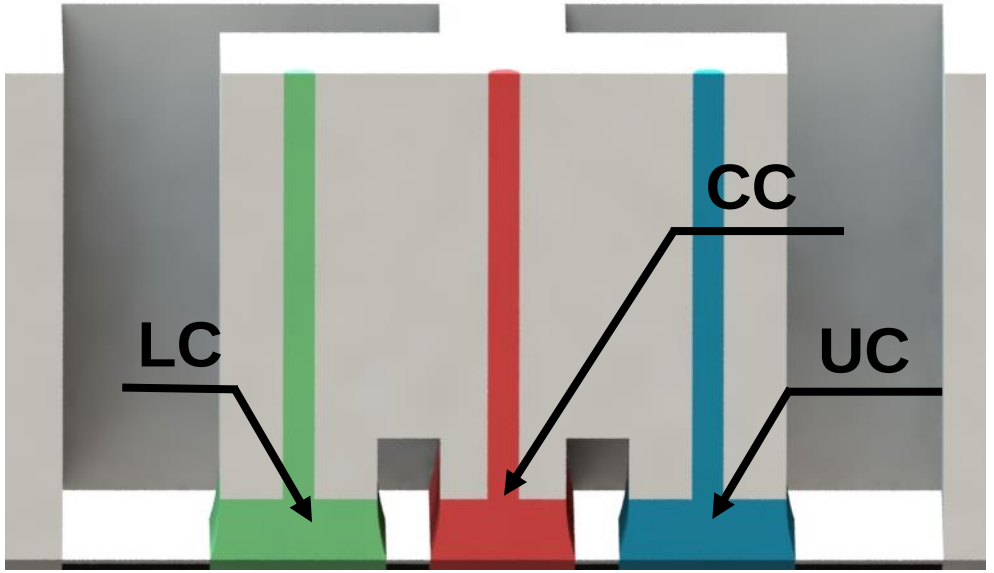
- Microfluidic device design



Fabricated Device



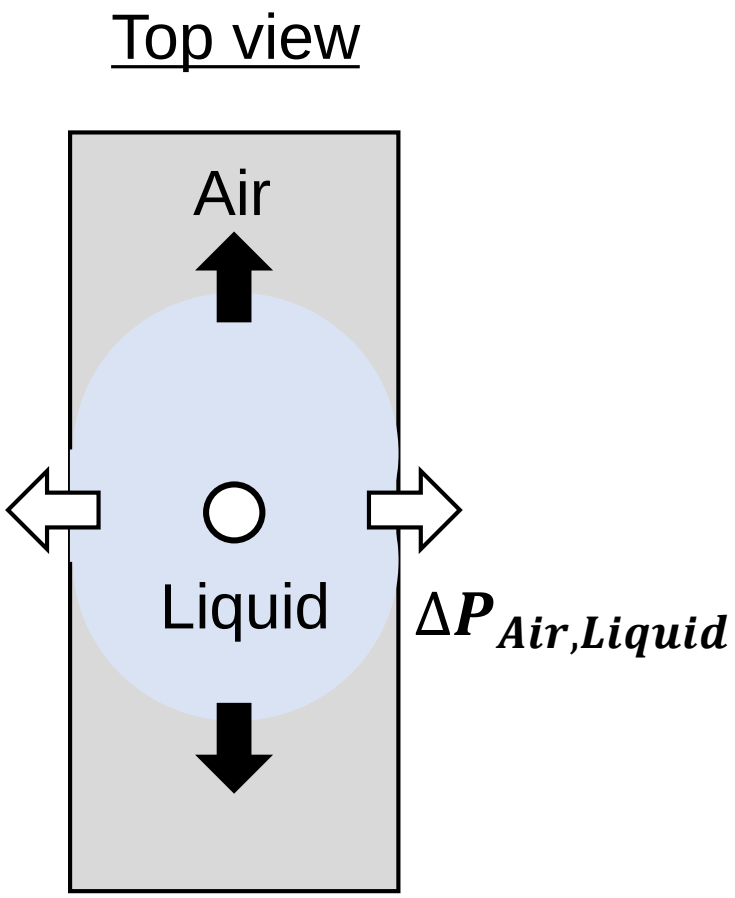
Bottom View

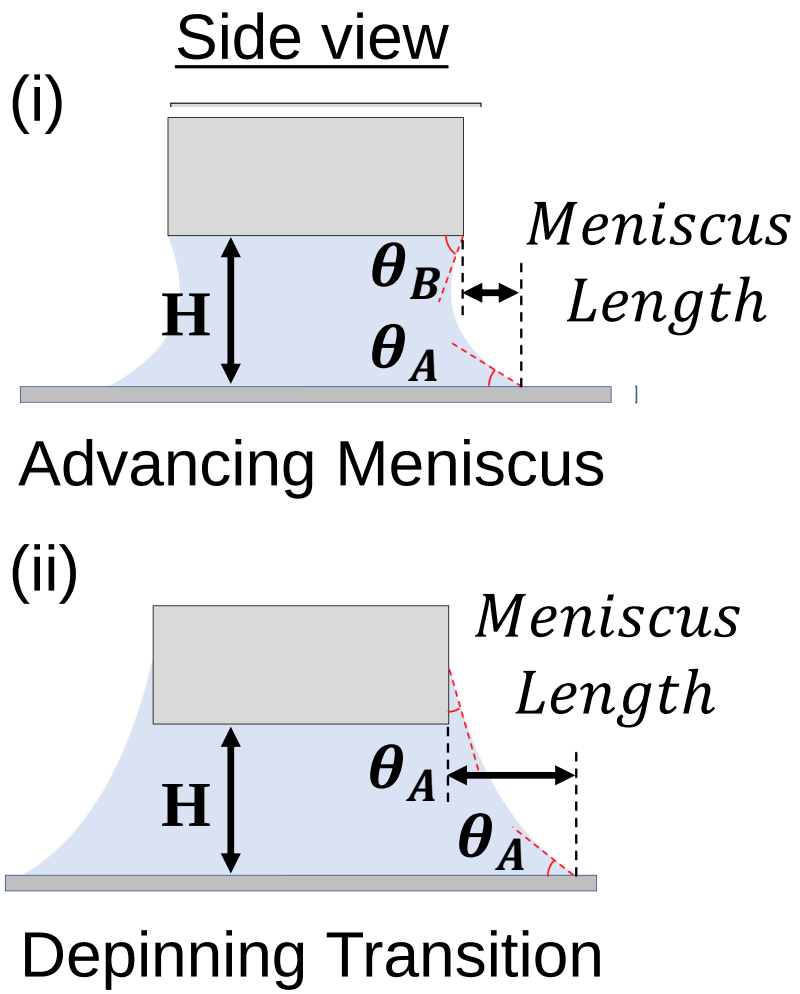


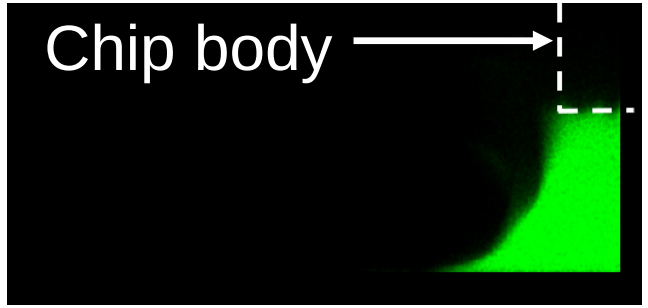
Section A-A′

Selective patterning using spontaneous capillary flow

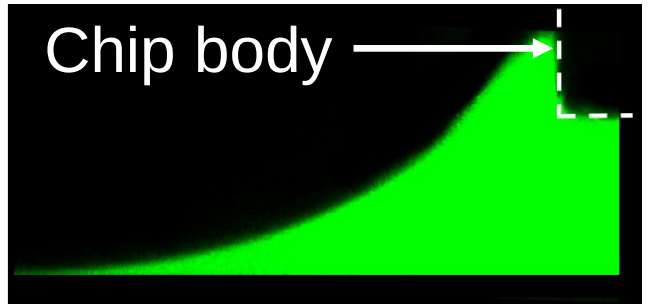
- **Proper volume range estimation**







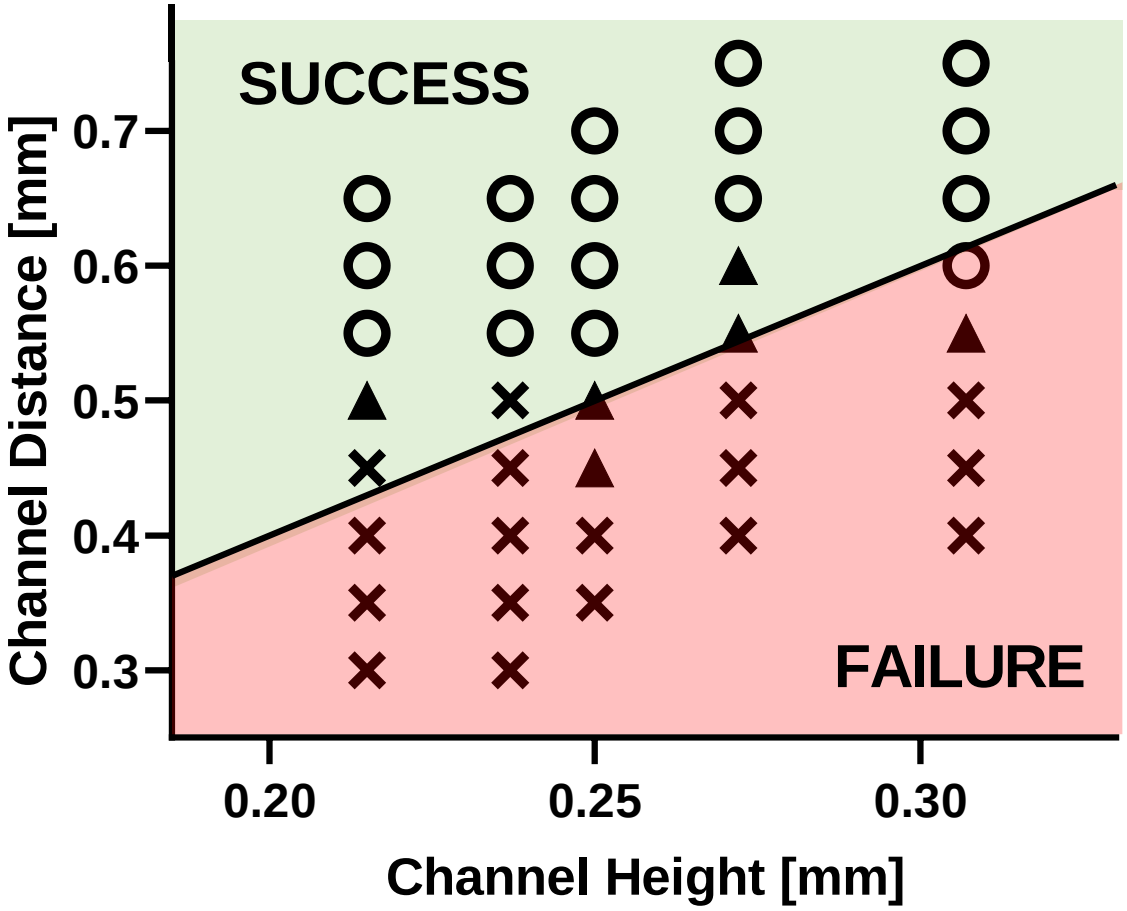
Patterned Volume = 10 µl



