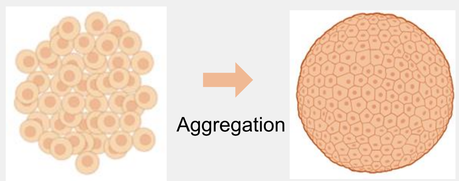
The Development of a High-efficiency spheroid fabrication platform using superhydrophobic surfaces

Suji Kim , Junmo Lee, Hyejune Yang, Taeyeon Kim , Dayoung Yu

가톨릭대학교 THE CATHOLIC UNIVERSITY OF KOREA

Introduction

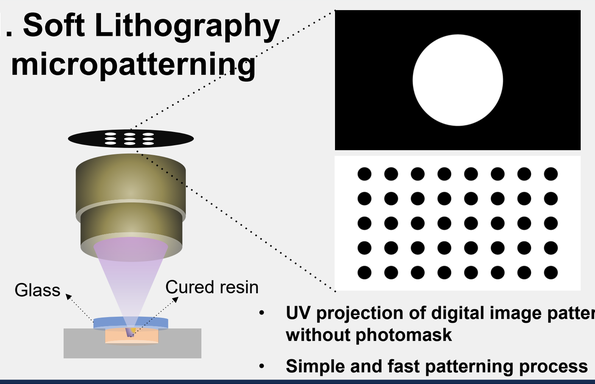
3D spheroid platform



- Cell to cell, cell to ECM interaction
- Mimic in-vivo tumor cell environment

→ Enhance cell functionality and survival

1. Soft Lithography micropatterning



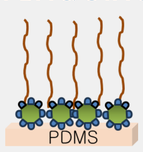
- UV projection of digital image patterns without photomask
- Simple and fast patterning process

2. Surface Modification

PDA coating

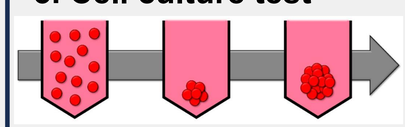
- Inspired by mussel adhesion mechanisms
  Can stably adhere to PDMS
- Provides hydrophilicity to the surface
  → Reduces hydrophobicity

PDA & STA coating



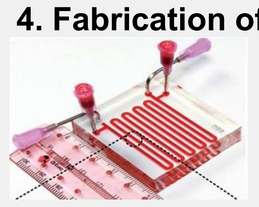
- Maximizes hydrophobicity by increasing the water contact angle
- Utilizes the excellent secondary surface modification properties of PDA

3. Cell culture test



- The surface provides a favorable environment for cell adhesion and spheroid formation

4. Fabrication of Microfluidic device



- Fabrication of Pipe Channel Molds using 3D printing
- PDMS into the mold and hardening to fabricate the microfluidic device

Methods

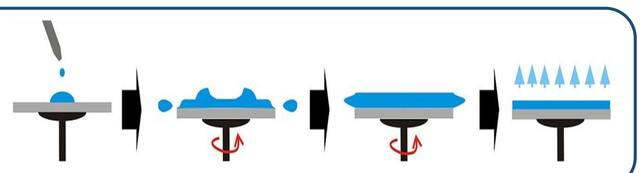
Soft lithography

Patterning & Developing

- fabrication technique to create micro-patterns
- transferring patterns from a photomask to PDMS(Polydimethylsiloxane) through a molding process

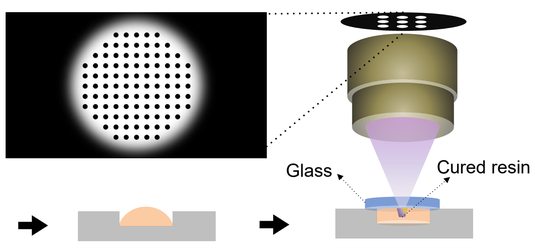
Spin coating

- HMDS (Hexamethyldisilazane)

