

Oil phase air transport for under-liquid biofabrication with microbial nanocellulose

Yi LU,¹ Yeedo Chun,¹ Xiaoya Su,¹ Xueting Shi,¹ Marina Mehling,¹

Milad Kamkar,² Gerald G. Fuller,³ Orlando J. Rojas ^{1,4}



¹ University of British Columbia

² University of Waterloo

³ Stanford University

⁴ Aalto University

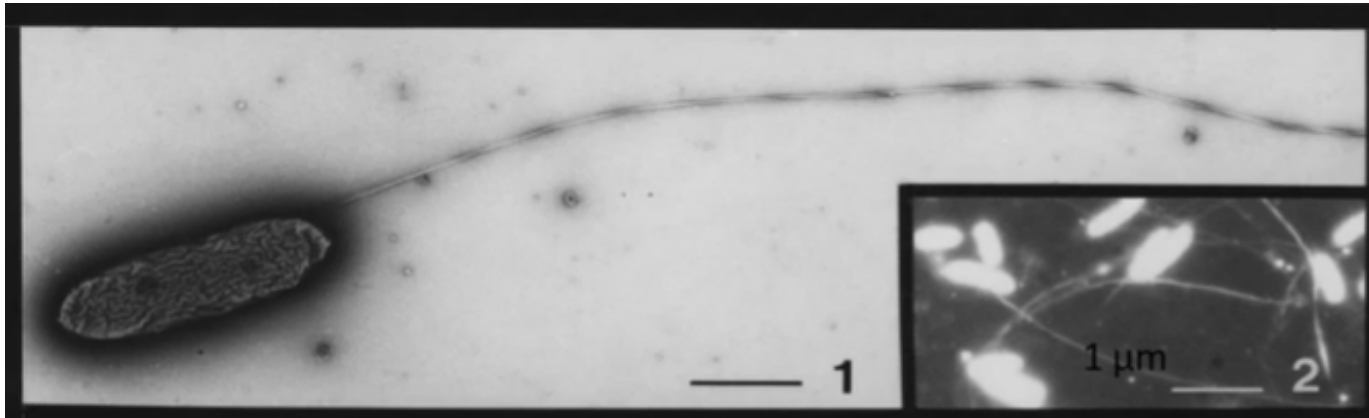


12-16 JUNE 2023 • VANCOUVER, B.C. CANADA

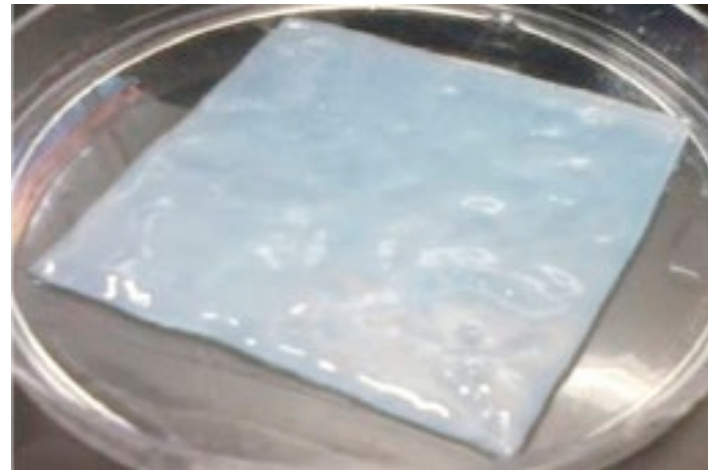
Bacterial cellulose (BC)



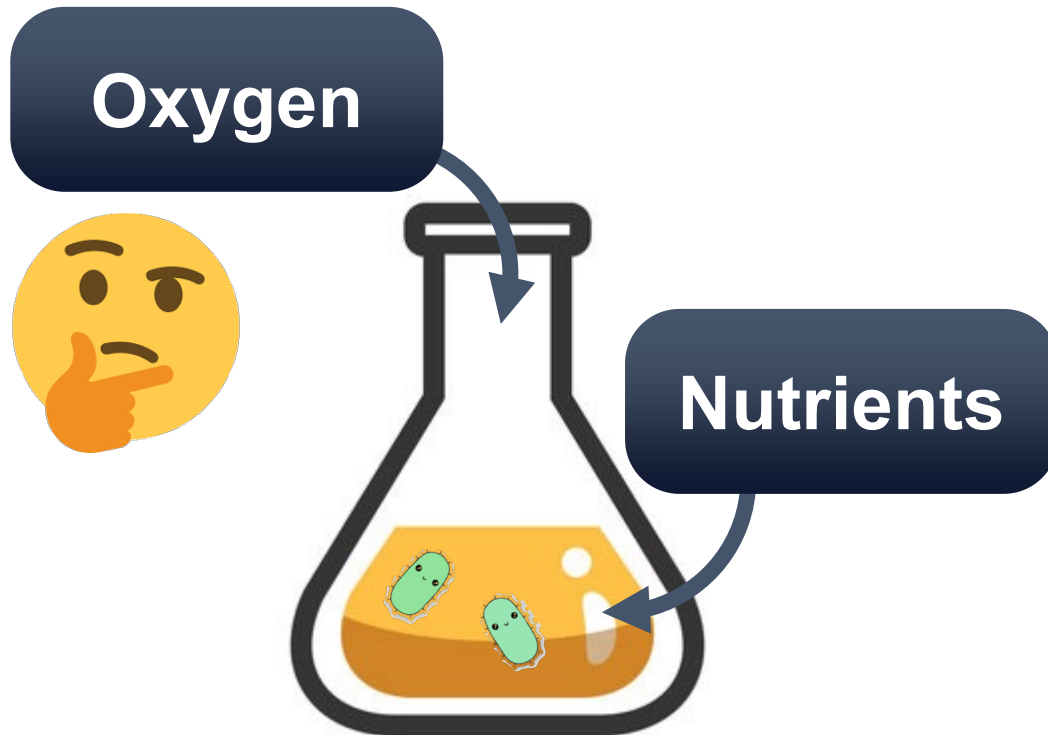
First reported by
A.J. Brown, in 1886.



- High-purity
- High degree of crystallinity
- Strong mechanical properties
- High water-holding capacity