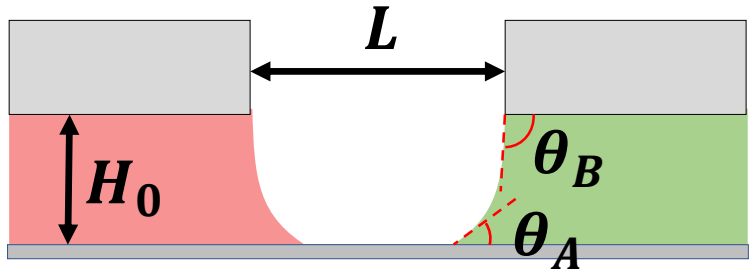
Patterned Volume = 16 µl

Selective patterning using spontaneous capillary flow

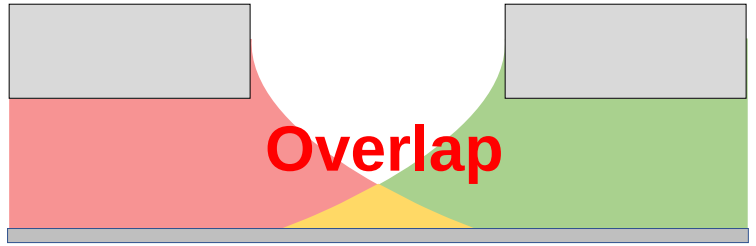
- **Design rule for selective patterning**



Success



Failure



$$L = H_0 tan\left(\frac{\theta_B}{2} - \frac{\theta_A}{2}\right) < H_0$$

Reconstituting fundamentals of BMCT on a chip

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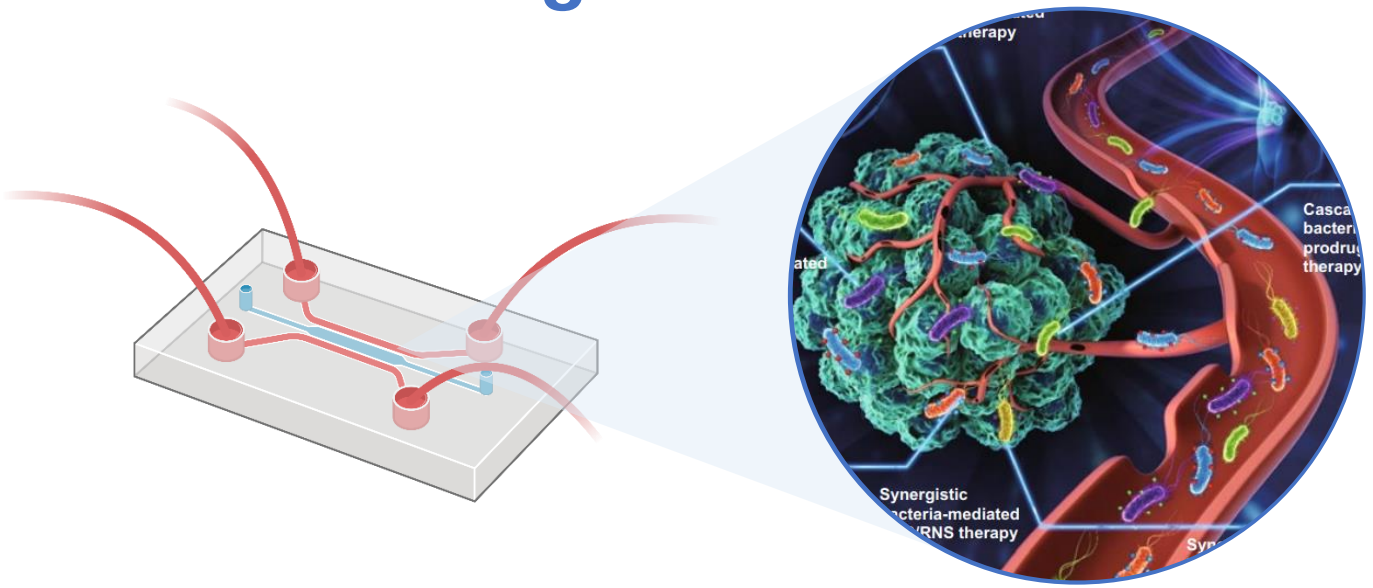


