Bioreactor Design and Bacterial Cellulose Synthesis in Blood Vessel Shape

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Fundamentals and Objectives

Bacteria like *Komagataeibacter* are able to synthesise cellulose [1]. Bacterial cellulose (BC) is a bio-based material suited for diverse applications such as wound dressings, prosthetics, vegan sausage casing or clothes. It is biocompatible, can grow in any shape, has a high-water holding capacity and high tear resistance [2]. These properties strongly motivate the development of cellulose-based materials for medical applications. Multiple publications deal with the production of BC in shapes of human organ parts e.g., cardiac valves or blood vessels. The aim of this work was to design a bioreactor and produce tube-shaped BC for instance for transplant purposes in the future.

Material and Methods

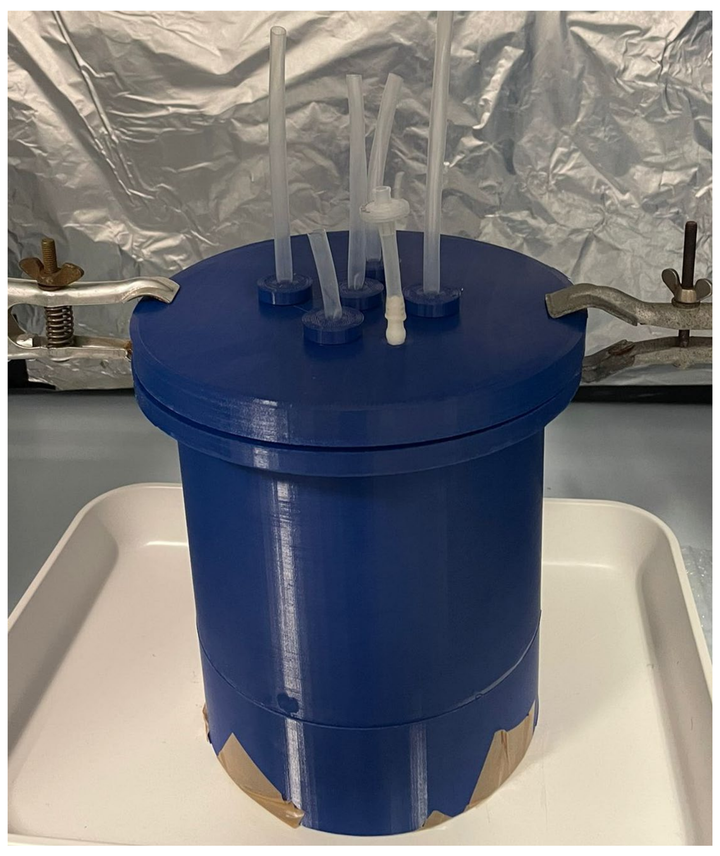

Fig. 1: 3D-printed bioreactor

Bacteria like *Komagataeibacter rhaeticus* produce cellulose to create an advantage regarding their oxygen uptake. The bacterial cellulose helps them float and stay on the surface of the media. To create this interface in the shape of tubes, oxygen permeable silicone tubes were used. The inside of the tubes is filled with air and the outside is located inside the culture broth.

A small bioreactor was designed using Shapr3D-software as well as 3D-printed in PETG (Fig. 1) for the production of BC-tubes of a maximum length of 160 mm in five replicates. The silicon tubes were attached at the top and at the bottom of the reactor. The bioreactor was semi-sterilized by spraying it with 70% ethanol. *Komagataeibacter rhaeticus* was cultivated statically for seven days at 29 °C in standard Hestrin-Schramm media.

Results

The model of the designed bioreactor is shown in Fig. 2. A slight contamination was detected on day 7 (Fig. 3). Results demonstrated that three out of five silicone tubes showed encasing BC-synthesis on their surfaces (Fig. 4, 5).