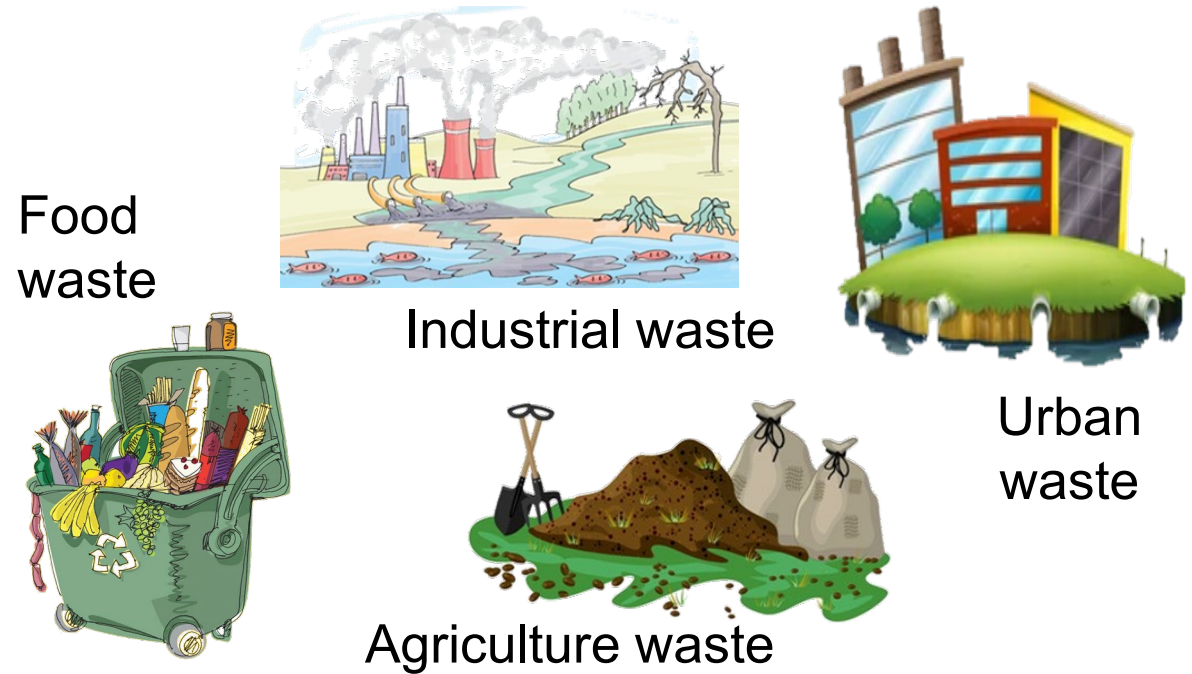
BC cultivation



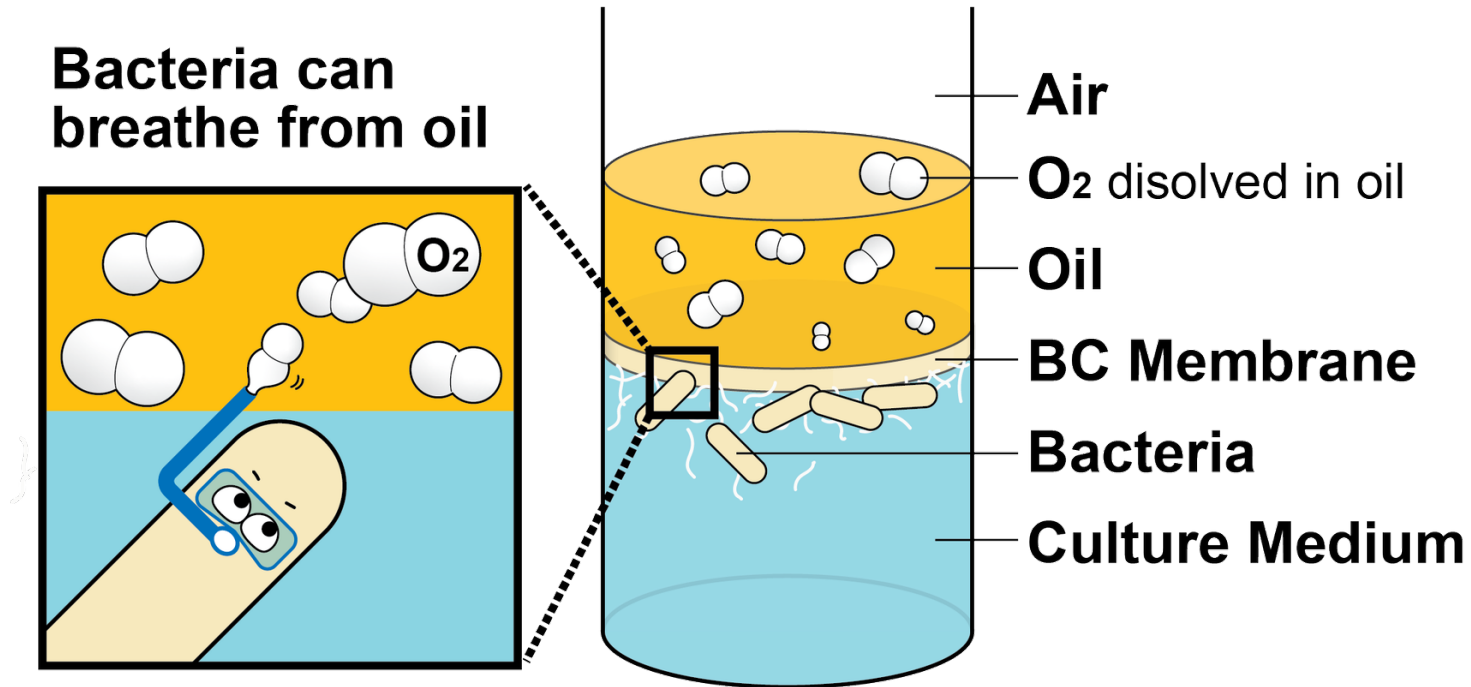
Laboratory: Standardized culture medium



Industry & Circular economy



Oil can act as an oxygen transportation medium

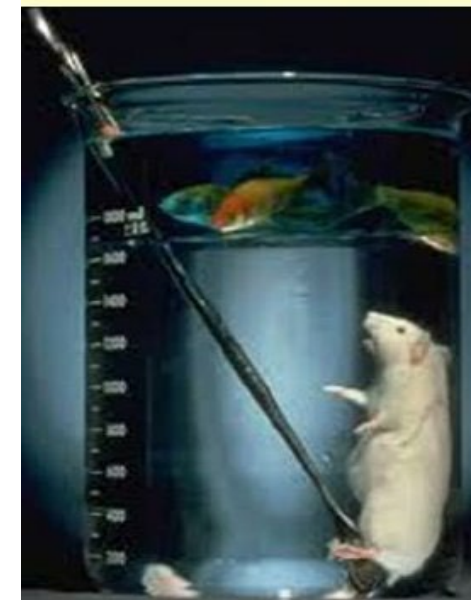
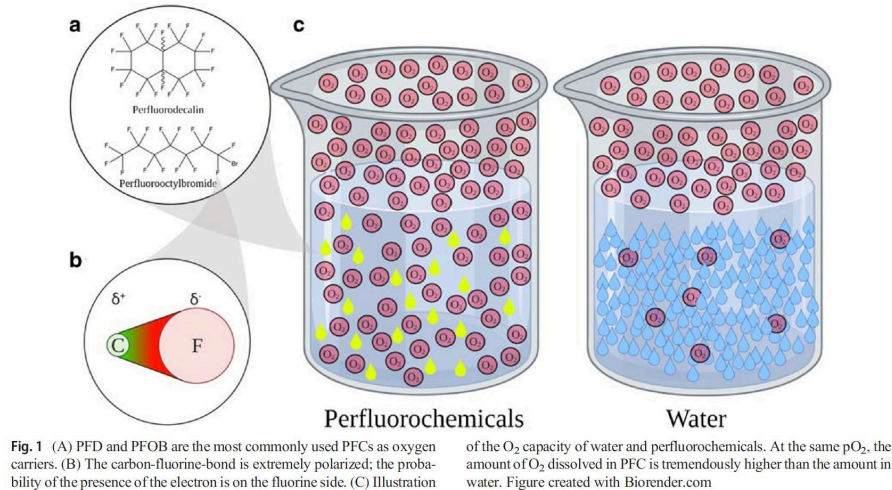


Under-liquid biofabrication

Perfluorocarbon (PFC) oil

- Exceptional oxygen dissolving and delivery capacity
— Gas-like liquids

Dissolved O₂: 200 H₂O molecules : 1 O₂ molecule
1/5 PFC molecules: 1 O₂ molecule



Stabilized Electronically Modified Oxygen Derivatives in a Bio Inert PFC Matrix,
DOI: 10.13140/2.1.1910.8648