X. Lou, et al., *Nano-Micro Letters* (2021)

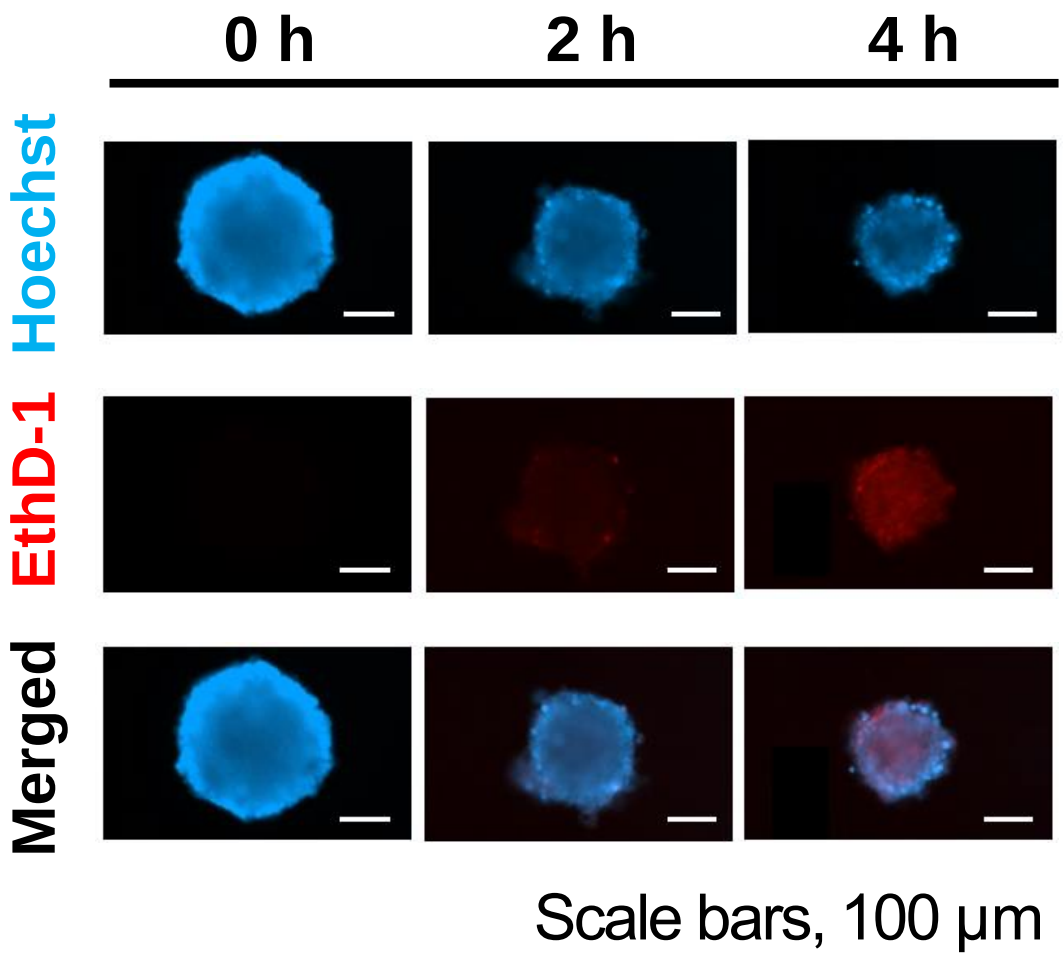
Concepts to be emulated

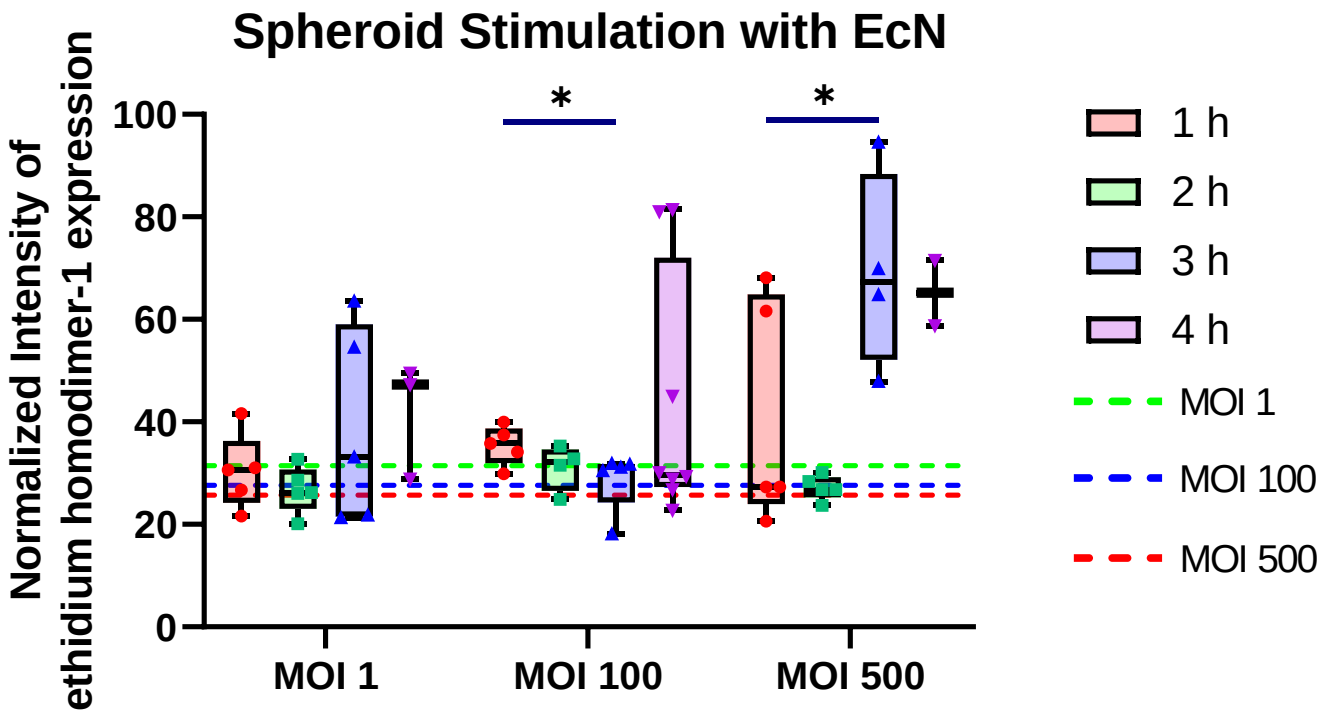
- Basics of bacteria-colonized tumor microenvironment (TME)
- Immunostimulation in TME

Designing the microfluidic device → **Modeling bacteria infection** → **On-chip TME generation**

