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Novel method for the generation of tissue-engineered vascular grafts based on a highly compacted fibrin matrix

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ABSTRACT

The generation of tissue-engineered blood vessel substitutes remains an ongoing challenge for cardiovascular tissue engineering. Full biocompatibility and immediate availability have emerged as central issues for clinical use. To address these issues, we developed a technique that allows the generation of highly stable tubular fibrin segments. The process is based on the compaction of fibrin in a custom-made high-speed rotation mold. In an automated process, fibrin is precipitated from plasma by means of the Vivostat® system. Following application to the rotating mold, the fibrin was compacted by centrifugal force and excess fluid was pressed out. This compaction results in increasing cross-links between the fibrin fibrils and a corresponding significant increase of biomechanical stability up to a burst strength of 230 mm of mercury. The molding process allows for a simultaneous seeding procedure. In a first *in vivo* evaluation in a sheep model, segments of the carotid artery were replaced by tissue-engineered vascular grafts, generated immediately prior to implantation ($n = 6$). Following subjection to the body's remodeling mechanisms, the segments showed a high structural similarity to a native artery after explantation at 6 months. Thus, this technique may represent a powerful tool for the generation of biomechanically stable vascular grafts immediately prior to implantation.

Statement of significance

Fibrin has previously been shown to be suitable as a matrix for the seeding of different celltypes and for that reason was widely used as scaffold in different fields of tissue engineering. Nevertheless, fibrin's lack of stability has strongly limited its application. Our study describes a novel moulding technique for the generation of a highly compacted fibrin matrix. Using this approach, it was possible to optimize the engineering process of tubular fibrin segments to provide bioartificial vascular grafts within one hour with sufficient stability for immediate implantation in the arterial system. Thus, this technique may represent a powerful tool to get closer to the ultimate aim of an optimal bioartificial vascular graft.

1. Introduction

The most influential factors which have driven forward the rapid and substantial progress of vascular surgery over the last decades are closely related to the development of synthetic vascular bypass materials namely polyethylenterephthalate (Dacron) in the 1950s and expanded polytetrafluorethylene (ePTFE) in the 1970s. Following the clinical introduction of those bypass materials, appropriate vascular prostheses became readily available off the shelf [1,2]. However, although those materials do not cause immediate harm to the recipient's body, slow and progressive alterations, i.e. (re-)stenosis by intimal hyperplasia as well as thromboembolic and infectious complications, continue to restrict their clinical application to large-caliber vascular replacement (reviewed in [3]). Thus, despite intense research conducted on the development of alternative and improved synthetic bypass materials, the use of autologous vessels, e.g. the radial artery and the greater saphenous vein, are still the method of choice for small-caliber ($\leqslant$5 mm) vessel replacement. Unfortunately, these biological vessels fall short of providing an "optimal"

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solution, because of their limited quantitative availability and occasional poor quality [4].

Therefore, the generation of small-caliber vascular grafts remains a major challenge for vascular tissue engineering (TE). The ideal graft would combine the advantages of both, synthetic and autologous grafts and overcome their respective disadvantages. Over recent years, a number of different approaches have been described which have applied various biological or alloplastic scaffold materials with varying levels of efficacy [5,6]. Here, biocompatibility and immediate availability have emerged in particular as crucial factors for clinical use [7].

Fibrin has been identified as an almost ideal scaffold material, because it enables cellular attachment, proliferation and three-dimensional arrangement. It has a high biocompatibility and can easily be isolated from blood in an autologous approach. One major disadvantage lies in its poor biomechanical stability. Different strategies have been developed to achieve higher mechanical strength, such as *in vitro* maturation processes, the addition of stabilizing polymers or guiding meshes such as polyethylenterephthalate or polycaprolactone [8–12]. However, these methods are either expensive and time-consuming, or associated with an impaired biocompatibility which could potentially induce an immunological reaction against the scaffold.

In our current work, we report on a novel method for the generation of seeded, highly stable fibrin tubes. The basic principle is based on the assumption that centrifugal forces inside a high-speed rotating mold lead to the compaction of fibrin fibrils with an increase of cross-links and a higher biomechanical stability in the resulting fibrin tubes. Additional improvement by the addition of factor XIII and seeding with endothelial cells (LOEC) and smooth muscle cells (LOSMC) was evaluated. Both cell types were differentiated from circulating precursor cells isolated from peripheral blood. In this paper, we report on the technical details of the developed technique and the first results from an initial *in vivo* series in a sheep model, in which a segment of the carotid artery was replaced.

2. Methods

2.1. In vitro experiments

In vitro experiments were carried out to evaluate the influence of centrifugal forces on the structure and compaction of fibrin fibrils and its effect on the biomechanical stability of the resulting fibrin tubes.

