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Controlled fabrication of triple layered and molecularly defined collagen/elastin vascular grafts resembling the native blood vessel

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ABSTRACT

There is a consistent need for a suitable natural biomaterial to function as an arterial prosthesis in achieving arterial regeneration. Natural grafts are generally obtained by decellularization of native blood vessels, but batch to batch variations may occur and the nature/content of remaining contaminants is generally unknown. In this study we fabricated a molecularly defined natural arterial graft from scratch resembling the native three layered architecture from the fibrillar extracellular matrix components collagen and elastin. Using casting, moulding, freezing and lyophilization techniques, a triple layered construct was prepared consisting of an inner layer of elastin fibres, a middle (porous) film layer of collagen fibrils and an outer scaffold layer of collagen fibrils. The construct was carbodiimide cross-linked and heparinized. Characterization included biochemical/biophysical analyses, scanning electron microscopy, micro-computed tomography, (immuno)histology and haemocompatibility. Burst pressures were up to 400 mm Hg and largely conferred by the intermediate porous collagen film layer. The highly purified type I collagen fibrils and elastin fibres used did not evoke platelet aggregation in vitro. Suturability of the graft in end to side anastomosis was successful and considered adequate for in vivo application.

1. Introduction

Coronary artery and peripheral vascular disease cause high mortality in Western societies [1]. The conventional treatment is surgery to restore blood flow using autologous or synthetic grafts. The autologous vein is the gold standard for lower limb bypass surgery, but 30% of patients lack sufficient material [2]. In these patients synthetic small diameter grafts are used, e.g. formed of extended polytetrafluoroethylene (ePTFE) or polyester (Dacron), but this may result in occlusion due to thrombosis, often manifesting itself within 5 years [3]. One of the approaches used to overcome these problems is cellularization of the conduits [4]. However, the processes of cell harvesting and cell culture are time consuming and costly, which presents a drawback in the cell-based tissue engineering approach and rules out off the shelf availability. Therefore, research is focusing on the improvement of current conduits by the addition of bioactive molecules and on construction of new ones, e.g. those based on natural materials [5]. An example of the first is the coating of ePTFE with heparin to reduce platelet deposition and neointimal hyperplasia [6]. Also, coating of polymers with recombinant human elastin peptides may significantly increase graft patency and decrease fibrin deposition and embolism [7]. Furthermore, elastin-mimetic peptides coated on ePTFE have shown excellent haemocompatible properties as an arteriovenous shunt [8]. Accordingly, grafts completely composed of natural materials are now under investigation [9–11].

Natural grafts are generally obtained by decellularization of native blood vessels [12–14]. However, batch to batch variations may occur as the exact composition of extracellular matrix (ECM) components present within these grafts is unknown. In addition, unidentified remnant components (such as growth factors) may be present in unknown amounts. This problem may be overcome by controlled preparation of grafts using purified ECM molecules. For example, Berglund et al. constructed a blood vessel equivalent based on a type I collagen gel deposited around purified arterial elastin [15].

Type I collagen and elastin are the main ECM molecules present in the arterial wall and they supply the artery with strength and elasticity, respectively [16]. In the typical arterial three layered architecture elastin is found in intimal and medial sites and type I collagen within the media and adventitia. Blueprinting Mother

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Nature, we designed a molecularly defined natural graft composed of type I collagen fibrils and elastin fibres (Fig. 1). These proteins can be obtained in large quantities by applying suitable purification procedures [17,18]. Monolayered collagen–elastin constructs have been prepared [19–23] and their cytocompatibility for blood vessel-specific cells, e.g. smooth muscle cells and endothelial cells, demonstrated [20,23–25]. To match the arterial architecture and mechanical properties more closely, in this study we prepared a triple layered tubular construct using highly purified collagen fibrils and elastin fibres. In addition, we covalently attached heparin to the construct by cross-linking with 1-ethyl-3-dimethyl aminopropyl carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) [26], to increase thromboresistancy [27]. The construct was evaluated with respect to biochemical and mechanical parameters, blood compatibility, location of the components ((immuno)histology), (ultra)structure (scanning electron microscopy and microcomputed tomography) and handling/suturability.

2. Materials and methods

2.1. Materials

Unless stated otherwise, all chemicals were purchased from Merck Chemicals (Darmstadt, Germany).

2.2. Purification of type I collagen fibrils and elastin fibres

2.2.1. Type I collagen fibrils

Insoluble type I collagen fibrils were highly purified from bovine Achilles tendon [18]. Purification consisted of several washing steps of 24 h at 4 °C while shaking (75 rpm, TR-250, Infors HT, Bottmingen, Switzerland), with 0.1 and 1 M NaCl solutions, 4 M urea, 0.5 M acetic acid, acetone and ultrapure water (Biocel A10 grade MilliQ system, Millipore, Billerica, MA). After purification, type I collagen fibrils were lyophilized and stored under vacuum at −20 °C.