Bacterial infection decreasing cancer viability

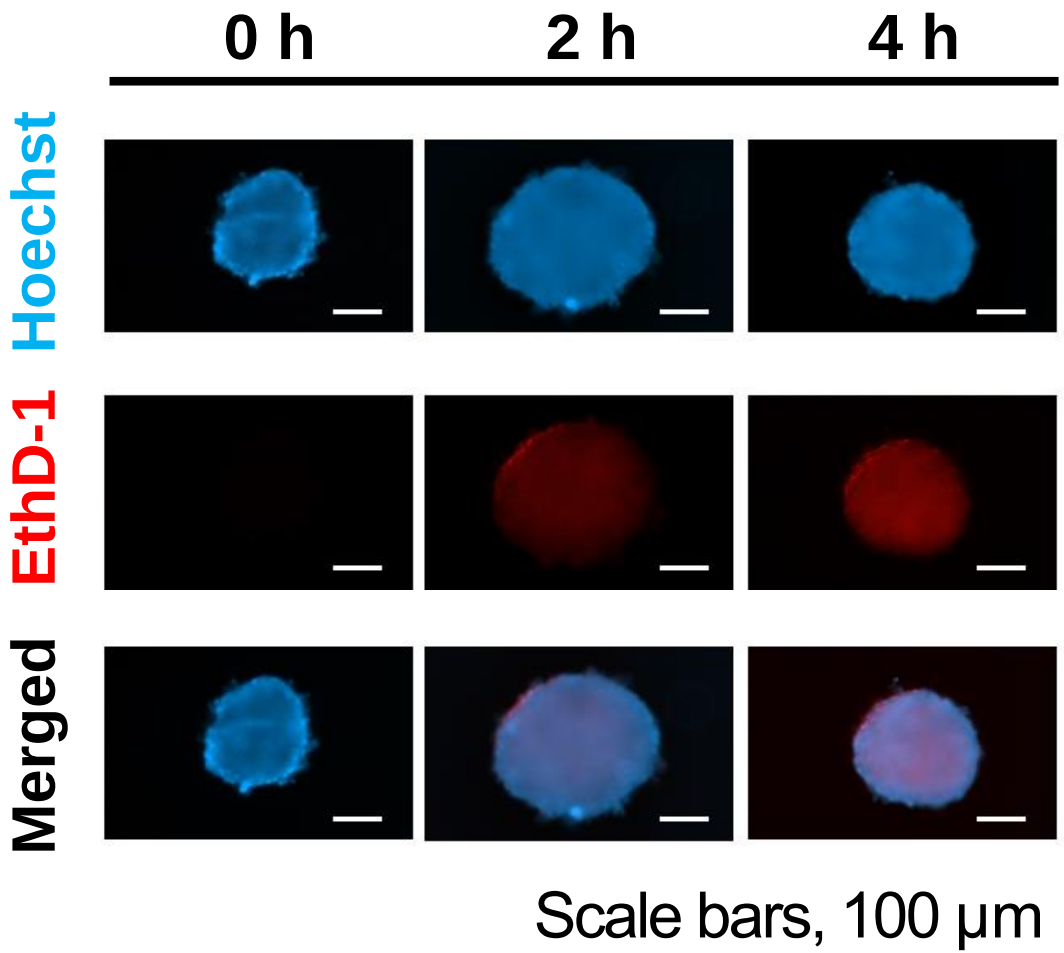
- MOI 500 *E.coli* Infection

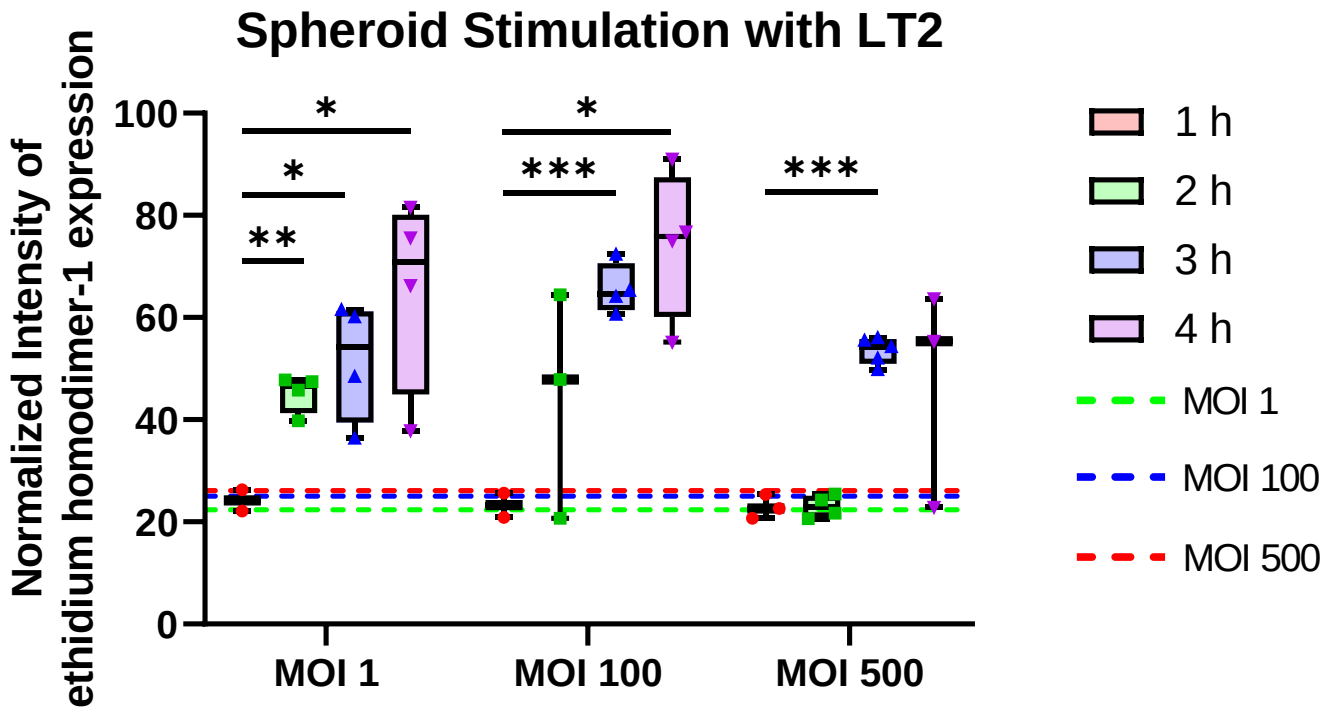




Virulent strain showing pronounced cytotoxicity trend

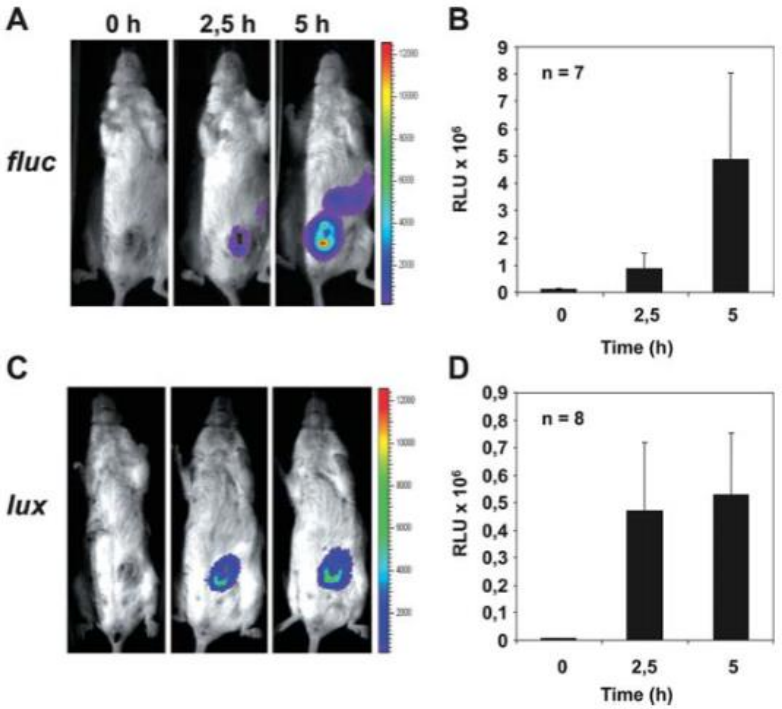
- MOI 500 *S.typhimurium* Infection



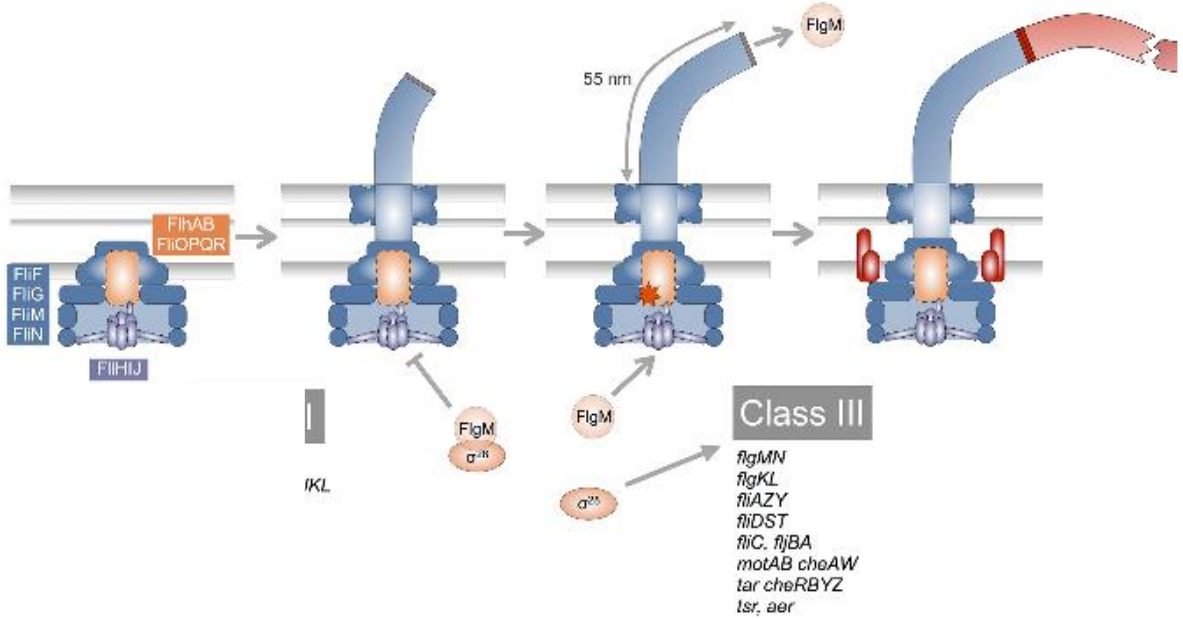


Engineering bacteria with a therapeutic payload