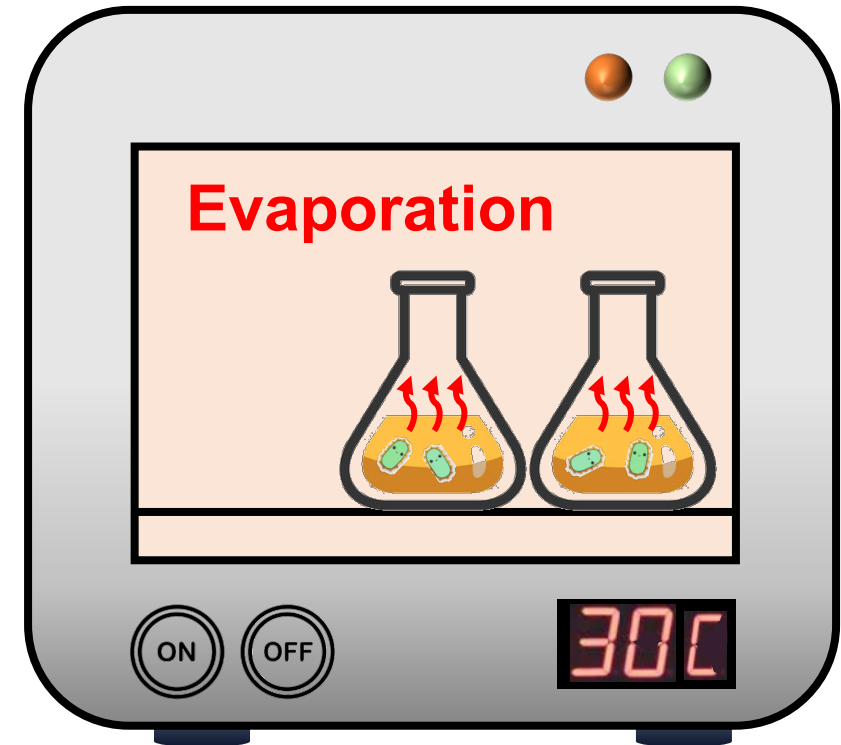
Motivations for under-liquid biofabrication

(i) Evaporation issue

- Difficulties in biosynthesizing small objects;
- Shrinking, Wrinkling, and Tilting;

A layer of non-volatile oil could eliminate evaporation;

Cultivation ~1–2 weeks



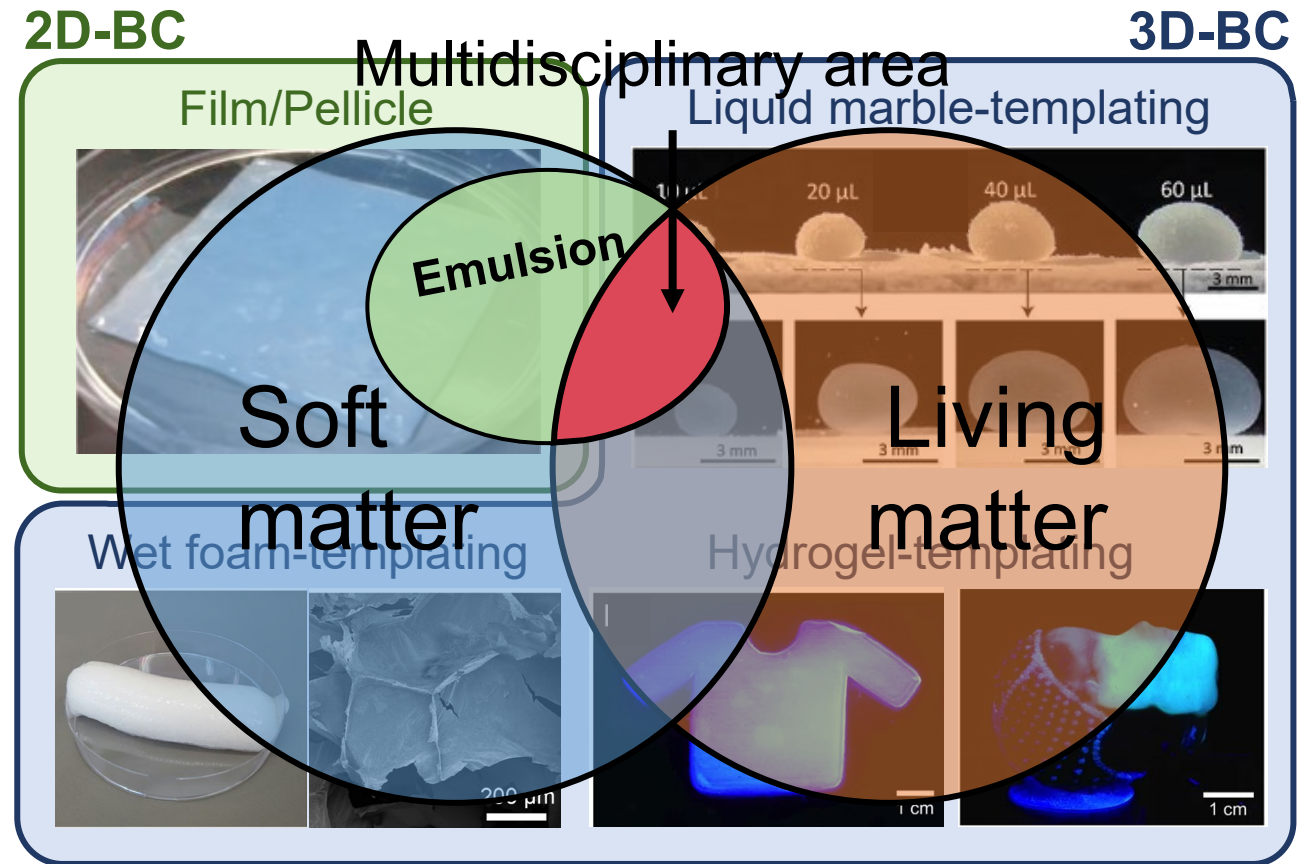
Motivations for under-liquid biofabrication

(ii) 3D biofabrication

- BC grows at the air-water interface;
- 3D biofabrication
→ The ability to shape the interface.

Oil-water interface is easier
to manipulate.

Emulsions

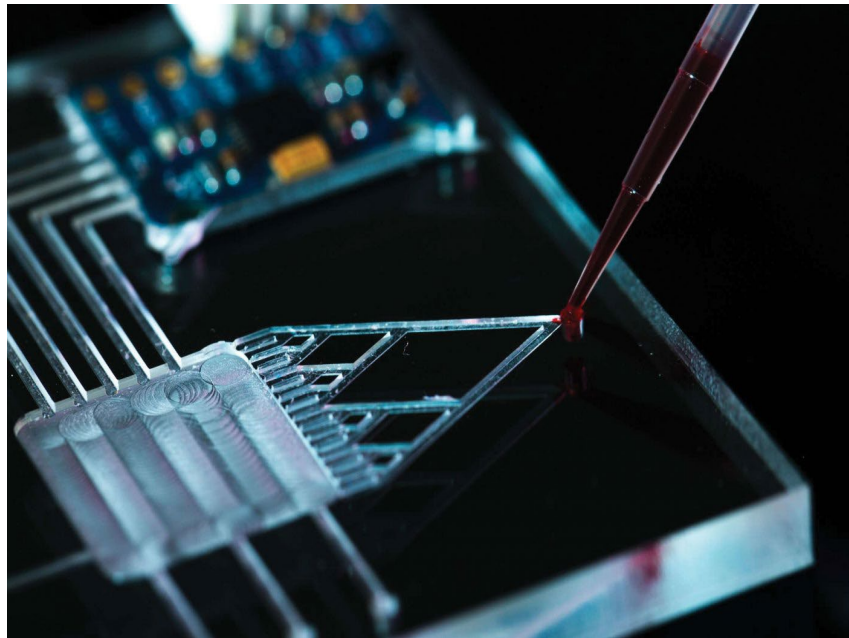


Rühs, P.A., et al., 2018. *npj Biofilms and Microbiomes*, 4(1), p.21.
Schaffner, M., et al., 2017. *Science advances*, 3(12), p.eaao6804.

Manoukian, O.S., et al., 2019. *Biomaterials for tissue engineering and regenerative medicine*.
Greca, L.G., et al., 2018. *Materials Horizons*, 5(3), pp.408-415.

