Technology Offer

Symbiotic Co-Culture for Static 3D Growth of Bacterial Cellulose-Based Living Materials

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Abstract

This invention introduces a novel co-culture system between Gluconacetobacter hansenii and Chlamydomonas reinhardtii, enabling static incubation of bulk bacterial cellulose (BC) with complex, tunable 3D geometries. Traditional BC production is limited to thin pellicles formed at the air-liquid interface due to oxygen constraints. This technology harnesses the oxygen-generating capacity of microalgae, which remain suspended in the medium through the use of bacterial cellulose nanocrystals (BCNCs). These nanocrystals prevent algal sedimentation and enable uniform oxygen distribution, thereby promoting homogeneous BC growth within the liquid medium. The resulting living material integrates viable microalgae and bacterial cells into a fibrous, robust matrix with self-healing, revivability, and scalability characteristics - ideal for developing engineered living materials and sustainable biocomposites.

Background

Conventional methods of bacterial cellulose production yield planar films due to oxygen limitation at the air-liquid interface. Though methods such as shaking incubation and aerosol spraying can increase oxygen diffusion, they often compromise the integrity, scalability, and shape flexibility of the material. Embedding living cells into BC matrices for engineered living materials is hindered by the difficulty of maintaining high cell viability alongside robust mechanical properties. Furthermore, hydrogel-based approaches struggle with balancing mechanical strength and biological functionality. There is a need for a scalable, energy-efficient, and biologically harmonious strategy to fabricate 3D BC-based materials that maintain structural and biological performance.

Technology

This invention presents a biomanufacturing strategy that uses an artificial symbiotic system between *Gluconacetobacter hansenii* and *Chlamydomonas reinhardtii* to enable volumetric, static production of bacterial cellulose (BC) with embedded microalgae. Typically, BC grows only as thin films at the air-liquid interface due to oxygen limitations. Here, BC-derived nanocrystals (BCNCs) are introduced into the co-culture medium, preventing sedimentation of microalgae by stabilizing them in suspension. These suspended algae continuously generate oxygen, which is essential for the biosynthesis of cellulose fibers by *G. hansenii*. The microbial cells are distributed uniformly within the matrix, creating a robust, living bio-composite.

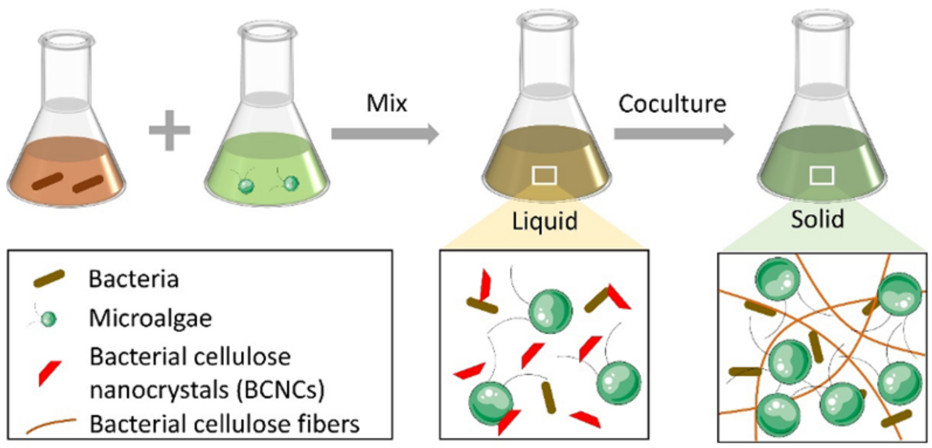

Figure 1: Schematic of Coculture Strategy for Composite Formation. Bacteria, microalgae, and BCNCs are mixed to form a liquid suspension, which transitions into a solid fibrous composite via static coculture.

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The process is optimized with 0.03 w/v% BCNCs, a 40:60 volume ratio of microalgal (TAP) to bacterial (HS) medium, and 0.3 w/v% NaOH to adjust pH for microalgae viability. Under these conditions, the composite achieves a wet thickness of ~4.0 mm and microalgae viability of ~83% after 4 days of static incubation. The final material possesses self-healing capability and retains high mechanical integrity even after drying, enabling its use in load-bearing or functional environments. Importantly, the material geometry is fully determined by the culture vessel, allowing programmable 3D shaping without complex fabrication tools - demonstrating a scalable, sustainable approach to engineered living material production.

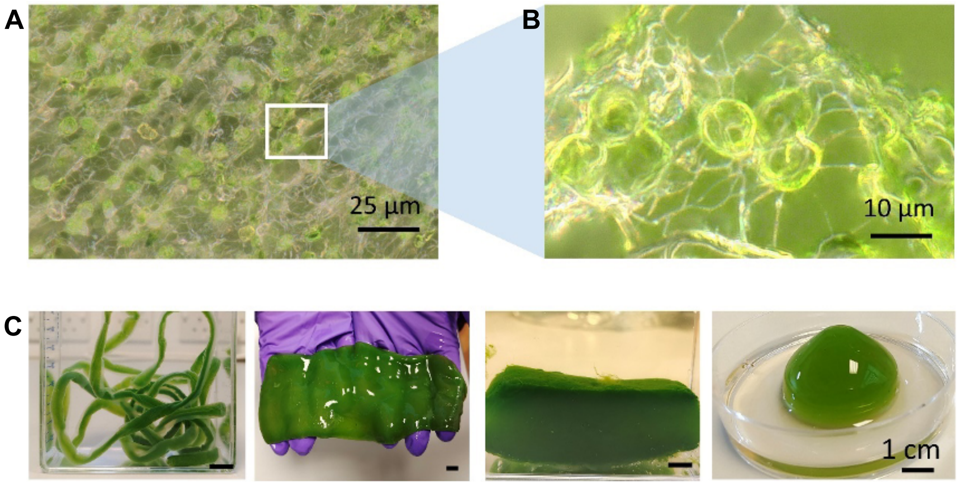

Figure 2: Structure and Morphology of Bacterial Cellulose–Microalgae Composites. **(A–B)** Optical microscopy images showing microalgae encapsulated within the fibrous bacterial cellulose network. **(C)** Macroscopic images of bio-composites with diverse 3D shapes, demonstrating geometric tunability and structural integrity.

Advantages

- **Volumetric Growth Under Static Conditions**: Enables production of thick bacterial cellulose structures without agitation, overcoming diffusion limitations.
- **Integrated Biological Functionality**: Microalgae remain viable (~83%) within the matrix, continuously producing oxygen and supporting co-culture health.
- **Template-Free 3D Shaping**: Material conforms to the shape of any container, allowing customizable geometries without molds or scaffolding.
- **Self-Healing and Revivability**: Damaged composites can regenerate upon re-incubation; biological activity can resume after dry storage.
- **Mechanical Robustness with Biological Integration**: Offers tensile strength and flexibility comparable to native BC, even with embedded cells.

Potential applications

- **Engineered Living Materials**: For building responsive, self-sustaining bio-interfaces or smart bio-hybrid systems.
- **Tissue Engineering**: As oxygenating, biocompatible scaffolds for regenerative medicine and organoid culture.
- **Photobioreactors**: Compact systems with immobilized algae for bioenergy, CO_2 capture, or high-value metabolite production.
- **Sustainable Packaging and Textiles**: Functional biocomposites with inherent biological properties.
- **Space Biomanufacturing**: Lightweight, regenerative materials for extraterrestrial habitats and life-support systems.

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