- **Loading *S.typhimurium* with IFN-β**



H. Loessner, et al., *Cellular Microbiology* (2007)



M. Erhardt, et al., *PLoS Genetics* (2014)

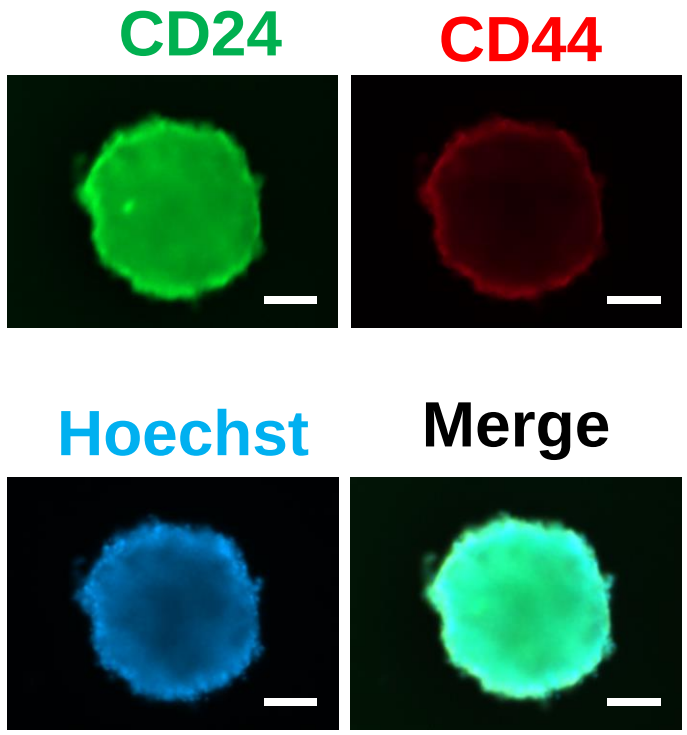
Remote control of gene expression by the use of L-arabinose as inducer

Protein secretion through FlgM tagging

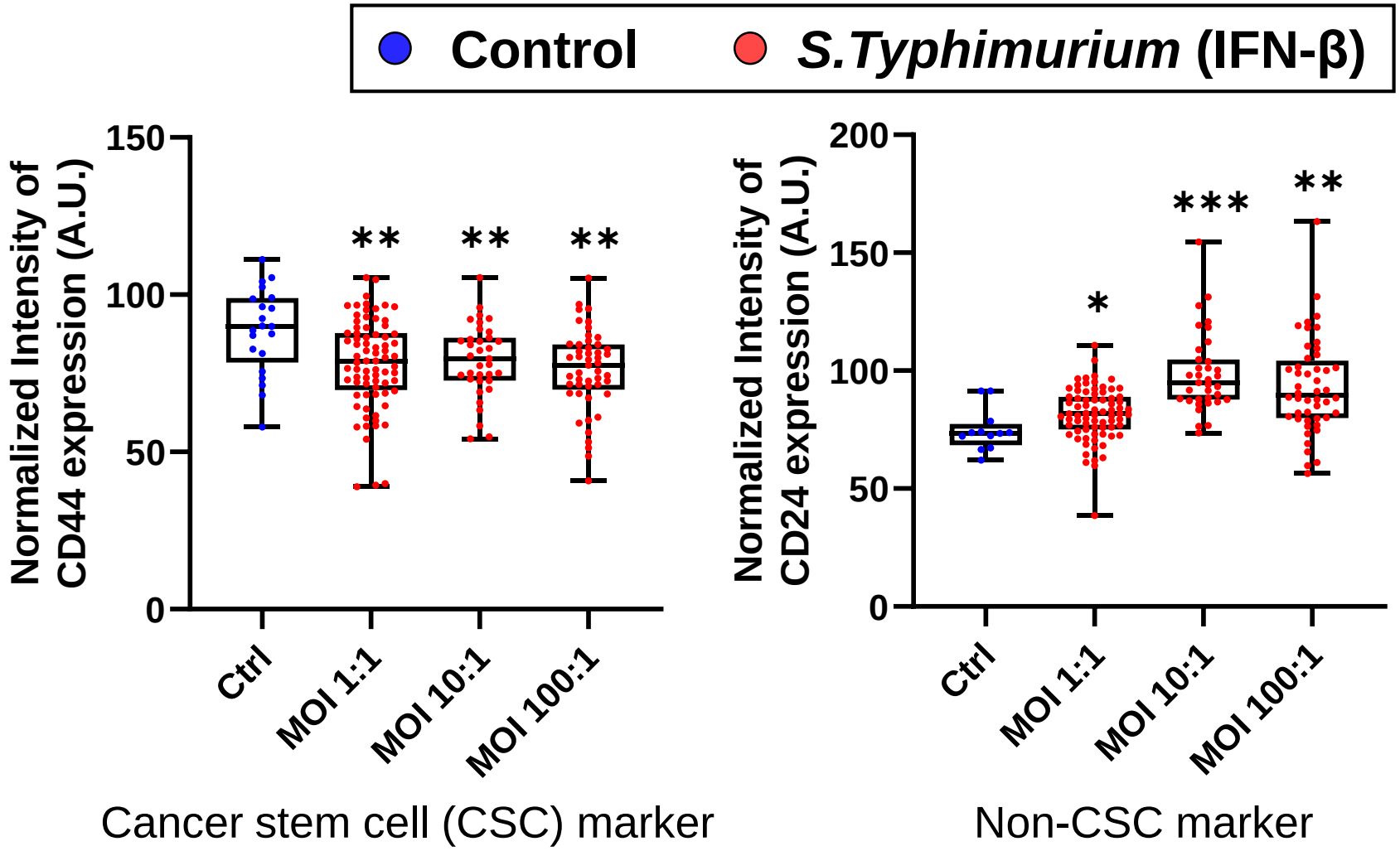
Spheroids reflecting properties of bacterial stimulant