Motivations for under-liquid biofabrication

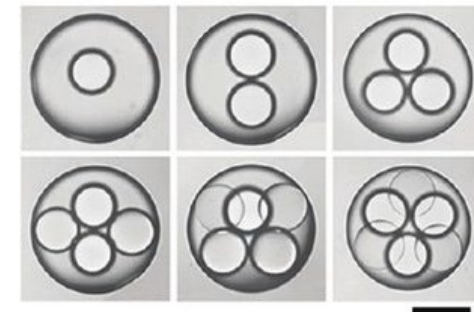
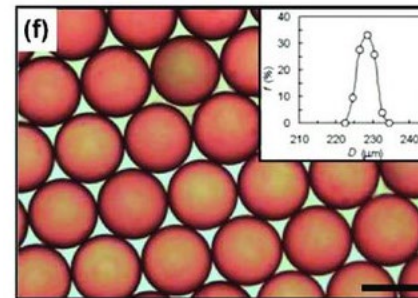
(iii) Continuous production



Microfluidic devices

“Microbial Farm”-on-a-chip

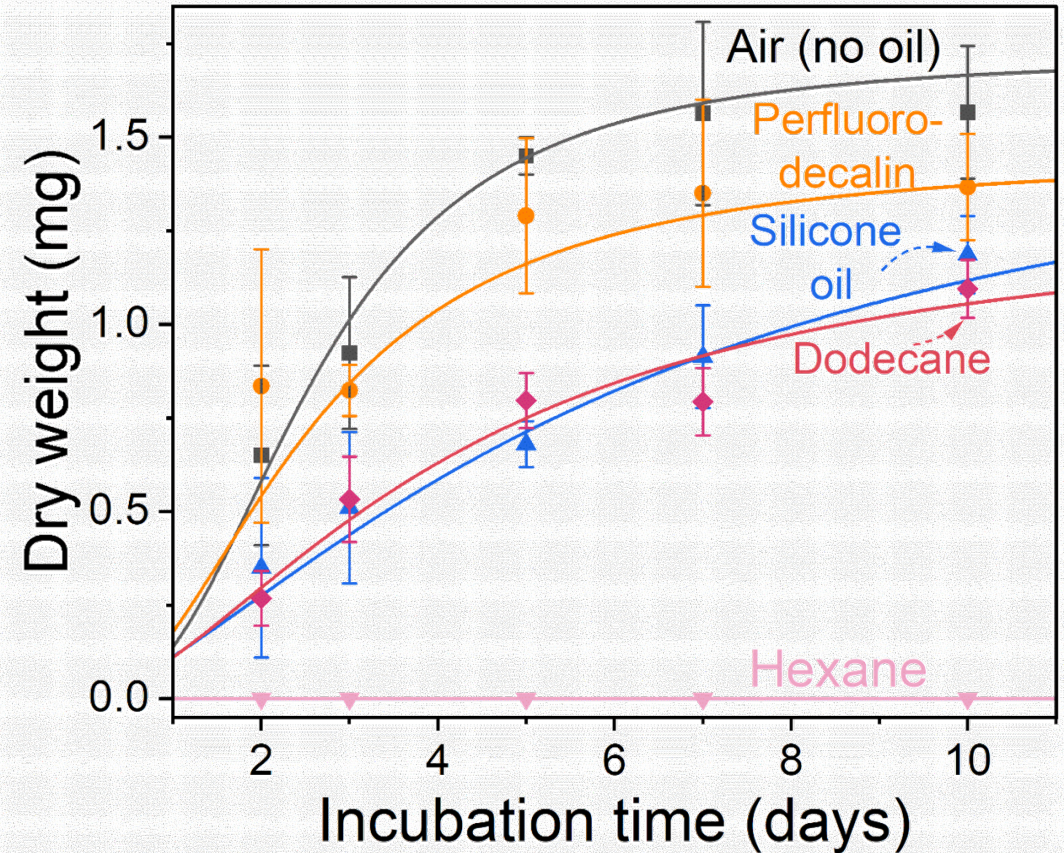
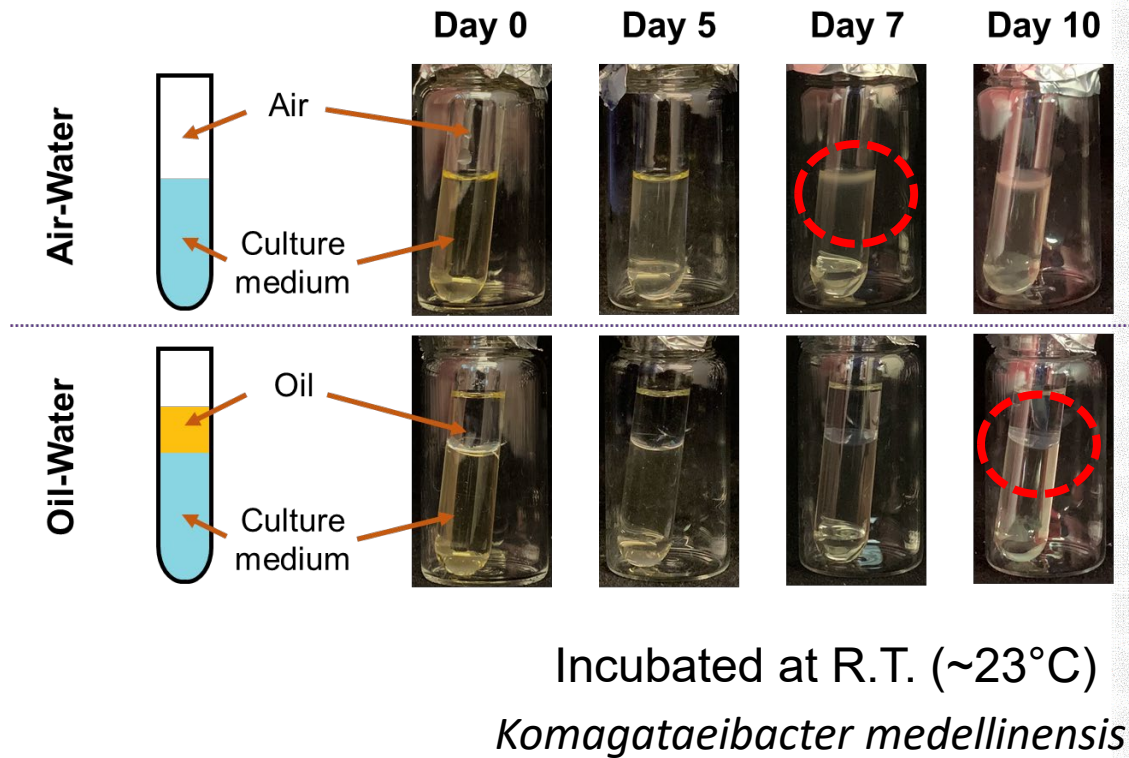
- Massive continuous production;
- Homogeneous sizing;
- Multi-compartment design.



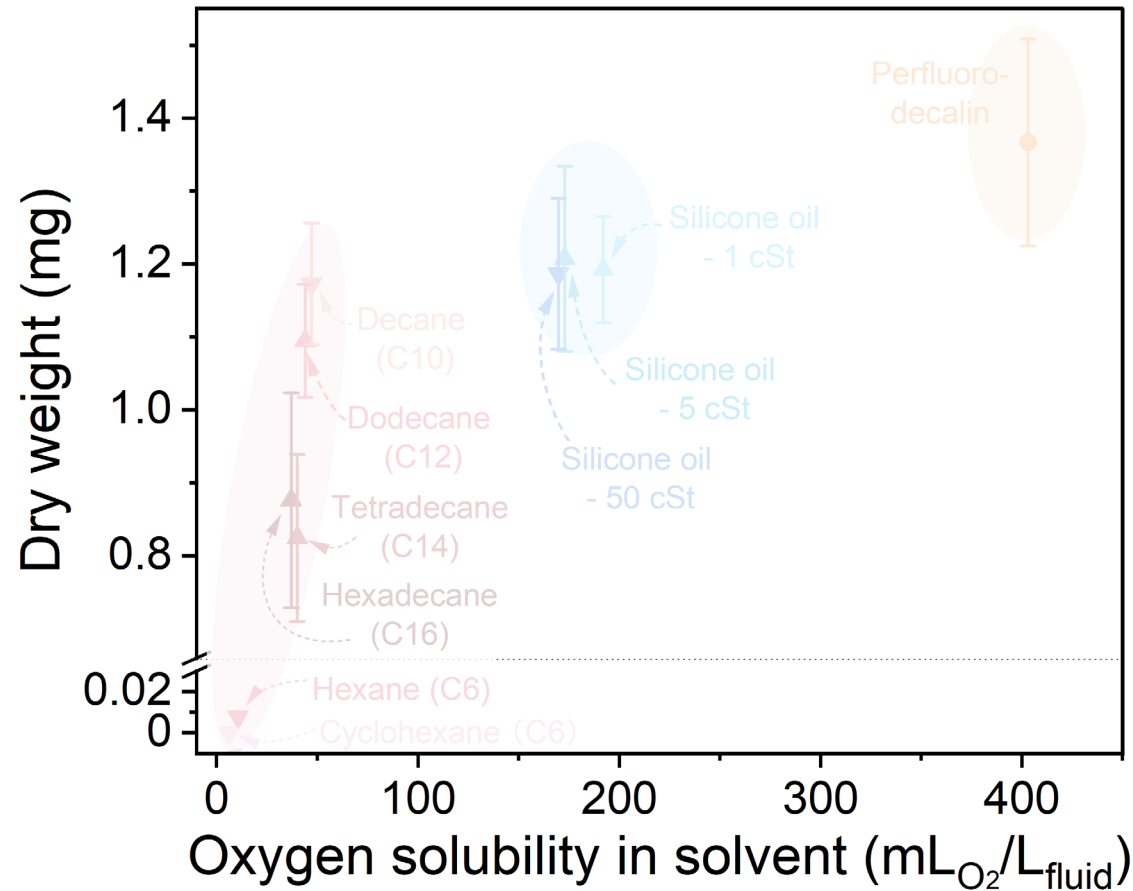
<https://www.genengnews.com/insights/microfluidics-stands-on-the-shoulders-of-microfab-giants/>

