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Decellularized human placenta chorion matrix as a favorable source of small-diameter vascular grafts

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ABSTRACT

Biomaterials based on decellularized tissues are increasingly attracting attention as functional alternatives to other natural or synthetic materials. However, a source of non-cadaver human allograft material would be favorable. Here we establish a decellularization method of vascular tissue from cryopreserved human placenta chorionic plate starting with an initial freeze–thaw step followed by a series of chemical treatments applied with a custom-made perfusion system. This novel pulsatile perfusion set-up enabled us to successfully decellularize the vascular tissue with lower concentrations of chemicals and shorter exposure times compared to a non-perfusion process. The decellularization procedure described here lead to the preservation of the native extracellular matrix architecture and the removal of cells. Quantitative analysis revealed no significant changes in collagen content and a retained glycosaminoglycan content of approximately 29%. In strain-to-failure tests, the decellularized grafts showed similar mechanical behavior compared to native controls. In addition, the mechanical values for ultimate tensile strength and stiffness were in an acceptable range for *in vivo* applications. Furthermore, biocompatibility of the decellularized tissue and its recellularizationability to serve as an adequate substratum for upcoming recellularization strategies using primary human umbilical vein endothelial cells (HUVECs) was demonstrated. HUVECs cultured on the decellularized placenta vessel matrix performed endothelialization and maintained phenotypical characteristics and cell specific expression patterns. Overall, the decellularized human placenta vessels can be a versatile tool for experimental studies on vascularization and as potent graft material for future *in vivo* applications.

Statement of significance

In the US alone more than 1 million vascular grafts are needed in clinical practice every year. Despite severe disadvantages, such as donor site morbidity, autologous grafting from the patient's own arteries or veins is regarded as the gold standard for vascular tissue repair. Besides, strategies based on synthetic or natural materials have shown limited success. Tissue engineering approaches based on decellularized tissues are regarded as a promising alternative to clinically used treatments to overcome the observed limitations. However, a source for supply of non-cadaver human allograft material would be favorable. Here, we established a decellularization method of vascular tissue from the human placenta chorionic plate, a suitable human tissue source of consistent quality. The decellularized human placenta vessels can be a potent graft material for future *in vivo* applications and furthermore might be a versatile tool for experimental studies on vascularization.

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1. Introduction

In the US alone more than 1 million vascular grafts are needed in clinical practice every year [1]. Currently, autologous grafting from the patient's own arteries or veins is regarded as the gold standard for vascular tissue repair. However, this method has several disadvantages as it requires multiple surgical procedures including procedures on healthy tissue [2]. In addition, in almost 30% of the cases, the necessary amounts of autologous material may not be available [3,4]. As an alternative, vascular prostheses based on synthetic polymers such as polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE) are used in clinics. In particular, small-caliber grafts with an inner diameter below 5 mm have limited success due to reported adverse healing reactions, including surface thrombogenicity [5,6].

Biomaterials based on decellularized tissues are increasingly attracting attention as a functional alternative to other natural or synthetic materials [7,8] because these decellularized materials are naturally derived and inherently support various cell functions. For instance, proteins of the extracellular matrix and the arrangement of tissue fibers have been proven to support cell migration and proliferation, including gene expression and cell–cell interactions [9–11]. Despite these obvious advantages, tissue engineering approaches using decellularized material are often limited due to batch-to-batch variations of the accessible allograft raw material [12]. Furthermore, the less favorable use of xenografts may elicit significant immune response in the recipient due to differences in the primary structure of the residual proteins in the ECM [13,14]. We hypothesize that the vasculature of the human placenta, in particular the placental chorionic plate, has great potential as a material source for human vascular allografts for a wide range of inner diameter sizes. Human placenta, an organ which develops jointly from the mother and the baby during pregnancy, is generally discarded after birth. According to the U.S. census figures from 2013, there were more than 3.9 million births in the United States [15]. Therefore, the placenta is likely to be the most easily accessible raw material of human tissue with consistent quality and without additional harm to the donor [16].

In this study, we established a method to obtain efficiently decellularized small-diameter (<4 mm) vessel grafts isolated from the human placental chorionic plate. The decellularization process, in general a combination of mechanical, chemical and enzymatic approaches, has to be accompanied by adequate analytics [17,18] to guarantee the effectiveness of the process and thus the characteristics of the final structure. Consequently, the removal of cells and cellular debris and the conservation of the decellularized tissue matrix in terms of composition and ultrastructure, were evaluated by histology, scanning electron microscopy analysis and an array of biochemical quantification methods. Moreover, biocompatibility of the decellularized tissue and its ability to serve as an adequate substrate for recellularization strategies using primary endothelial cells were shown. This indicates that this biomaterial could be a versatile tool for experimental studies on vascularization and a potent graft material for future *in vivo* applications.