- **IFN-β decreasing stemness of 4T1 tumor spheroids**

LT2 Stimulated Spheroid



Scale bars, 100 μm

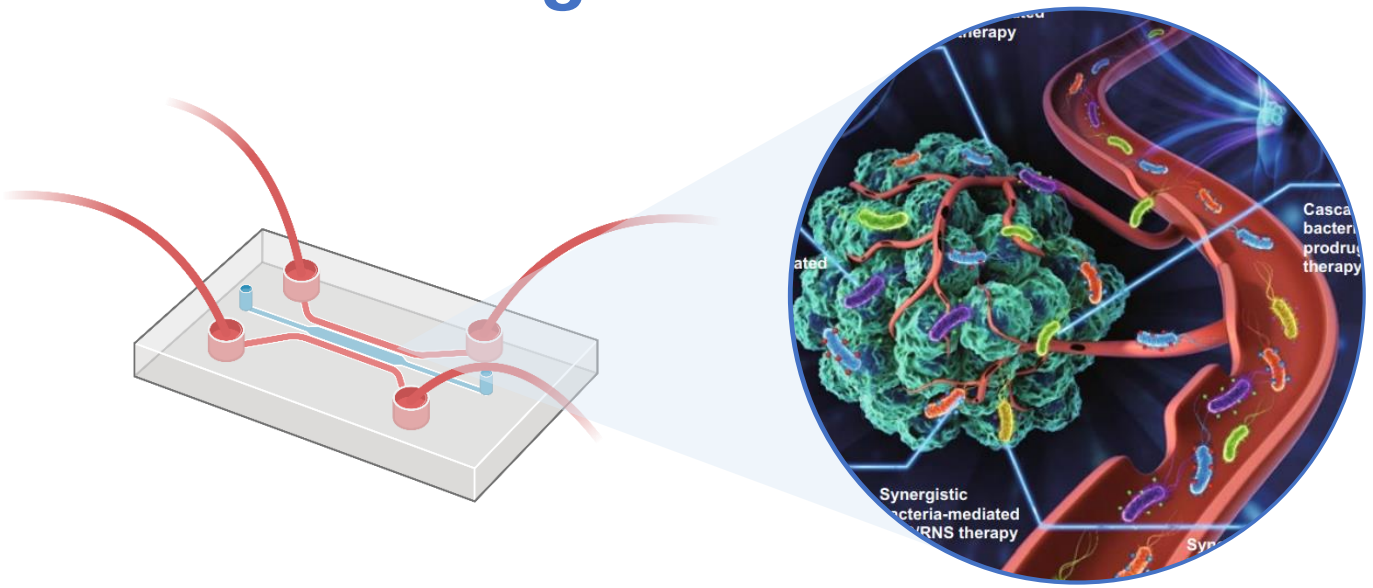


Cancer stem cell (CSC) marker

Non-CSC marker

Reconstituting fundamentals of BMCT on a chip

- **Research goal**



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Concepts to be emulated

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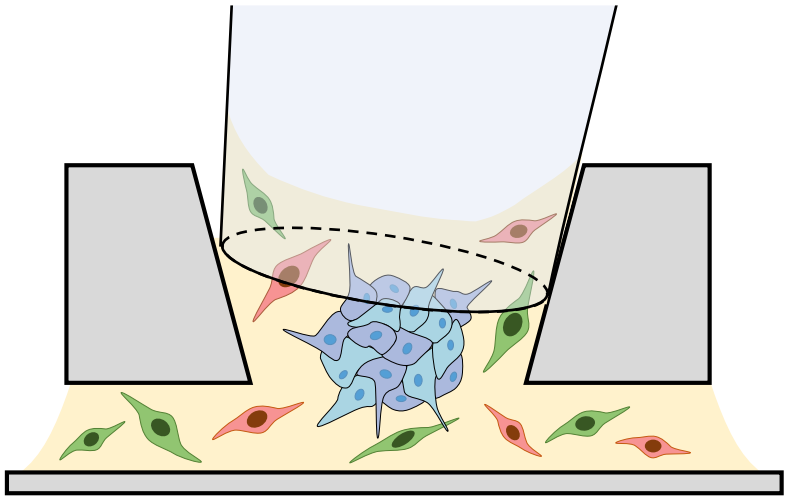
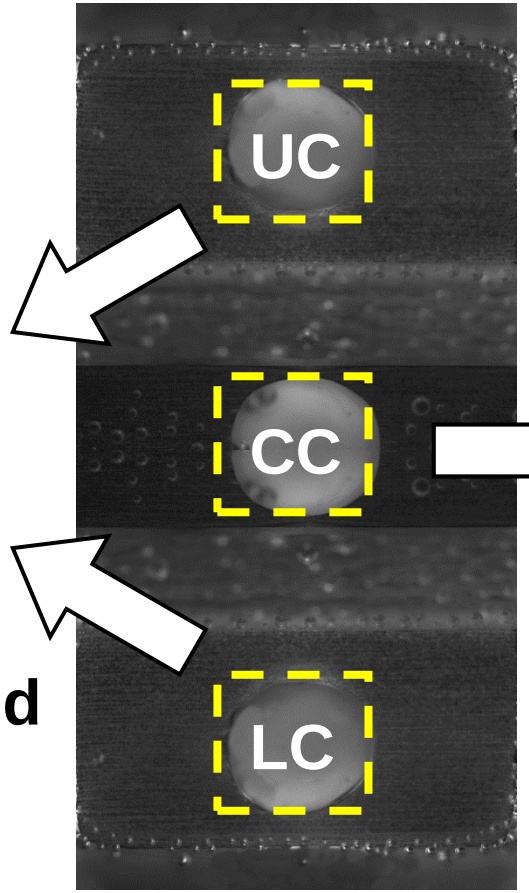
- Immunostimulation in TME

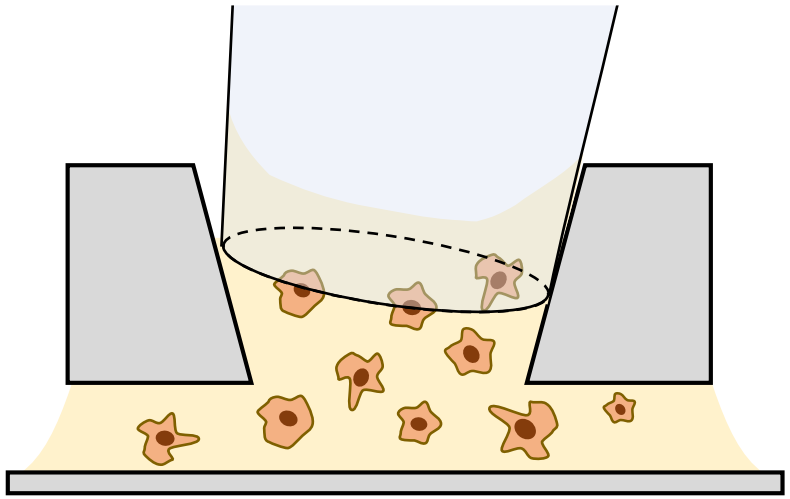
Designing the microfluidic device → **Modeling bacteria infection** → **On-chip TME generation**