He, F., et al., 2019. *Advanced Materials Technologies*, 4(6), p.1800687.

Under-liquid biofabrication of BC films



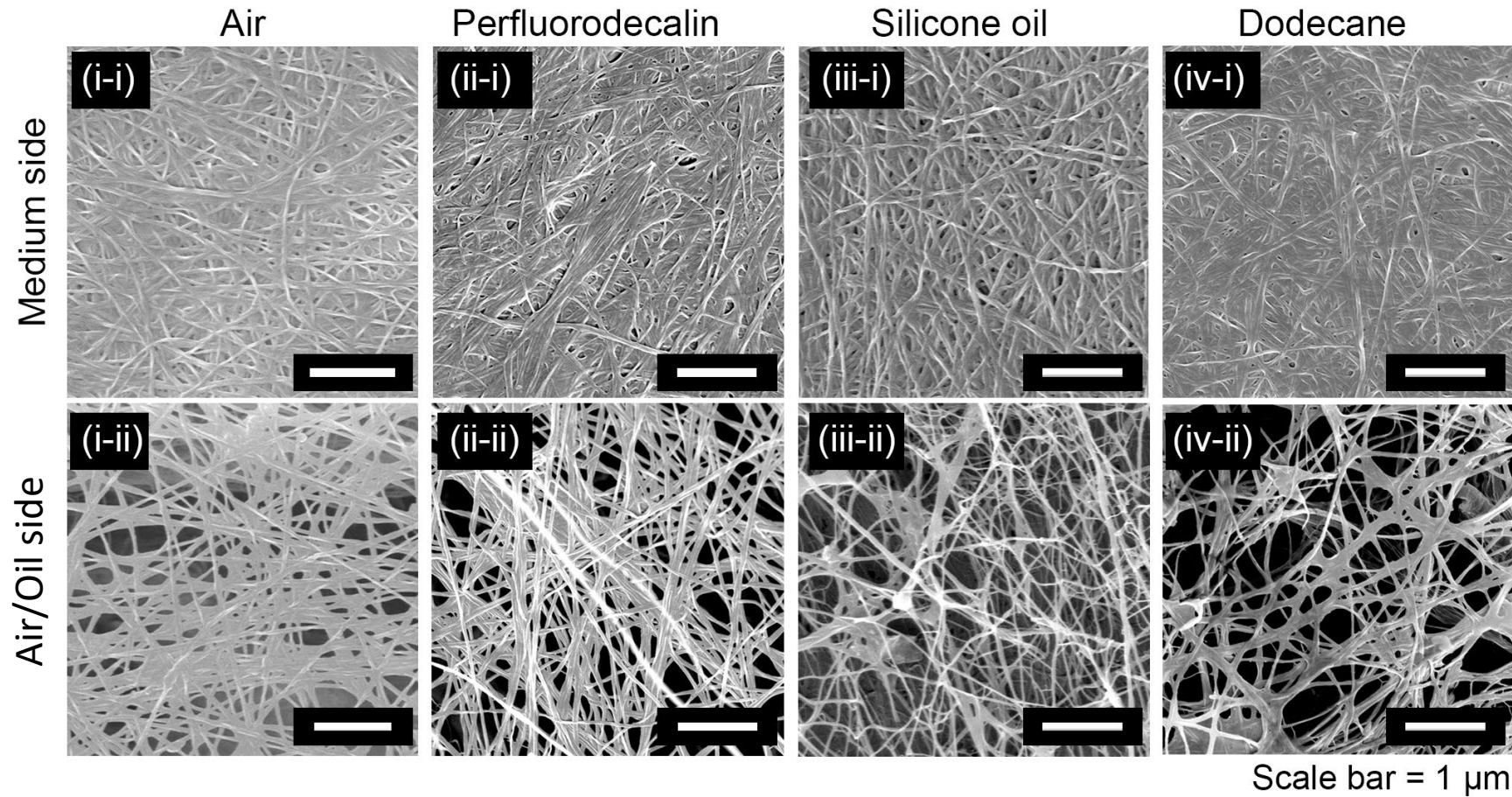
BC growth vs. Oxygen solubility



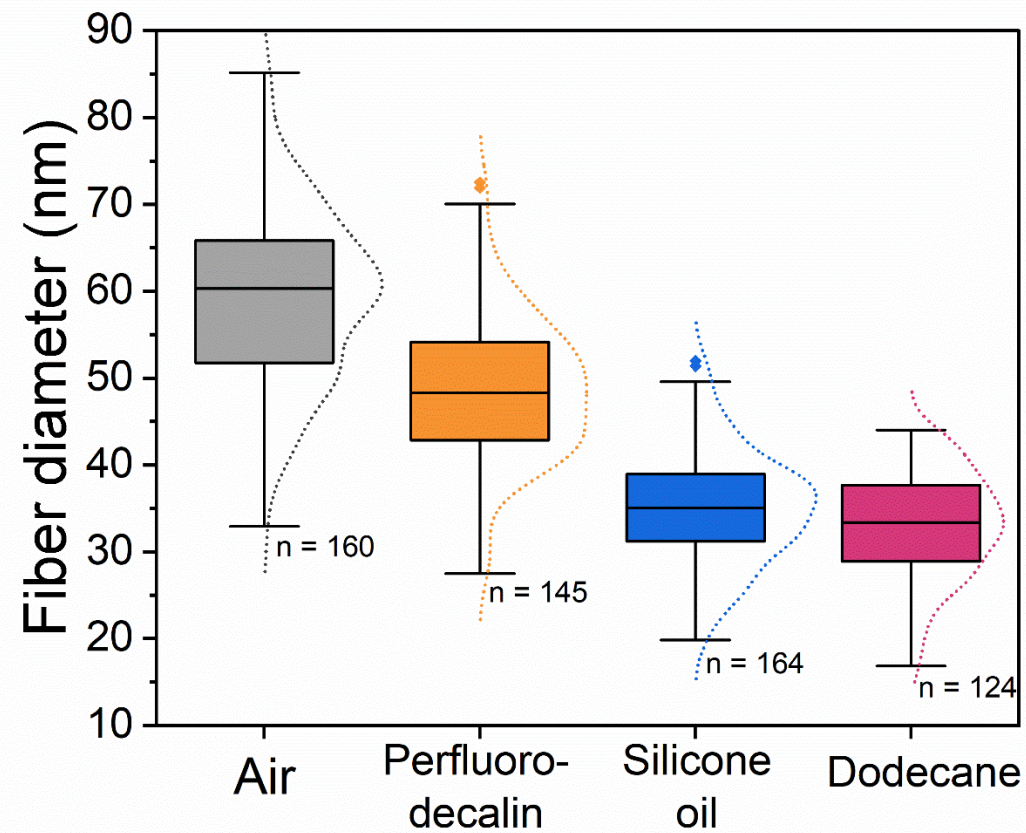
- **PFC**: Best
 - Biomedical compatible
 - Expensive
- **Silicones**: Works well
 - Negligible viscosity impact at < 50 cSt
 - From the literature: More viscous (10⁵ cSt) → Much reduced dry weight ^[1]
- **Hydrocarbons**: Depends on chain length
 - C10 & C12 appear to be the best so far

[1] Binelli, M.R., et al., 2022. *Biomaterials Advances*, 141, p.213095.

