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In vivo application of tissue-engineered blood vessels of bacterial cellulose as small arterial substitutes: proof of concept?

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ABSTRACT

Background: Tissue-engineered blood vessels (TEBVs) represent an innovative approach for overcoming reconstructive problems associated with vascular diseases by providing small-caliber vascular grafts. This study aimed to evaluate a novel biomaterial of bacterially synthesized cellulose (BC) as a potential scaffold for small-diameter TEBV.

Methods: Small-diameter blood vessels with a supramolecular fiber network structure consisting of tubular hydrogels from biodesigned cellulose were created using Gluconacetobacter strains and Matrix reservoir technology. BC tubes (length: 100 mm, inner diameter: 4.0–5.0 mm) were applied to replace the carotid arteries of 10 sheep for a period of 3 mo to gain further insights into (a) functional (*in vivo*) performance, (b) ability of providing a scaffold for the neoformation of a vascular wall and (c) their proinflammatory potential, and the (d) technical feasibility of the procedure.

Results: Preoperative analysis revealed a bursting strength of the grafts of approximately 800 mm Hg and suture retention strength of 4–5 N. Postexplantation analysis showed a patency rate of 50% ($n = 5$) and physiological performance of the patent grafts at 4, 8, and 12 wk postoperatively, compared with native arteries. Histologic analysis revealed a neoformation of a vascular wall–like structure along the BC scaffold consisting of immigrated vascular smooth muscle cells and a homogeneous endothelialization of the inner graft surface without signs of prothrombogenic or inflammatory potential. Scanning electron microscopy revealed a confluent luminal endothelial cell layer and the immigration of vascular smooth muscle cells into the BC matrix.

Conclusions: BC grafts provide a scaffold for the neoformation of a three-layered vascular wall exhibit attractive properties for their use in future TEBV programs for cardiovascular surgery.

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1. Introduction

Atherosclerotic vascular disease is still the major cause of mortality in Western societies [1]. Every year more than 600.000 surgical procedures such as coronary artery bypass grafts are performed worldwide using small-diameter vascular grafts with a diameter <6 mm. Although autologous vessels remain the standard for small-diameter vascular grafts, many patients do not have vessels suitable for use because of vascular disease, amputation, or previous harvest. Moreover, this method requires an additional surgical procedure during surgery [2]. Despite the initial success by Matsumoto *et al.* [3] using expanded polytetrafluoroethylene for small caliber arterial bypass, further investigations revealed clinically poor medium- to long-term patency of synthetic grafts (expanded polytetrafluoroethylene, Dacron), when used in small-diameter vascular surgery [4]. Tissue engineering has emerged as a promising approach to address the shortcomings of current options. As an interdisciplinary field, it applies the principles of engineering life sciences and medical research to the development of biological substitutes to restore, maintain, or improve tissue functions [5]. Our research group focuses on the investigation of bacterial cellulose (BC) as a potential scaffold for the application as small-diameter vascular grafts. BC is synthesized extracellularly in form of nanosized fibrils by the bacterium *Acetobacter xylinum* [6]. Because of the hydrogen bonds of the hydroxy groups holding the cellulose chains in place, BC presents a high degree of crystallinity, low solubility, and poor degradation of cellulose *in vivo* [7]. BC has been characterized as a biomaterial with high mechanical strength and enormous water retention values [8–10]. After having analyzed the *in vivo* performance of BC grafts in a rat model, preliminary *in vivo* results in large animals of our group in a first feasibility study revealed a 3-mo patency rate of 87.5% after unilateral implantation of BC grafts with a length of 10 mm without any signs of acute thrombosis or stenosis [10,11]. With regard to these promising results, we aimed to create grafts fulfilling the requirements for a potential clinical use in a high pressure circulatory system, especially in terms of expanded length and adequate suture retention and bursting strength. We aimed to analyze the *in vivo* performance of these grafts with regard to (a) technical feasibility, (b) functional performance (c) the ability of providing a scaffold for the neoformation of a vascular wall, and (d) their proinflammatory potential.

2. Materials and methods

2.1. Graft formation

The formation of BC tubes used in this study was obtained by the cultivation of *Gluconacetobacter xylinus* strains (DSMZ 14666) of the culture collection controlled by the Polymet Jena Association (Polymet Jena e.V., Jena, Germany) and deposited by the German Resource Centre for Biological Material (DSMZ, Braunschweig, Germany). The biosynthesis of BC grafts was performed in a standard Hestrin-Schramm culture medium, pH 5.0 [12] using matrix technology: the sterile culture medium is inoculated with the cellulose generating bacteria. Afterward, the bacteria are cultivated in a special matrix apparatus as previously described [10,11]. Using this construction, one BC graft could be produced over a cultivation period of 7 d. As the analysis of the water absorption capacity for our grafts was between 100 and 120 times more than its dry weight, the BC grafts consist of 1% cellulose providing a nanofibril network and 99% water. For our experiments, grafts with a length of 100 mm, an internal diameter of 3.0–4.0 mm and a wall thickness of 2–3.5 mm have been created. BC grafts were purified by boiling in 0.1 M NaOH at 60°C for 4 h. Afterward, the material was steam sterilized (1 bar, 120°C) for 20 min and stored in 0.9% NaCl until its implantation. The grafts were not treated with any additional chemical or pharmacological substance.

2.2. Measurement of bursting strength and suture retention strength

Suture retention strength of the tissue-engineered vascular grafts was measured using a mechanical tester and 6-0 prolene sutures (Ethicon Endo-Surgery Europe GmbH, Dülmen, Germany). The testing was carried out at room temperature. Specimens with similar dimensions as the implanted grafts and a single suture (set at 6 mm distance from the specimen's edge) were measured, and tensile forth was applied until complete rupture. The bursting strength was measured by cannulation of the vascular constructs and pressurization with 0.9% NaCl. Hydrostatic pressure was started at 200 mm Hg and increased by 10 mm Hg steps until vessel failure or rupture.

2.3. In vivo *experiments*

The experimental study has been approved and supervised by the local animal welfare commissioner according to German Animal Welfare Law. Ten female sheep (Texel sheep), aged 6–7 y with a body weight of 46–50 kg received preoperative humane care in compliance with the guidelines of the European Convention on Animal Care. The sheep were clinically healthy and received an anthelmintic treatment 3 wk before surgery. The animals were housed in a group and had *ad libitum* access to hay and water. Twenty-four hours before surgery, the animals received carprofen (2 mg/kg body weight (bwt), subcutaneously) to ensure preemptive analgesia. Before surgery, the sheep were sedated with xylazine (0.02–0.1 mg/kg bwt, intramuscularly). Thereafter, they where anesthetized with an intravenous injection (V. cephalica antebrachii) of propofol 2% (Disoprivan 2%, Emulsion; Astra Zeneca, Germany) and bolus injection of buprenorhine (0.005–0.0.1 mg/kg bwt, intravenously, Temgesic; Schering-Plough, Kenilworth, NJ). After endotracheal intubation, mechanical ventilation (Fabius; Dräger, Germany) was performed with a 0.5 FiO_2 to maintain a pCO_2 between 30 and 50 mm Hg and a pO_2 above 100 mm Hg. Maintenance of anesthesia was obtained *via* admixing 1.5%–2% isoflurane to the air or O_2 flow. In addition, a stomach tube was administered to avoid ruminal tympanites. Effectiveness of anesthesia was monitored by testing the inter-claws-reflex of the animals.