Bacteria-colonized TIME mimetic coculture model

- Schematic for cell culture approach





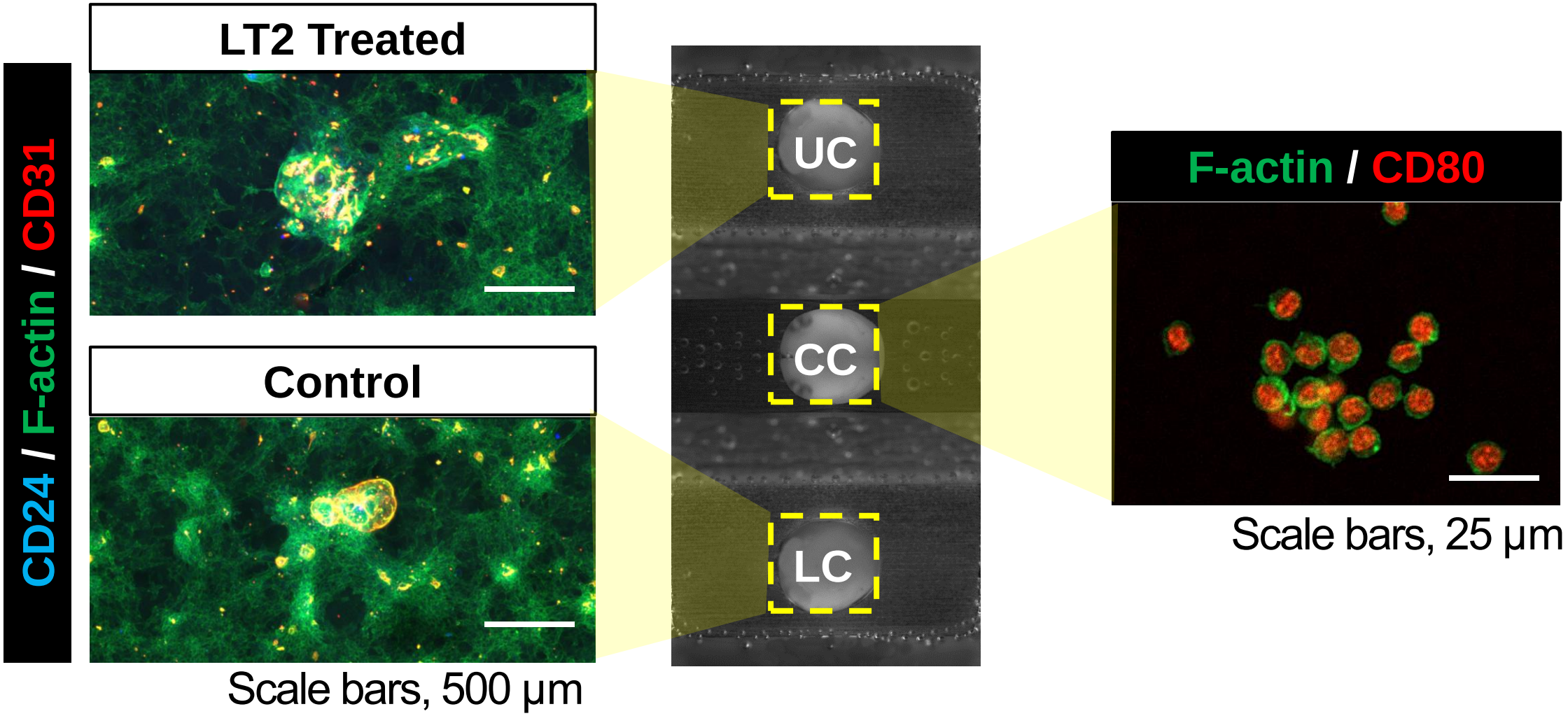


Stimulated or control spheroid
+ NIH/3T3
+ HUVECs

RAW264.7 cells

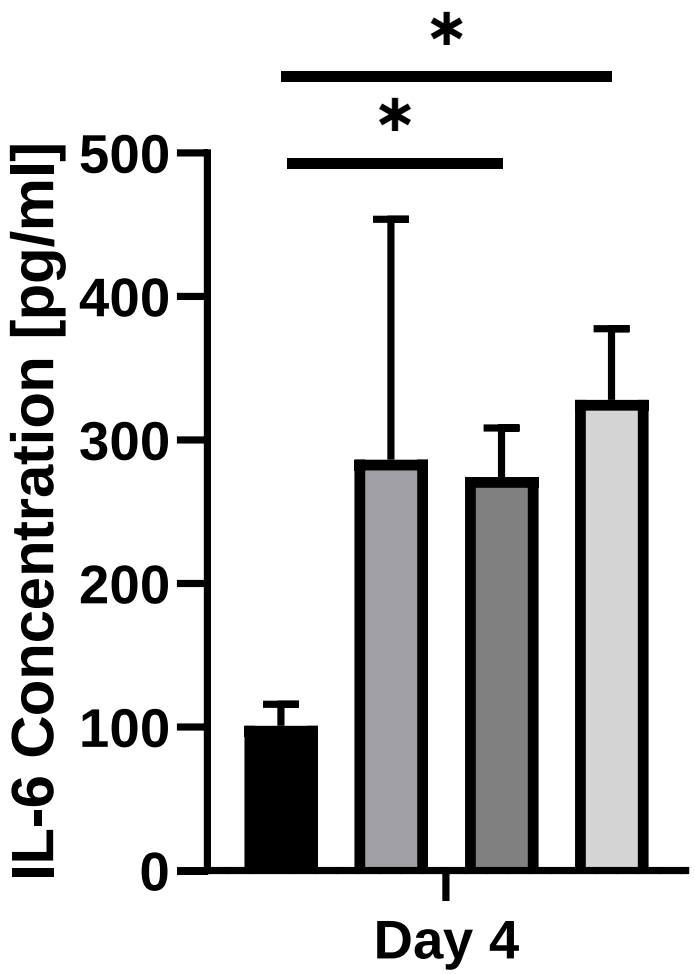
Bacteria-colonized TIME mimetic coculture model

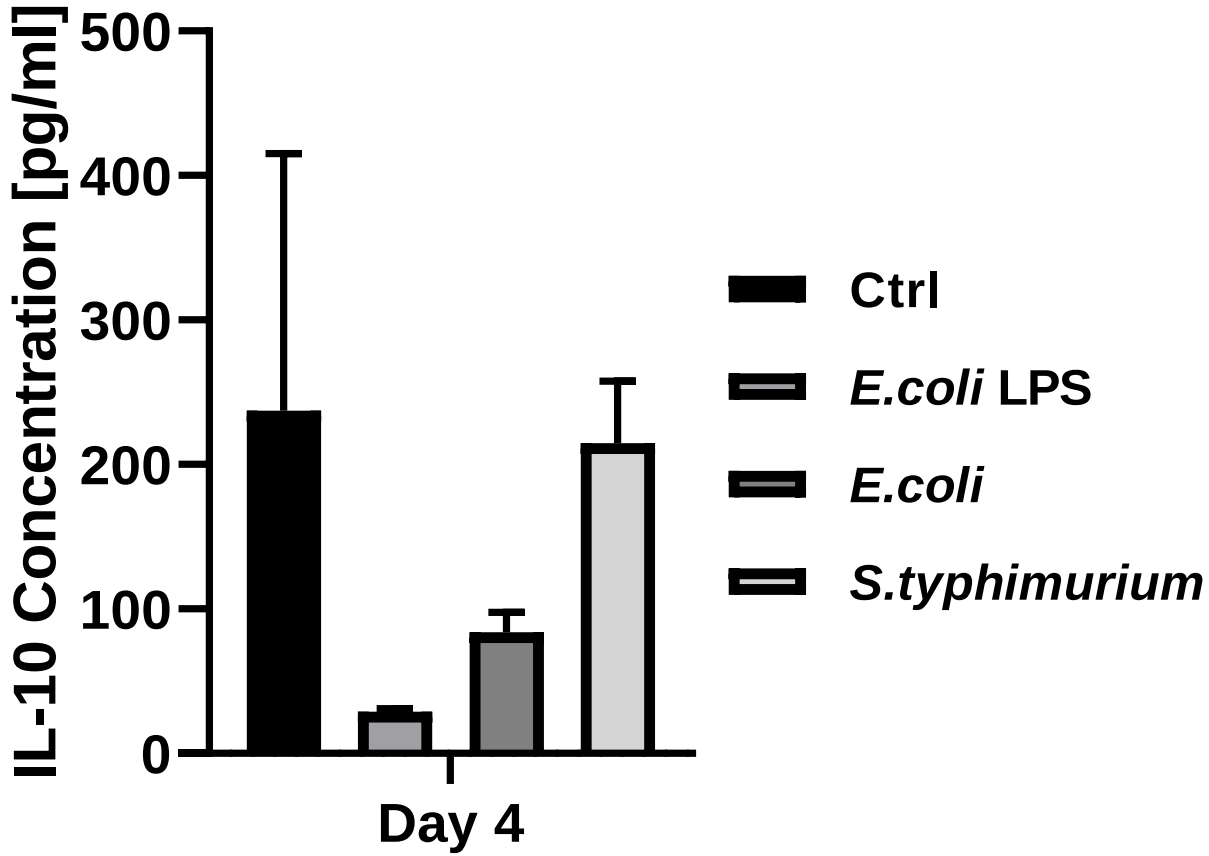
- Representative fluorescence images of each well