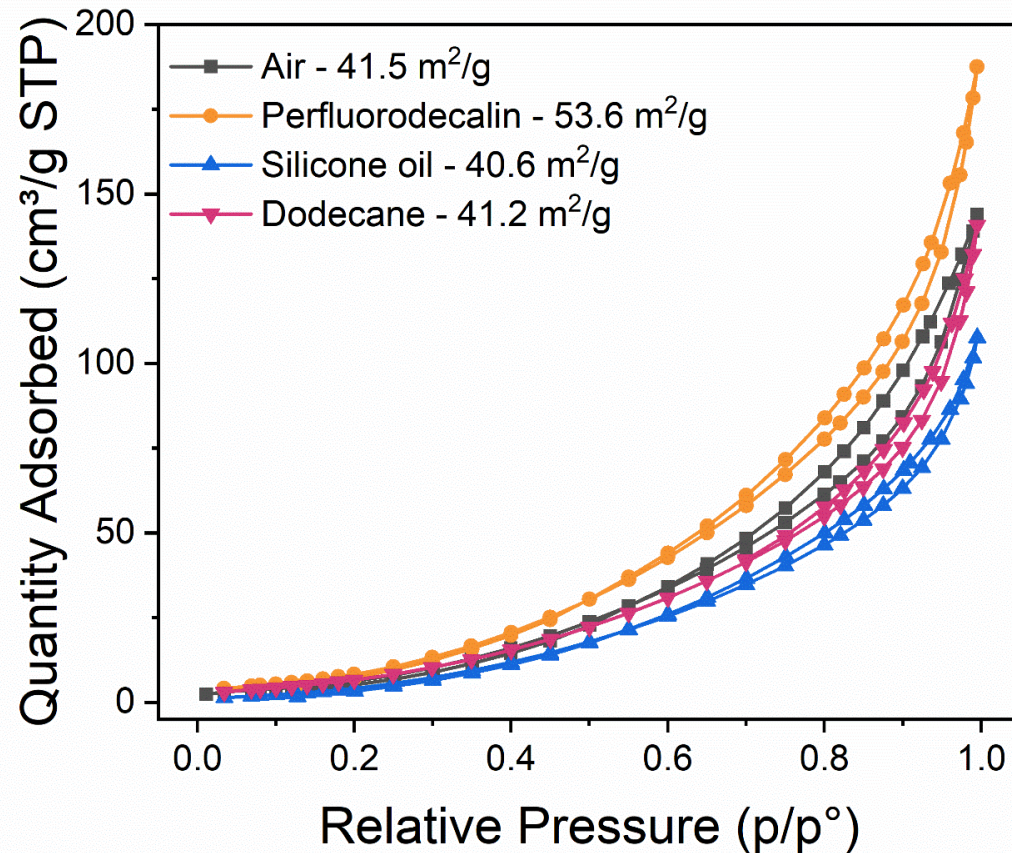
BC film morphology



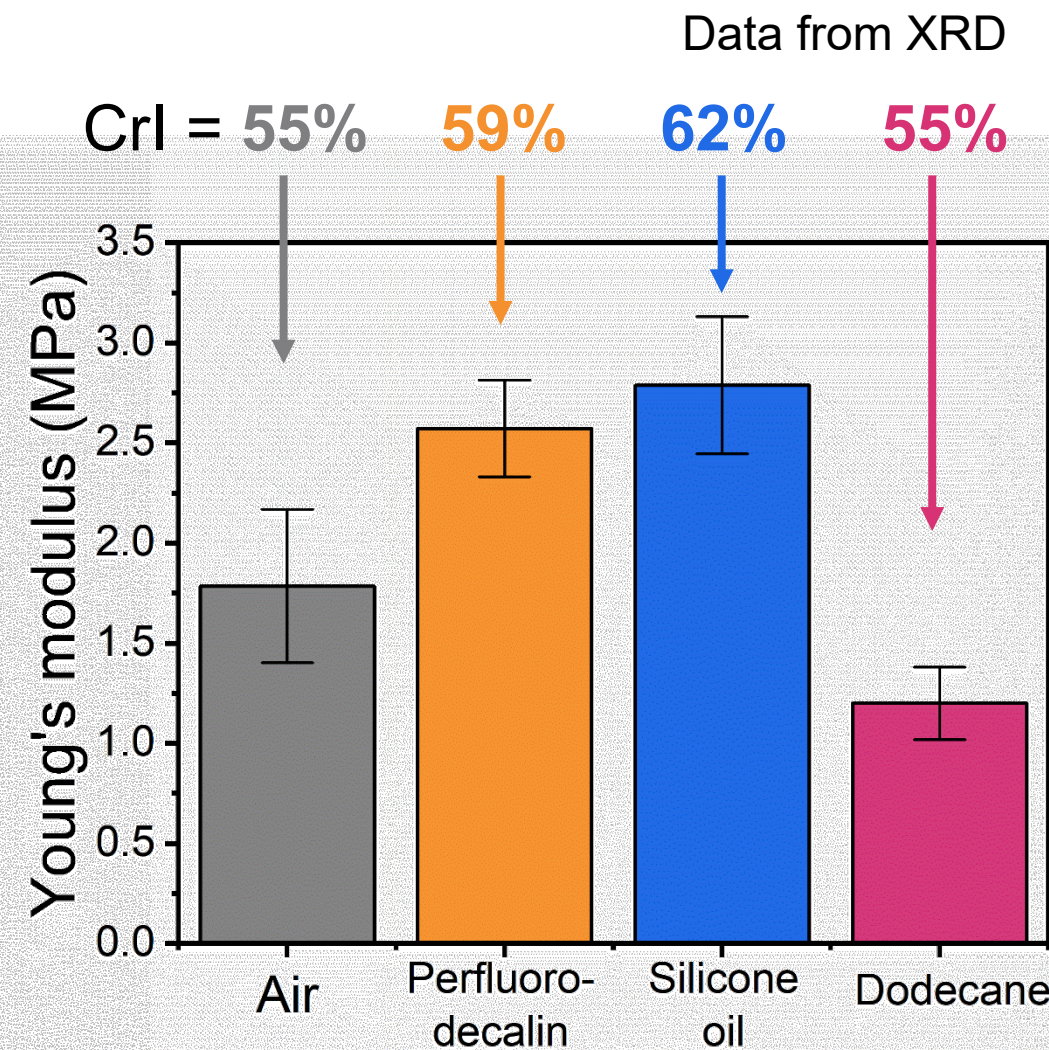
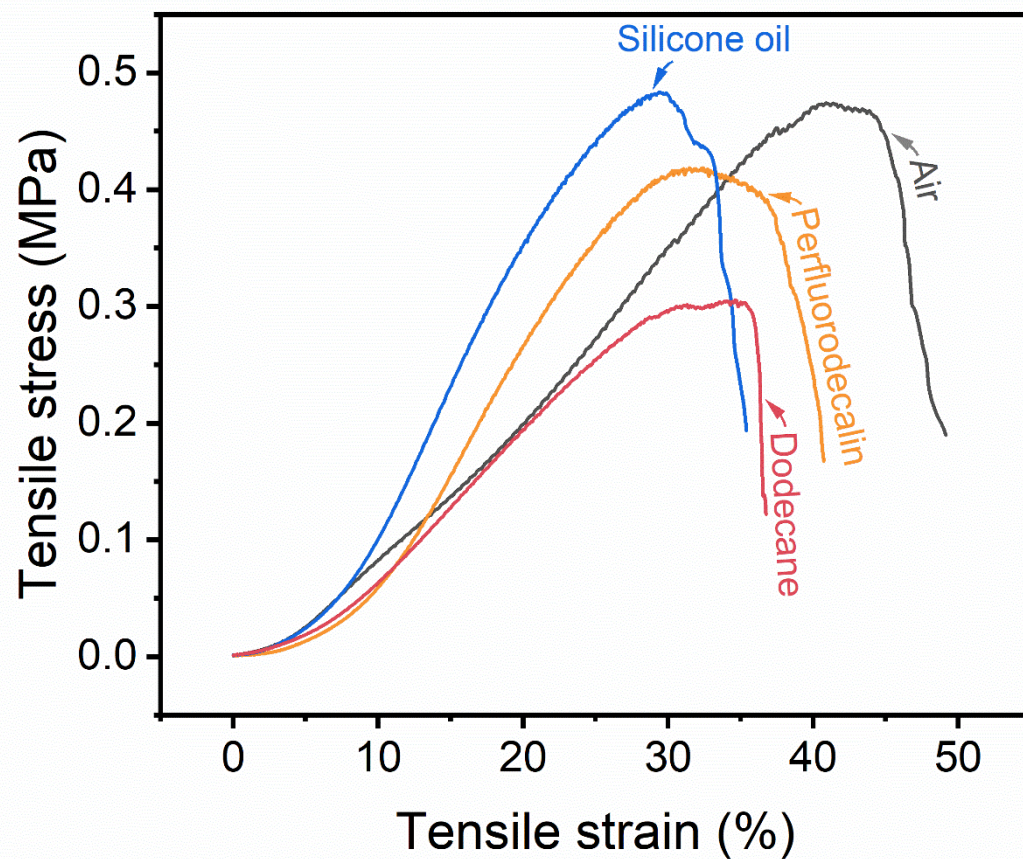
Fiber diameter: Air > PFC > Silicone $\approx$ Dodecane