2.1.1. Fibrin precipitation

Fibrin was precipitated from fresh frozen plasma concentrates (FFP), which were obtained from the blood bank at Hannover Medical School since those were not suitable for clinical use. A commercial available two-stage system (Vivostat®, Denmark) was used, which enables: (i) the precipitation of a fibrin preparation in an automated process from FFP or whole blood and (ii) the application of the precipitated fibrin preparation. The automated process of the Vivostat®-processor has already been described in detail [13]. In brief: a custom-made cartridge was filled with 100 ml plasma and placed in the processor unit. During centrifugation biotinylated batroxobin was added. Batroxobin, snake venom produced by a species of viper, acts in a way similar to thrombin by cleavage of fibrinopeptide A from fibrinogen. The resulting acid-soluble fibrin I-oligomer accumulated on the wall of the cartridge. After removal of the remaining plasma, the fibrin-I oligomer was dissolved in 3.5 ml sodium acetate (0.2 M, pH4) and collected in a syringe connected to the top of the cartridge. The acidic pH of the precipitation prevents premature clotting. The concentration of the fibrin I-oligomer was determined from the optical absorbance of the precipitate and automatically adjusted to a desired concentration of 20 mg/mL.

Fibrin tubes were engineered by simultaneous application of the fibrin precipitation with an activating carbonate/bicarbonate buffer (pH10) (Fig. 1B) in a ratio 7:1 in a high speed rotating casting mold. The mixture of both solutions neutralizes pH-value, so that endogenous prothrombin was activated to thrombin, starting the polymerization of the precipitated fibrin I-oligomer to fibrin. Additionally, thrombin activated endogenous factor XIII catalyzing cross-linking in the resulting fibrin [14].

2.1.2. Technical composition of the manufacturing set up and generation of fibrin tubes

The high speed rotating casting mold was driven by an electric motor and consisted of an outer brass tube (Fig. 1C-4 and C-5) with two removable Teflon® half shells inside (Fig. 1D-9), enclosing a 100 mm long and 7 mm wide central cavity. Three pairs of drill holes (diameter 0.3 mm) within the brass tube allowed for the drainage of excess fluid. Teflon® cuffs (Fig. 1D-10) narrowed the lumen at both ends, preventing fibrin loss during the manufacturing process. Drill holes within these cuffs (diameter 4 mm at the proximal and 3 mm at the distal end) allowed for the insertion of the applicator lancet and air vent at the distal tail.

The Vivostat®-application pen (Fig. 1C-6) was placed in a lancet and mounted on a mobile slide driven by a separate electric motor (Fig. 1C–7), which allowed for its adjustable movement. The application pen was placed within the rotating mold and pulled out at a defined speed during the application of the fibrin precipitate. The application pen itself contained four separate channels for: (i) the application of the fibrin precipitation, (ii) the application of the pH10-solution, (iii) a co-delivery channel for the application of factor XIII and/or a cell suspension, and (iv) an air supply to transport/spray the applied solutions. Both the mold and the lancet were autoclaved prior to use to guarantee sterility.

Three experimental groups were defined (for details see Table 1): In *group-1* fibrin tubes were generated without other additives at five different rotation velocities (ranging from 1000 to 15,000 rpm). The g-force resulting from the rotation velocity was calculated by the formula: centrifugal force (g) = $1119 \times 10^{-5} \times r \times \mathrm{rpm}^2$ (r = radius of the mold in cm) [15].

In *group-2* fibrin tubes were generated with addition of 300 units of coagulation factor XIII (Fibrogammin, CSL Behring, Germany) to evaluate its potential extra stabilizing effect on the fibrin. Factor XIII was suspended in 5 ml calcium chloride-solution (CaCl, 0.04 M) and applied via the separate co-delivery channel.

In *group-3* fibrin tubes were generated with addition of factor XIII and additional seeding with LOSMC and LOEC. In detail, LOSMC (1×10^6 cells) were suspended together with 300 units factor XIII in 5 ml CaCl-solution (0.04 M) and added via the co-delivery channel. Afterwards, LOEC (6×10^5 cells) suspended in 1 ml CaCl-solution were sprayed on the luminal surface during rotation of the mold. Rotation for a further 20 min followed to achieve LOEC attachment to the luminal surface.

For comparative analysis, fibrin matrices ($n = 5$) were generated under static conditions by filling 1.2 ml of the fibrin precipitation with pH10-solution (ratio 7:1) in each chamber of a chamber slide (Nunc, Denmark).

2.1.3. Isolation and expansion of late outgrowth endothelial and smooth muscle cells

Both LOEC and LOSMC were isolated and differentiated from the monocyte fraction of ovine blood samples taken prior to these experiments as previously described [16]. In brief, blood was taken under sterile conditions from sheep's jugular vein (black-headed