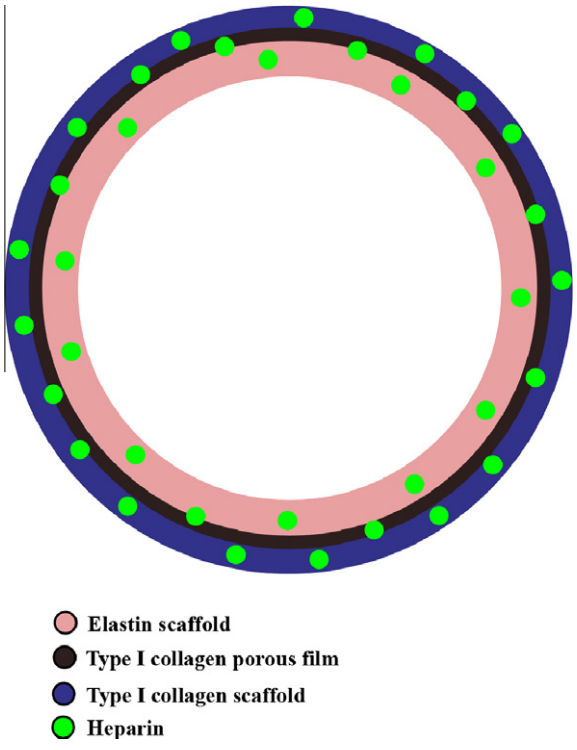

Fig. 1. Schematic representation of the design of an acellular vascular graft constructed from natural materials mimicking the native blood vessel. It contains a luminal layer of elastin fibres, a supportive middle layer of type I collagen porous film and an outer layer of porous type I collagen scaffold to support cellular influx. Heparin may be covalently coupled using EDC/NHS cross-linking to prevent blood coagulation.

2.2.2. Elastin fibres

Elastin fibres were purified from equine ligamentum nuchae [17]. The purification consisted of several washings steps while stirring with 1 M NaCl, the organic solvents ethanol, chloroform/methanol (Labscan, Dublin, Ireland) (2:1), acetone and ether and 8% CNBr in formic acid (under gaseous nitrogen conditions), demineralized water, 4 M urea and 1 M 2-mercaptoethanol, followed by trypsin digestion (10,000 U/g, 4 h, 37 °C). After purification the elastin fibres were stored under wet conditions at −20 °C.

2.3. Construction of triple layered vascular grafts

Homogenization of collagen and elastin suspensions in 0.25 M acetic acid was performed at 4 °C (on ice) with a Potter–Elvehjem device (Louwers Glass and Ceramic Technologies, Hapert, The Netherlands) with an intervening space of 0.35 mm. Generally about 10 strokes were performed to achieve homogenization. Collagen suspensions were deaerated by means of centrifugation at 250*g* for 15 min at 4 °C. Tubular grafts were prepared according to the schematic representation in Fig. 2. In total, over 40 triple layered grafts were fabricated in a controlled manner.

2.3.1. Elastin grafts

Monolayered elastin grafts were prepared by the homogenization and moulding of a 3.2% elastin/type I collagen suspension (98:2, collagen was added as a glue, see Section 3) in 0.25 M acetic acid, after which the suspension was left for at least 5 min to achieve deaeration. The homogenized and deaerated suspension (3 ml) was placed in polystyrene moulds with an inner diameter of 10 mm and with mandrels (Ø 4 mm) of polyether ether ketone (PEEK). After spacer placement (for mandrel fixation), the moulds were frozen in liquid nitrogen and lyophilized (Zirbus, Bad Grund, Germany).

2.3.2. Addition of porous collagen film layer to elastin grafts

A porous collagen film layer was added to increase the mechanical strength of the graft in order to withstand (physiological) pressures. Therefore, after lyophilization, elastin grafts were wetted by dipping in demineralized water and semi-dried around the mandrels for 20–30 min at room temperature. Entrapped air bubbles were removed with a 25 G needle. Then the mandrels with semi-dried elastin grafts were submerged in the moulds in homogenized and deaerated 0.8% type I collagen suspension in 0.25 M acetic acid (2–2.5 ml), frozen for 4 h at −20 °C with a fixed mandrel and lyophilized. The elastin–collagen grafts obtained were wetted in demineralized water and fully dried around the mandrels into tubular films in an oven (Stuart Scientific, Staffordshire, UK) for 16 h at 25 °C.

2.3.3. Addition of porous collagen layer

After drying the mandrels with the fully dried elastin–collagen films were submerged in the moulds in homogenized 0.8% collagen suspension in 0.25 M acetic acid (3 ml). After mandrel fixation, the moulds were incubated for 4 h at 4 °C to facilitate adhesion of the collagen layer. Then the moulds were frozen for 16 h at −20 °C and lyophilized.

2.3.4. EDC/NHS cross-linking and attachment of heparin

After lyophilization the triple layered grafts were cross-linked using EDC and NHS [28]. Grafts were preincubated for 5 min in

ID	Title	Pages
2012	Controlled fabrication of triple layered and molecularly defined collagen/elastin vascular grafts resembling the native blood vessel	9