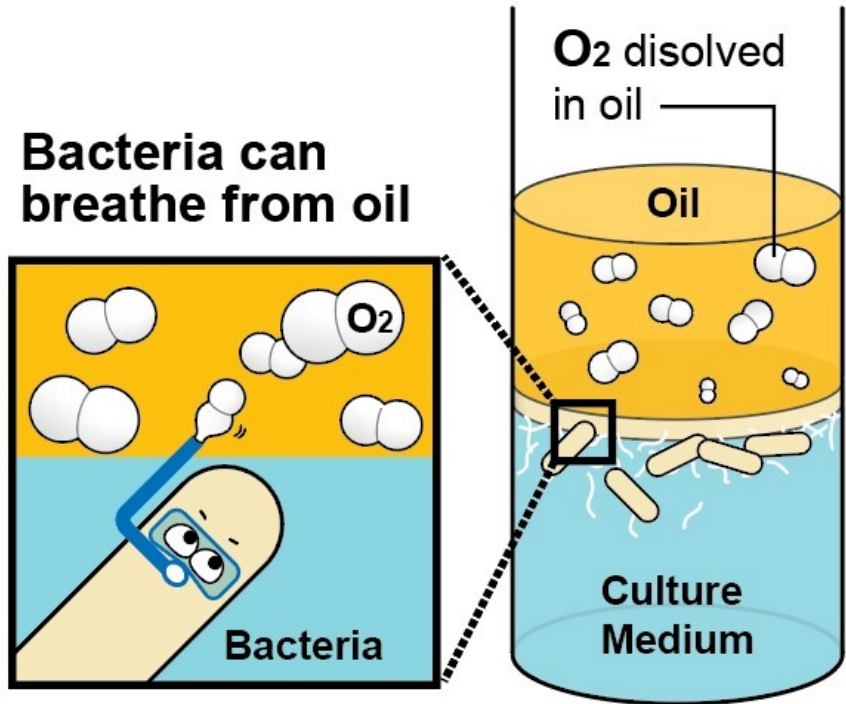
BET surface area: PFC > Air $\approx$ Dodecane $\approx$ Silicone



Mechanical properties

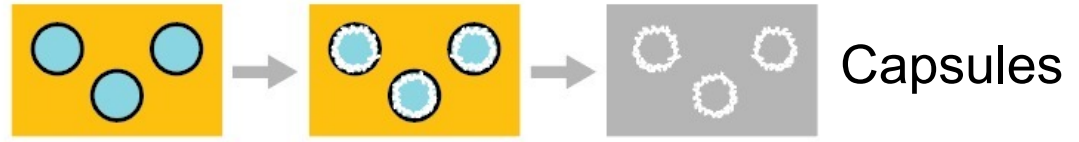


Road map for under-liquid biofabrication

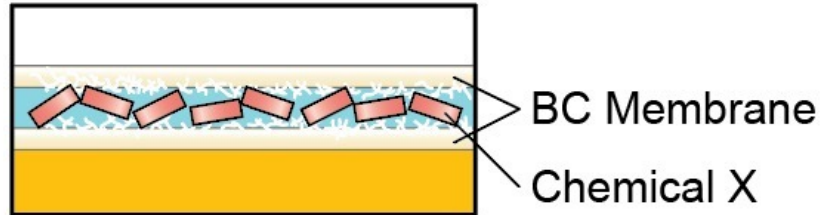


Application

W/O emulsion

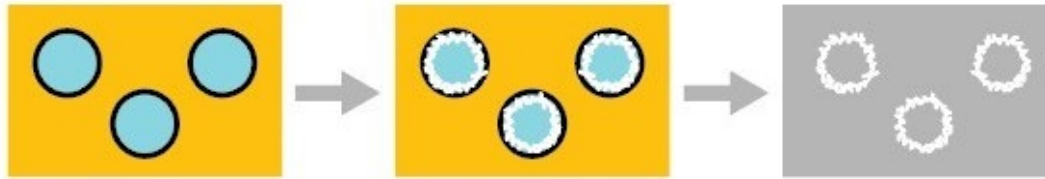


One pot bio fabrication of layered composites

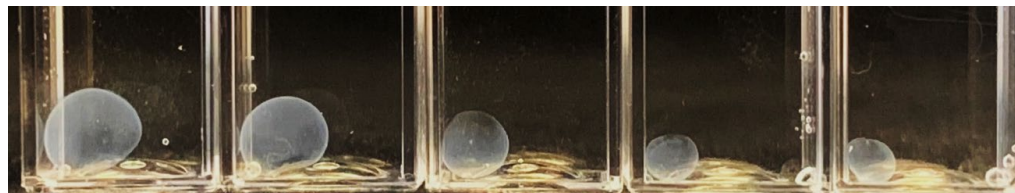
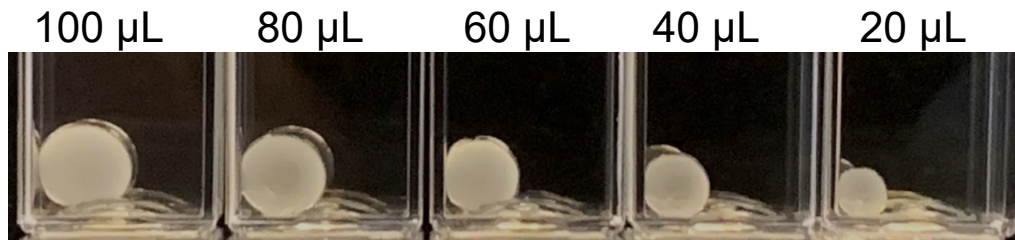


