CHARACTERIZATION OF SACRIFICIAL TEMPLATE-BASED 3D BACTERIAL CELLULOSE SCAFFOLDS FOR TISSUE ENGINEERING

Emilija Baltrukonytė[1], Milda Alksnė[1], Ieva Šimoliūnė[1], Daiva Baltriukienė[1], Egidijus Šimoliūnas[1]

[1]Vilnius University, Life Sciences Center, Biochemistry Institute, Vilnius, Lithuania

Background

Bacterial cellulose (BC) is a promising biomaterial for tissue engineering due to its excellent biocompatibility, high crystallinity, and nanofibrillar structure with strong water-retention capacity. However, native BC has a dense structure, which limits its use in scaffold fabrication with controlled porosity. To address this, sacrificial templating using 3D-printed constructs can be applied to create BC scaffolds with predefined geometry and interconnected pore networks

Objective

This study aimed to characterize bacterial cellulose scaffolds engineered with defined geometry and controlled porosity through a sacrificial templating strategy

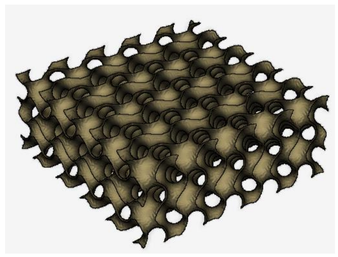


Fig. 1. *Example of sacrificial template structure.*

Sacrificial template and scaffold development

- A symbiotic culture of bacteria and yeast (SCOBY) derived from kombucha was used for BC cultivation
- Acrylonitrile butadiene styrene (ABS) was selected for 3D printing due to its widespread use and its solubility in acetone, which does not affect BC
- SCOBY was cultured on a sacrificial template pre-treated with hydroxypropyl cellulose and washed afterward. This treatment resulted in enhanced BC ingrowth
- Gradient sacrificial scaffolds with varying pore sizes were used to determine which pore sizes support BC ingrowth (Fig. 2)



Fig. 2*. Gradient sacrificial scaffold 3D printed using acrylonitrile butadiene styrene (ABS) polymer.*

- The results showed that pore sizes below 50 μm were too small to allow effective BC ingrowth (Fig. 3)
- Therefore, these smaller pore sizes will be excluded in further experiments



Fig. 3*. Gradient sacrificial scaffold 3D printed using acrylonitrile butadiene styrene (ABS) polymer.*

Scaffold characterization

- The surface morphology of the produced BC was analyzed using scanning electron microscopy (SEM), confirming its characteristic nanostructured architecture (Fig. 4).

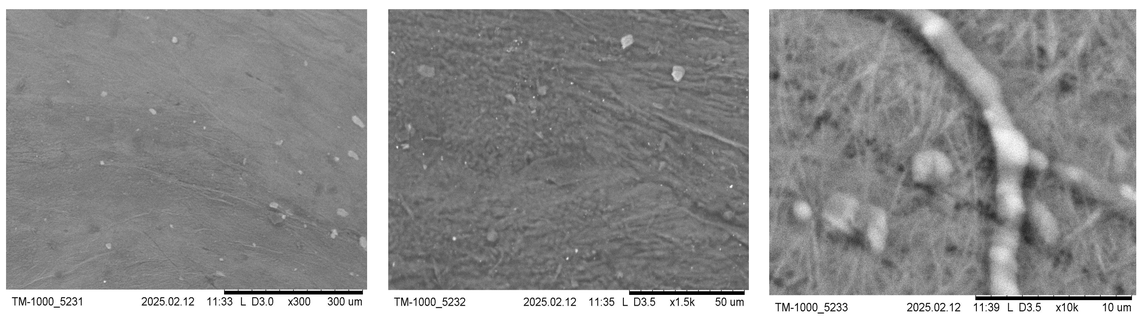


Fig. 4. *SEM images of the macromolecular structure of bacterial cellulose.*

- The BC scaffolds exhibited a water absorption capacity (WAC) of 2.99 ± 0.48 g/g and a water retention capacity (WRC) of 1.78 ± 0.44 g/g. This indicates high water uptake and effective retention, which are favorable for tissue engineering applications (Table 1.)