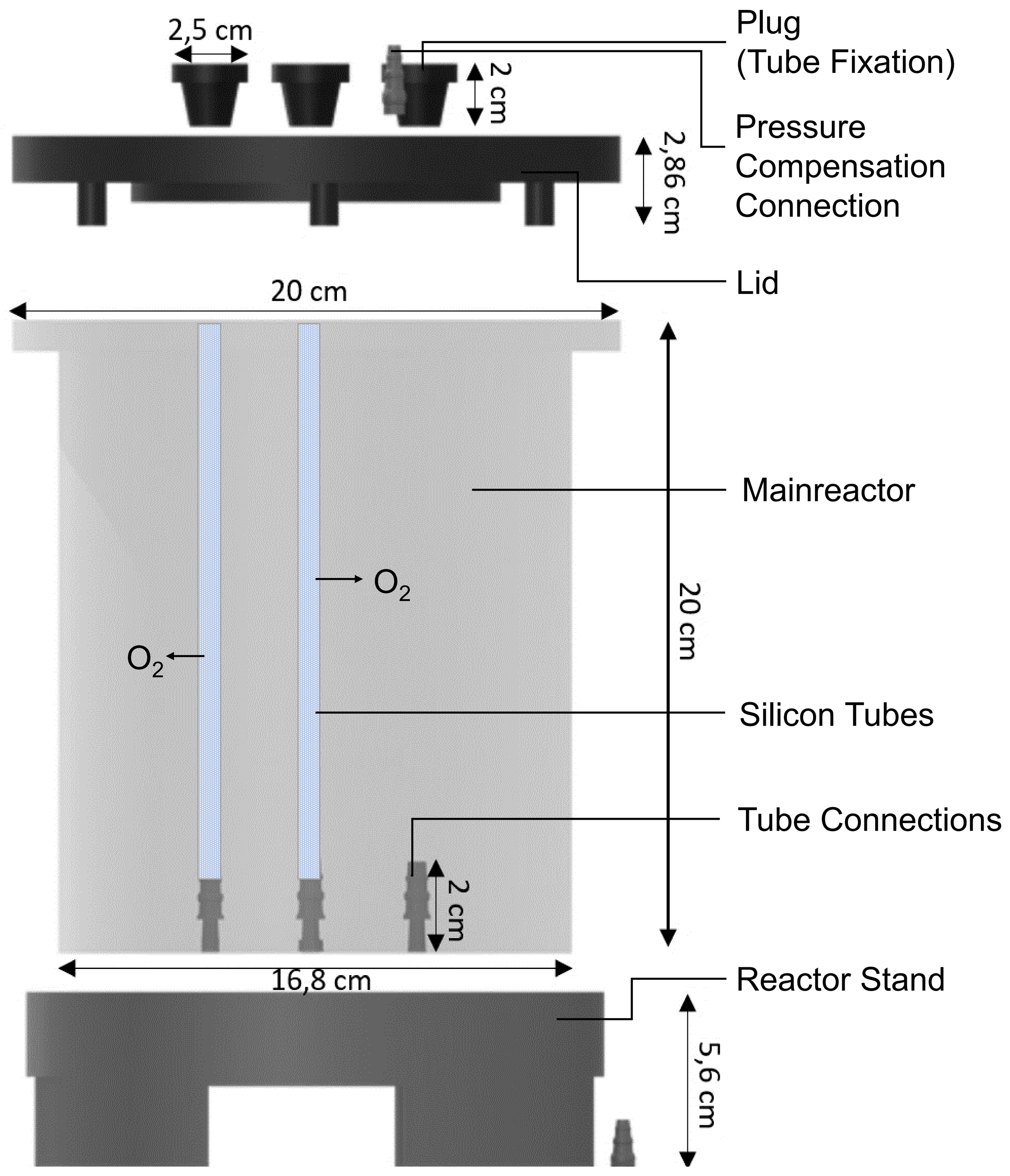

Fig. 2: Bioreactor Modell

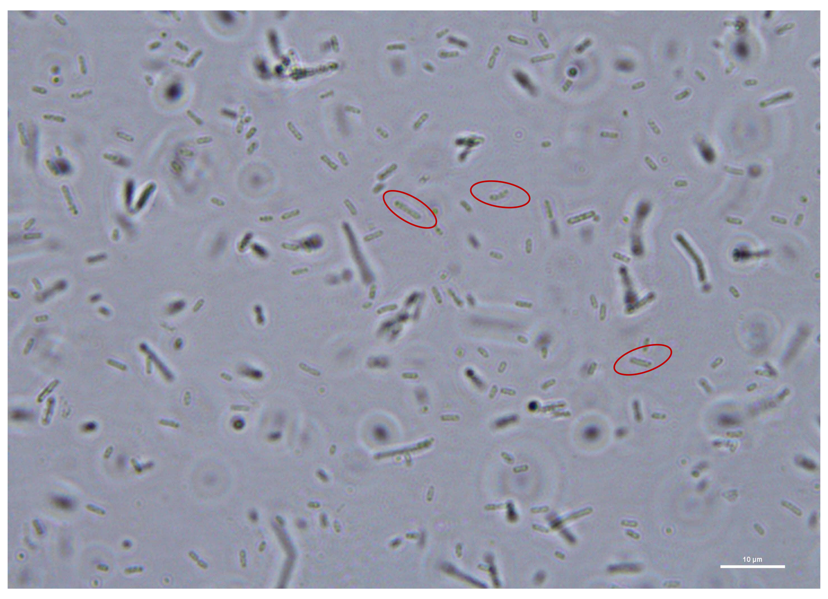

Fig. 3: Microscopic record of the cultur on day 7, *K. rhaeticus* is marked red– 1.000 x -Magn.

Fig. 4: BC on Silicon Tube

Fig. 5: BC Tubes

The biomass concentration reached 0.907 g L^{-1} on day seven, the average yield of destilled water washed BC was determined with 0.214 $g_{dry\ BC}\ L^{-1}$ and the water content of the cellulose was determined with 98,69 $\pm$ 0,16 % for all 3 tubes produced. Electron microscopic images clearly showed that the bacteria were incorporated in and connected by the BC (Fig. 6, 7).