BC Capsules

W/O emulsion



Capsules



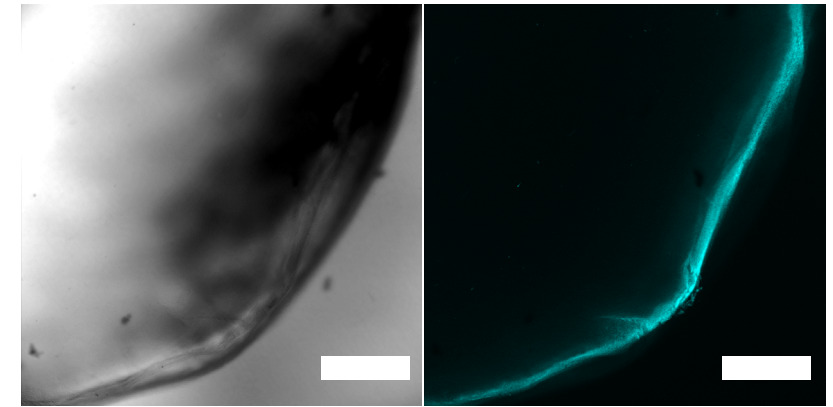
Decreasing droplet volume

Under-liquid
biofabrication of
BC capsules



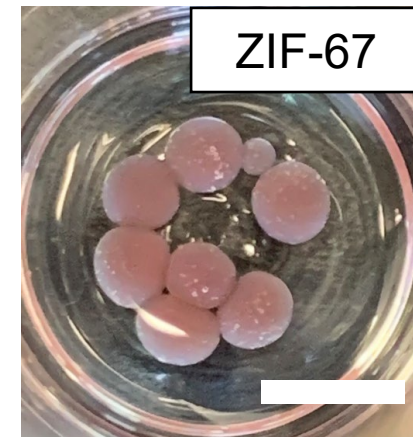
After bacteria/
protein removal
(NaOH, 60°C,
4 hr)

BC shell structure



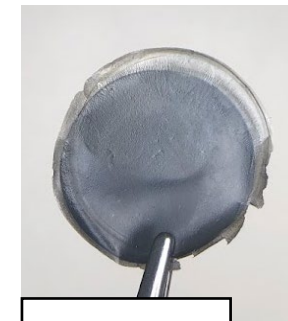
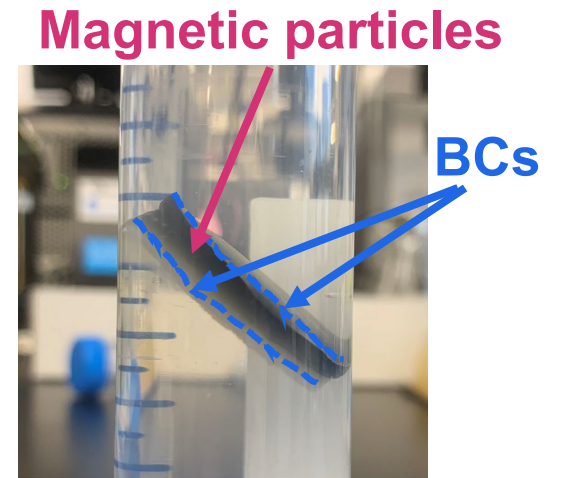
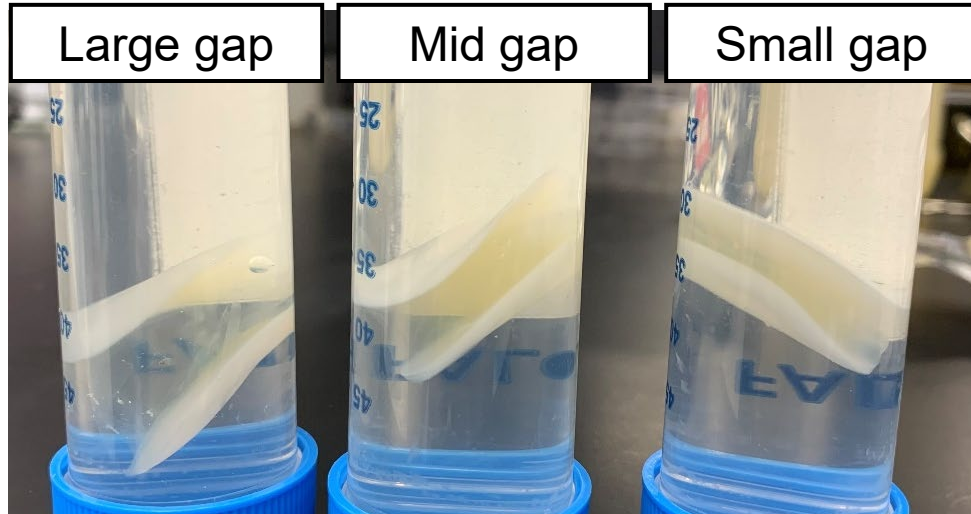
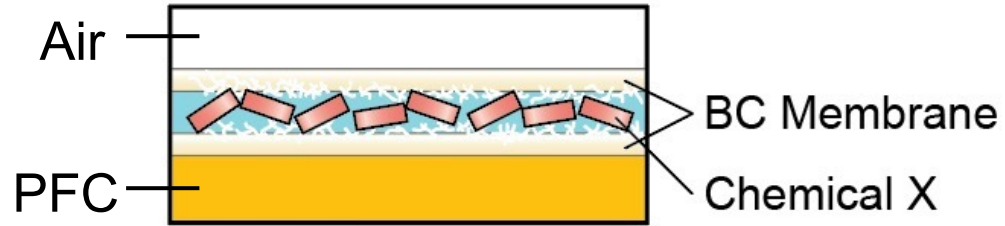
Scale bar = 500 μm

Encapsulation of MOFs



Scale bar = 10 μm

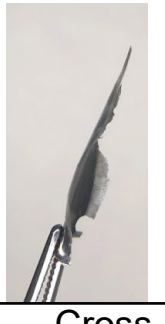
Three-layer BC composites



Air side



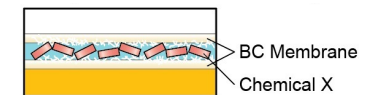
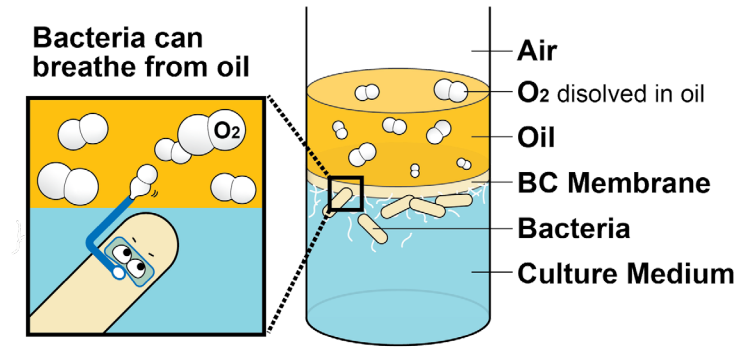
PFC side



Cross-section

Conclusions

- Under-liquid biofabrication provides a robust routine to produce 3D bacterial cellulose (BC)
- **PFC oil** > **Silicone oil** > **Hydrocarbon oil**
- Towards 3D biofabrication
 - Water-in-oil (w/o) emulsions: BC capsules
 - New interface: Three-layer BC composites





Dr. Yi Lu, yi.lu@ubc.ca   

Postdoctoral Research Fellow

The University of British Columbia

Acknowledgments

Thank You!



Prof. Orlando Rojas



Prof. Gerald Fuller



Y. Chun



X. Su



X. Shi



A. Takagi



M. Mehling



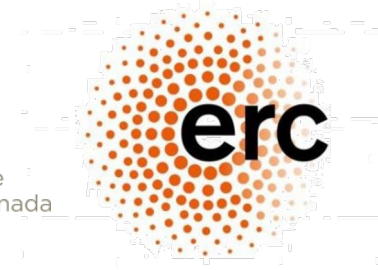
Prof. M. Kamkar



BiMat



Canada Excellence
Research Chairs
Chaires d'excellence
en recherche du Canada



INNOVATION
Canada Foundation
for Innovation
Fondation canadienne
pour l'innovation