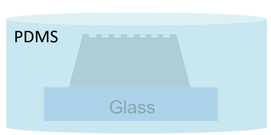


Digital photomask

1.7mm diameter gradient pattern



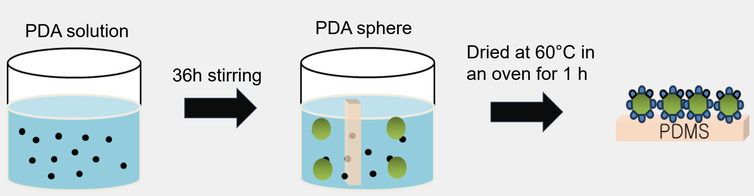
Hardening



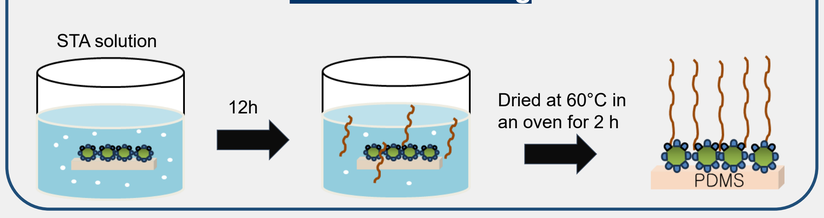
Mold detach

Surface modification

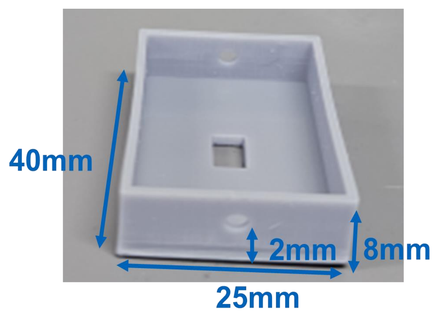
PDA coating

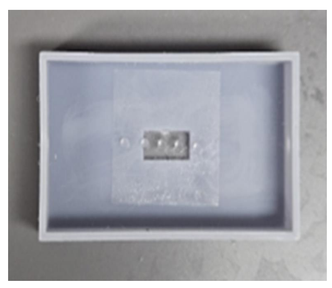


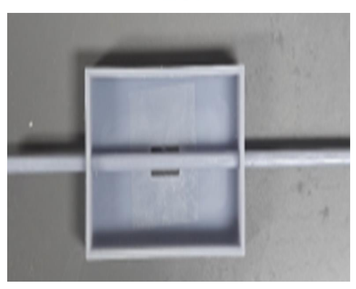
PDA&STA coating

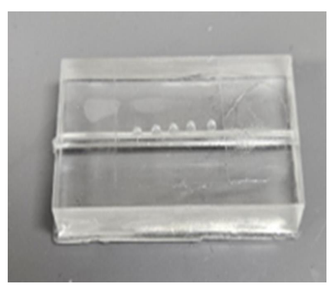


Microfluidic device





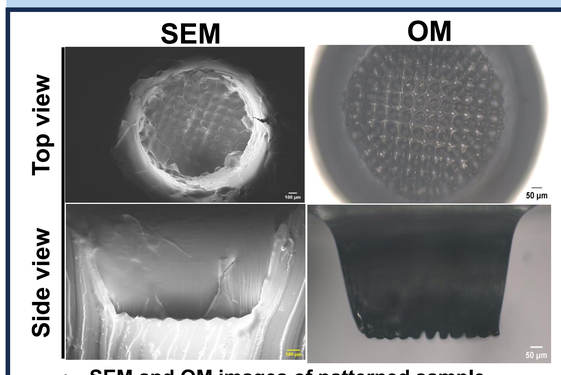




- 40mm x 25mm x 8mm container and 2mm diameter pipe channel was fabricated using a 3D printing DLP method
- micro-patterned substrate was placed on a channel mold, PDMS was poured, hardened, and detached → complete microfluidic device

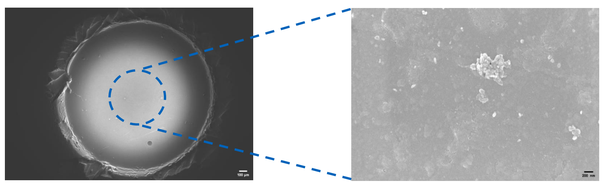
Result

Structural & surface confirmation



- **SEM and OM images of patterned sample** showed successful pattern transfer, resembling the photomask design