2. Materials and methods

2.1. Materials

If not indicated otherwise, all reagents were purchased from Sigma–Aldrich (Vienna, Austria) and are of analytical grade. All antibodies were obtained from BD Biosciences (Franklin Lakes, USA). Endothelial cell growth medium and bovine fetal calf serum (FCS) were bought from Lonza (Lonza, Basel, Switzerland).

2.2. Harvesting of human placenta

The ethics committee of Upper Austria gave approval for the use of this material collected by the Red Cross Hospital, Linz and informed consent was obtained from the donors. After the extraction of the placenta during caesarian section births, the placental vasculature was rinsed through the umbilical vein and arteries with Phosphate Buffered Saline solution (PBS) supplemented with additional heparin (50 IU/ml) and antibiotics (1% penicillin/streptomycin). After removal of the blood from the vessels, the whole placenta was frozen at −20 °C until further processing. All donors were serologically tested (HIV, HBV, HCV).

2.3. Preparation and decellularization of human vascular scaffolds from the placenta chorion

Prior to the decellularization of the placenta vessels, the whole placenta was thawed and single vessel parts, with an approximate length of 3–4 cm and with an inner diameter of 2–3 mm, were isolated from the chorionic plate with one end of the harvested vessel connected with a surgical thread to a 14GA BD Venflon™ (BD, Heidelberg) for the following perfusion steps. Then the vessels were subjected to a freeze–thaw step at −80 °C for 18 h and subsequently thawed in PBS solution at room temperature (RT). For the chemical decellularization, the vessels were connected with the Venflon to a custom-made recirculating perfusion system using a peristaltic pump (Minipuls Evolution, Gilson, Middleton) (Fig. 1A). The pulsatile perfusion was measured by a pressure sensor and adjusted to a range of 60–80 mmHg (8000–10,670 Pa). The vessels were perfused with hypertonic (1.2% NaCl) and hypotonic (0.4% NaCl) saline solutions for 30 min each to cause cell lysis by osmotic pressure. Afterwards, the samples were perfused with 1% Triton X-100 (Sigma, Vienna) and 0.02% w/w ethylenediaminetetraacetic acid (EDTA) in PBS for 24 h at room temperature. Before incubation in DNase I-solution (200 IU/ml, Roche) over night at 4 °C, the vessels were thoroughly washed with PBS for 3 h. For chemical sterilization, after several rinsing cycles in sterile PBS, the scaffolds were washed with 0.1% (w/v) peracetic acid (PAA) solution at pH 7.0, as described by Lomas et al. [19], in a 50 mL Falcon tube for 3 h at room temperature on a roller mixer (Type, Firma, Stadt, Land) at 30 rotations per minute (rpm). Finally the vessels were rinsed with 35 ml sterile PBS 3 times for 10 min and stored in sterile PBS at 4 °C before further use.

2.4. Quantification of matrix components, DNA residuals and statistics

Biochemical quantification methods were used to quantify DNA, glycosaminoglycan (GAG), and its collagen contents. All vascular scaffolds specimens were lyophilized, 10 mg dry ECM was weighed and digested with 3 IU/ml papain from *Papaya latex* (Sigma) in the presence of 20 mM L-Cystein (Sigma) in Papain buffer (75 mM NaCl, 27 mM Na Citrate, 0.1 M NaH_2PO_4, 15 mM EDTA, pH 6.0) at 60 °C before analysis. The measured values were normalized to the dry weight of the samples.

2.4.1. DNA quantification

To quantify the residual amounts of DNA on the decellularized scaffolds, Hoechst 33342 (Sigma) was used to quantify the remaining DNA. A Hoechst dye stock (10 mg/ml in dH_2O) was prepared, sterilized by filtration (0.22 μm, Rotilabo, Roth, Karlsruhe) and stored at 4 °C in the dark. Prior to each assay the working dye solutions were freshly prepared and diluted with assay buffer (2 M NaCl, 50 mM NaH_2PO_4, pH 7.4) to a final concentration of 5 μg/ml. Dilutions of DNA sodium salt from calf thymus (Sigma; dissolved in ddH_2O) were used to generate a standard curve. The samples/standards were pipetted in duplicates into 96-well black

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