However, the cellulose was too thin to measure it's tear resistance (Fig. 7). The detection, though in low abundance, of larger bacteria confirms a contamination.

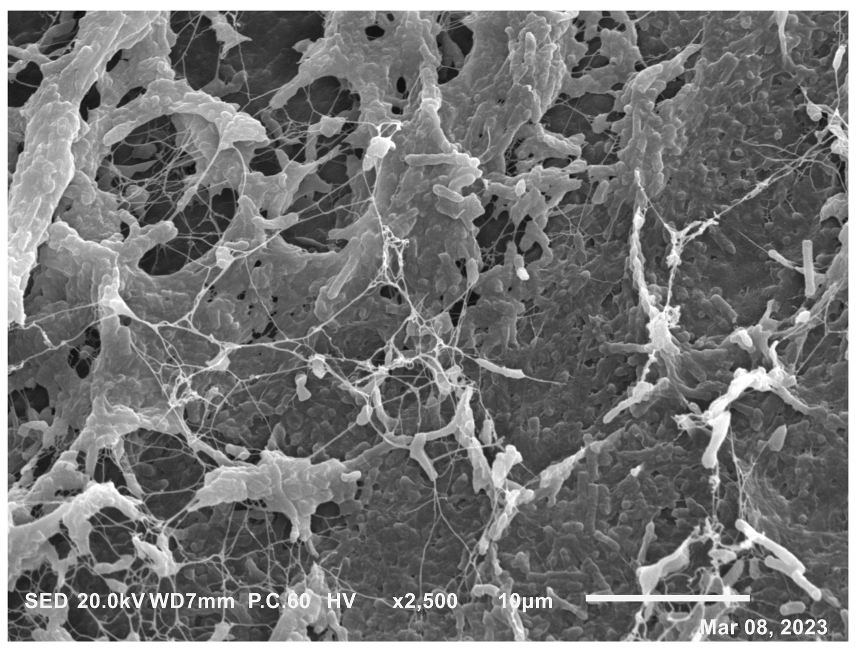

Fig. 6: Electronmicroscopic record of BC – 1.500x Magn.

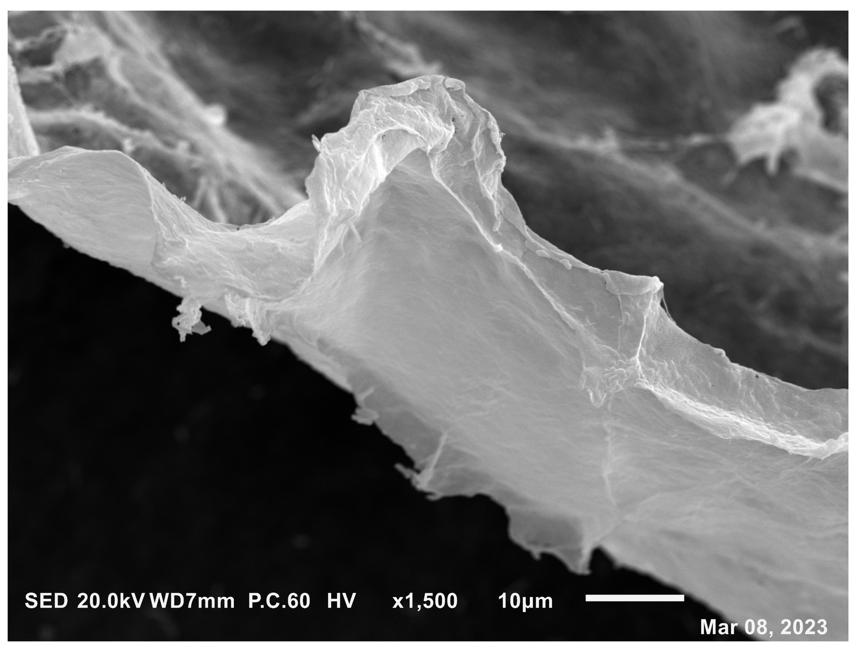

Fig. 7: Electronmicroscopic record of an overlapping region – 2.500x Magn.

Discussion and Outlook

These results demonstrate that the designed bioreactor is suitable for synthesizing BC-based tubes. However, data clearly indicate that the process and bioreactor need optimization in particular with respect to oxygen transfer and sampling options throughout the cultivation. Furthermore to prevent contamination and hence a better BC-production or thicker BC-layers, the decontamination process of the bioreactor needs to be improved using for example UV or gamma radiation and/or production of a new reactor using temperature-resistant material such as stainless steel or Inconel. A new way for sampling should be developed to identify and monitor process-influencing parameters. The BC-washing process should be optimized through the use of NaOH to remove the incorporated bacteria from the BC structure. Overall, the formed BC tubes need to be significantly stronger to find application as blood vessel substitutes, which is why future investigations of wall thickness, elasticity and tensile strength are planned.

Literature

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