Immunostimulation triggered by bacterial infection

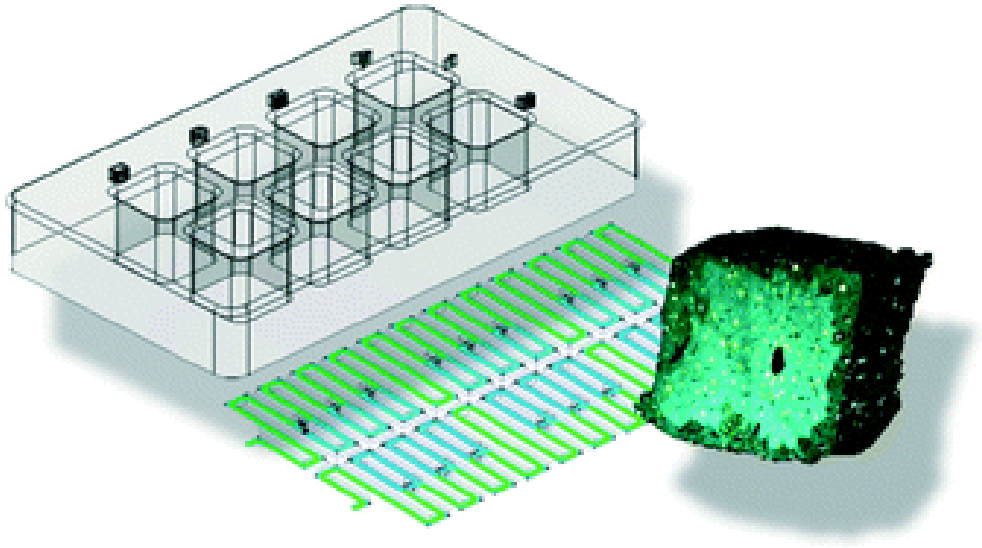
- ELISA results from cell culture media after 4 days



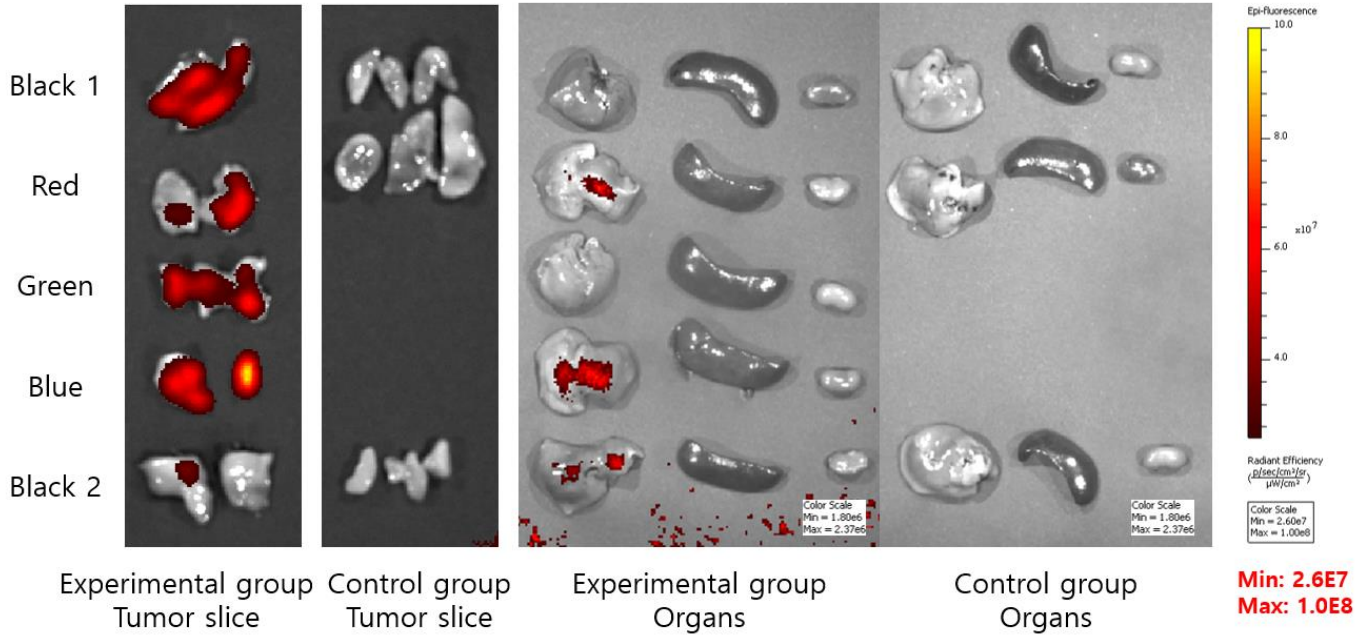


Future work

- **Incorporating advanced tissue models**



L. F. Horowitz, et al., *Lab on a Chip* (2021)

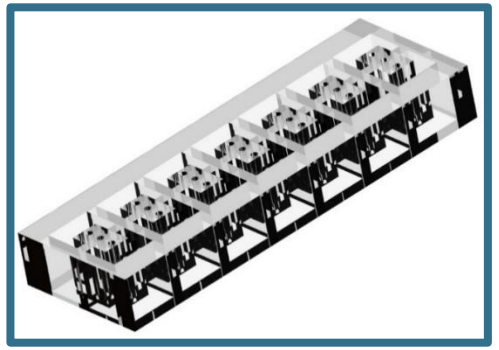


Tumor-Organ IVIS & Tumor-Organ

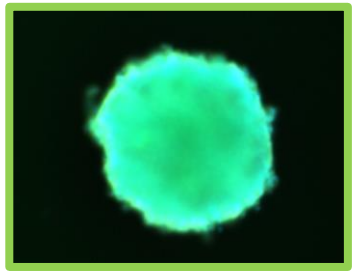
96 well-plate IVIS (Cy5)

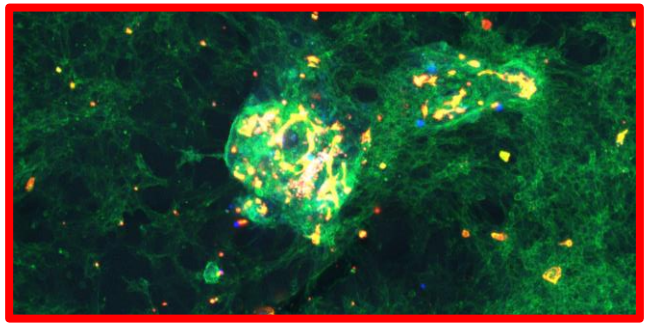
Conclusion

- Introduced a novel microfluidic platform that can recapitulate the key fundamentals of bacteria-cancer interaction
  - ✓ Optimization through laser cutting-based rapid prototyping technique
  - ✓ Design rule for selective patterning
  - ✓ User-friendly platform with straightforward design



- Demonstrated the effects of bacterial stimulation on tumor spheroid and corresponding pro-inflammatory response of macrophages experimentally
  - ✓ Currently working to incorporate primary cells for in-depth analysis.





Closing

Thank you

People with any questions are welcome to contact us

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Tel: +82-10-4596-5830