SEM image of PDA+STA PDMS suface

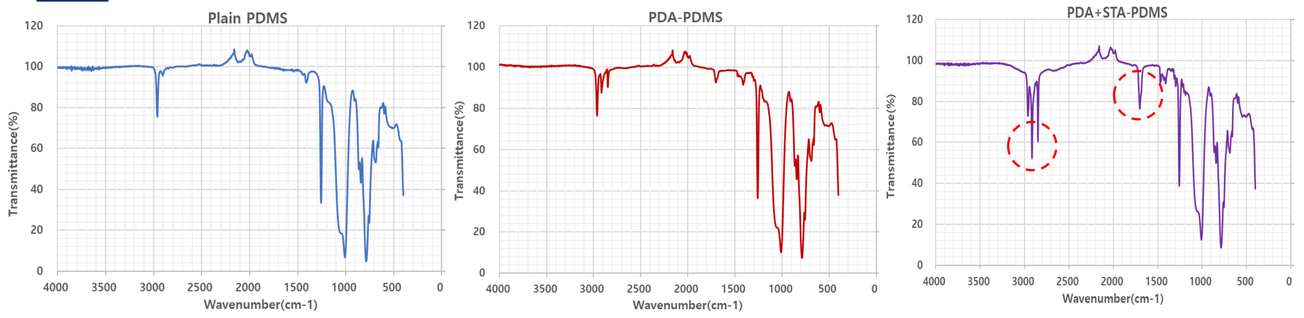


- confirmed the successful STA coating

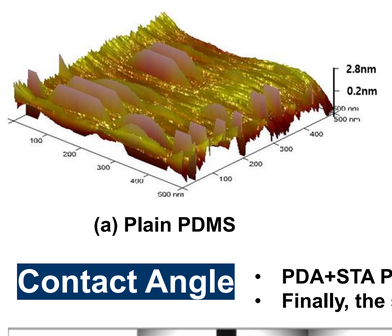
→ can enhance hydrophobicity of the PDMS surface

Surface structure analysis by PDA & STA surface modification

FTIR



- **Observe a specific peak of 1700-1750cm-1 and 2800-3000cm-1 in STA**
- **STA Successfully Binds to PDMS Surface to Strengthen Hydrophobicity.**



(a) Plain PDMS (b) PDA+STA PDMS

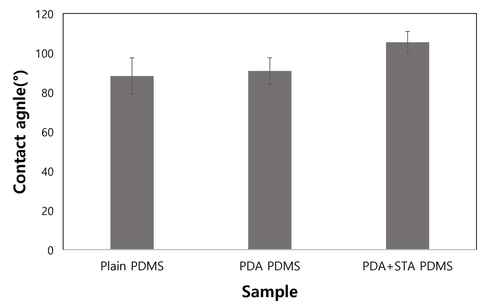
AFM

- By coating STA in surface, the surface height significantly increases from 2.8nm to 66.7nm.

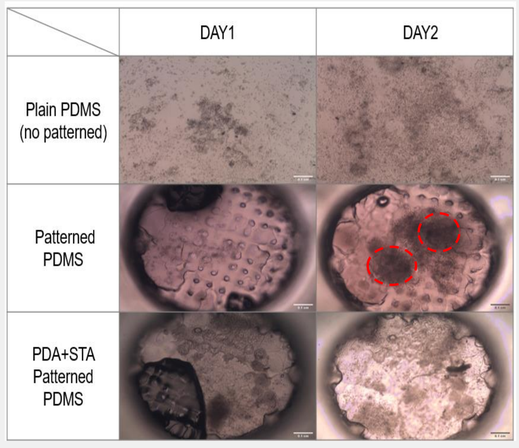
Contact Angle

- PDA+STA PDMS indicates contact angle above 100.
- Finally, the strengthening of hydrophobicity was confirmed by STA surface modification.

Droplet Image			
Sample	Plain PDMS	PDA-PDMS	PDA+STA-PDMS
Average(°)	88.23	90.8	105.4
Standard deviation(°)	9.23	6.76	5.5



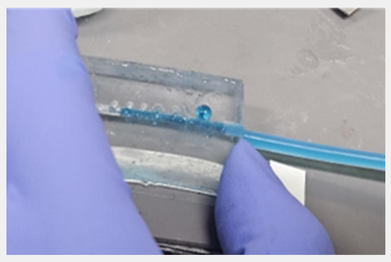
Cell culture test



Microscopic Images of Cell culture on Different PDMS sample over two days

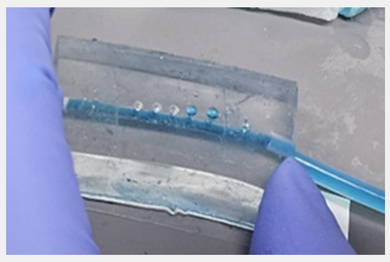
- spheroid formation was observed in some sample
- Patterns and surface modifications influenced cell attachment and spheroid formation on cell culture

Fluid flow evaluation



Microfluidic Device

- tested with methylene blue to confirm fluid flow through the channels



- While smooth flow was observed in the channel layer, fluid in the cell layer did not diffuse evenly, and backflow into the channel layer disrupted circulation.

Conclusion

The possibility of creating hydrophobic surfaces through soft lithography and PDA+STA surface modification was confirmed, establishing a foundation for 3D spheroid culture. Additionally, the fluidic performance of the microfluidic device with the hydrophobic surface was evaluated, suggesting potential directions for developing an optimal platform for efficient 3D spheroid culture. These results are expected to provide a significant foundation for applications in biomedical fields such as cancer research, drug screening, and tissue engineering.