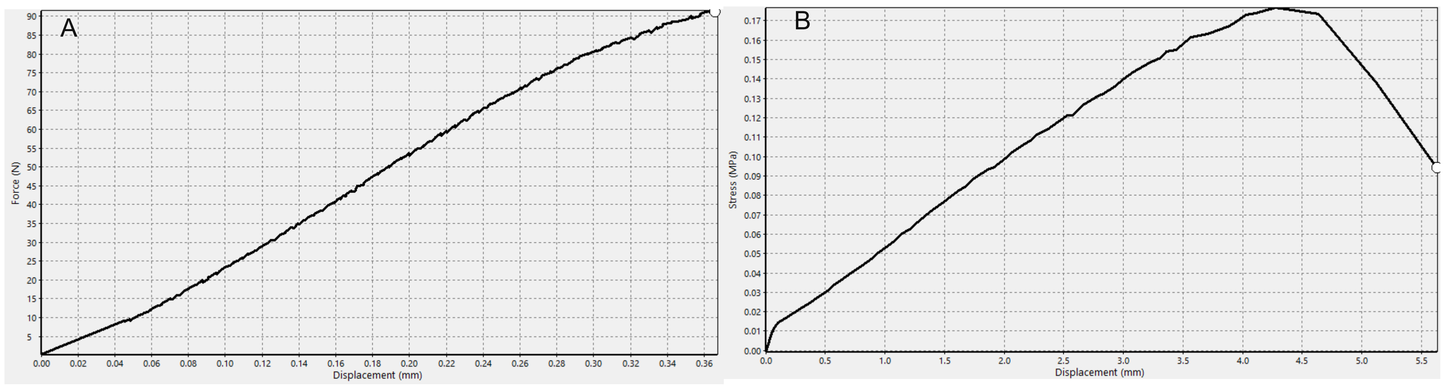
Table 1. *W*ater absorption and retention capacity

	WAC (g/g)	WRC (g/g)
Mean	2.99	1.78
SD	0.48	0.44

**n=5*

- Mechanical testing revealed distinct behavior of bacterial cellulose depending on its physical state
  - Dried BC scaffolds exhibited high tensile strength and stiffness, with moderate elongation at break, indicating a rigid and structurally stable material (Fig.5, A and C)
  - In contrast, never-dried (native) BC showed low tensile strength and stiffness but high elongation, reflecting a soft and highly deformable network (Fig.5, B and C)
- The measured mechanical properties fall within the range reported for bacterial cellulose, confirming the expected material behavior
- These results demonstrate that the mechanical properties of BC scaffolds are strongly dependent on their structural state, highlighting the importance of processing conditions for tailoring material performance.

Fig. 5*.* Mechanical characterization of BC scaffolds: (A) dried BC, (B) native BC, (C) summarized tensile properties.



C

Property	DRY BC	Native BC
Ultimate tensile strength (MPa)	57.0 ± 5.6	0.15 ± 0.03
Young's modulus (GPa)	4.31 ± 1.01	1.57 ± 0.61
Elongation at break (%)	26.5 ± 1.6	53.0 ± 5.0

**n=3*

Conclusions

Altogether, this work demonstrates a reproducible strategy for fabricating architecturally defined BC scaffolds, highlighting their potential for applications in regenerative medicine. The results suggest that pore size is an important factor in scaffold design, as smaller pores appeared to limit BC ingrowth and were therefore not considered for further development. Scaffold characterization confirmed a nanofibrillar structure with high water absorption and retention, indicating a porous and hydrophilic network. Mechanical properties depended on the structural state, with dried BC showing higher strength and stiffness, and native BC greater flexibility, consistent with literature.

For comments and suggestions please contact to emilija.baltrukonyte@gmc.vu.lt