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Vascular tissue engineering

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Cardiovascular disease is one of the main causes of death in the industrialized society. In 2009, 87.534 and 17.583 patients were hospitalized in the Netherlands for treatment of ischemic heart disease and peripheral arterial disease, respectively. In both patient populations, the mortality rates are approximately 12 % (1). For the treatment of cardiovascular disease, functional vascular grafts with an inner diameter less than 6 mm are needed. The availability and quality of autologous bypass grafts (e.g. mammary artery, saphenous vein) is frequently limited, especially in elderly patients. Although synthetic vascular grafts made from the polymers Dacron or Teflon perform reasonably well in large-diameter applications, these prostheses fail in small-diameter reconstructions due to thrombus formation and intima hyperplasia, hampering successful treatment of these patients.

Vascular tissue engineering

As shown during the last 10-15 years, tissue engineering is a promising technique to prepare functional small-diameter arterial grafts. Vascular smooth muscle cells (SMCs) of the patient are cultured and subsequently seeded in biodegradable porous tubular scaffolds. The resulting constructs are mounted in a pulsatile flow bioreactor and perfused for a period up to several weeks, during which the medial layer of the graft develops. Subsequently, autologous endothelial cells (ECs) are seeded on the luminal surface, after which the grafts are perfused for an additional period of several days (2). In alternative procedures, autologous stem cells have been used (e.g. mesenchymal stem cells from bone marrow). Moreover, for low pressure reconstructions such as the pulmonary artery, constructs have been implanted immediately after mesenchymal stem cell seeding (3).

The ideal scaffold for vascular tissue engineering is biocompatible, flexible, elastic and biodegradable. To facilitate formation of the medial layer of the graft, the pore structure should be interconnected to provide a three-dimensional space for adhesion, proliferation and differentiation of the cells, and to allow diffusion of nutrients and metabolic waste products. Moreover, the scaffold should maintain suitable mechanical properties until maturation of the newly formed vascular tissue (4, 5). The pulsatile flow bioreactor has to mimic the blood circulation in terms of flow rate, pressure and pulse frequency. The constructs are perfused with culture media optimized for the culturing of SMCs and ECs, respectively. In the case of stem cell seeding, the culture media can be supplemented with appropriate differentiation factors.

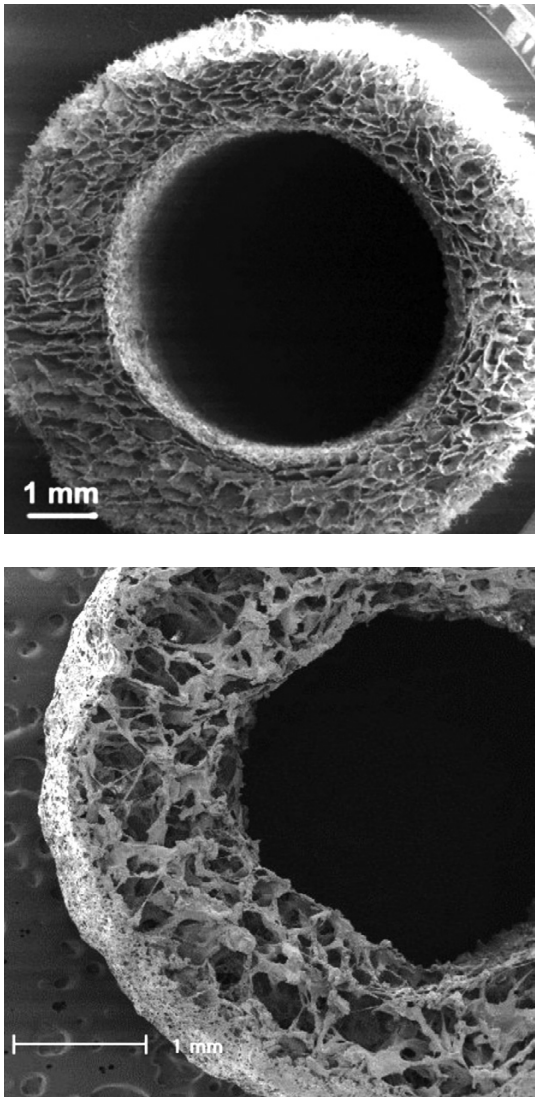

Figure 1. Scanning electron microscopy images of a collagen/elastin scaffold (top) and a PTMC scaffold (bottom).

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Approximately 10 years ago, we started with vascular tissue engineering using scaffolds prepared from collagen and elastin (6-8). These proteins are key components of the extracellular matrix in the natural vessel wall. Suspensions of collagen and elastin (1:1 w/w) were freeze-dried in annular molds, yielding tubular scaffolds with an inner diameter of 3 mm and with pore sizes of 130-150 µm and a porosity of 90 % (figure 1). To improve the mechanical properties, the scaffolds were cross-linked by means of a non-cytotoxic method introducing intermolecular peptide bonds. SMCs from umbilical cords were seeded in the scaffolds using a filtration seeding method and subsequently cultured during 14 days under dynamic conditions in a pulsatile flow bioreactor, or under stationary conditions as control. Dynamically cultured constructs contained 2.5 times more SMCs and the cellular expression of collagen type I mRNA was 2-fold increased, as compared to the controls. Only in the case of dynamic culturing, the SMCs were homogeneously distributed in the wall of the scaffolds. Although dynamic culturing significantly improved the mechanical properties of the constructs, the maximum strength in the radial direction was still 50-fold lower than that of a porcine carotid artery (9-12).

To improve the mechanical properties of the constructs, we started to use the synthetic polymer poly(trimethylene carbonate) (PTMC) as material for the preparation of scaffolds. PTMC shows excellent biocompatibility and is degraded in vivo by enzymatic surface erosion. Compliant and creep-resistant PTMC networks are obtained by means of γ-irradiation. Porous tubular PTMC scaffolds can be formed by dip-coating glass mandrels in a salt-containing polymer solution. After drying and subsequent leaching of the salt in water, an interconnected pore structure is obtained. In this way, tubular PTMC scaffolds were prepared with an inner diameter of 3 mm, an average pore size of 110 µm and a porosity of 85 %. To improve the cell seeding efficiency, a thin PTMC outer layer with a pore size of approximately 30 µm was dipped around the tubes (figure 1) (13,14). As determined by stress-strain measurements in the radial direction, these scaffolds were 10 times stronger than the collagen-elastin scaffolds. Moreover, the compliance of the tubular PTMC scaffolds was the same as that of porcine carotid arteries (15).

Also in the PTMC scaffolds, human SMCs were seeded by means of a filtration seeding method, after which the constructs were cultured during 14 days in a pulsatile flow bioreactor. In this case, we used a computer-controlled bioreactor, perfusing the constructs at a shear rate of 350 s^{-1} and pressures of 70-130 mm Hg at 70 pulses per minute (figure 2). Again, SMC numbers were significantly higher in dynamically cultured constructs, as compared to stationary controls, and now the strength of the constructs in the radial direction approached that of the porcine carotid artery (construct 0.5 MPa, artery 1.5 MPa) (16).

In conclusion, we have prepared a potentially useful medial layer of a small-diameter vascular graft by means of a tissue engineering approach. Currently, we

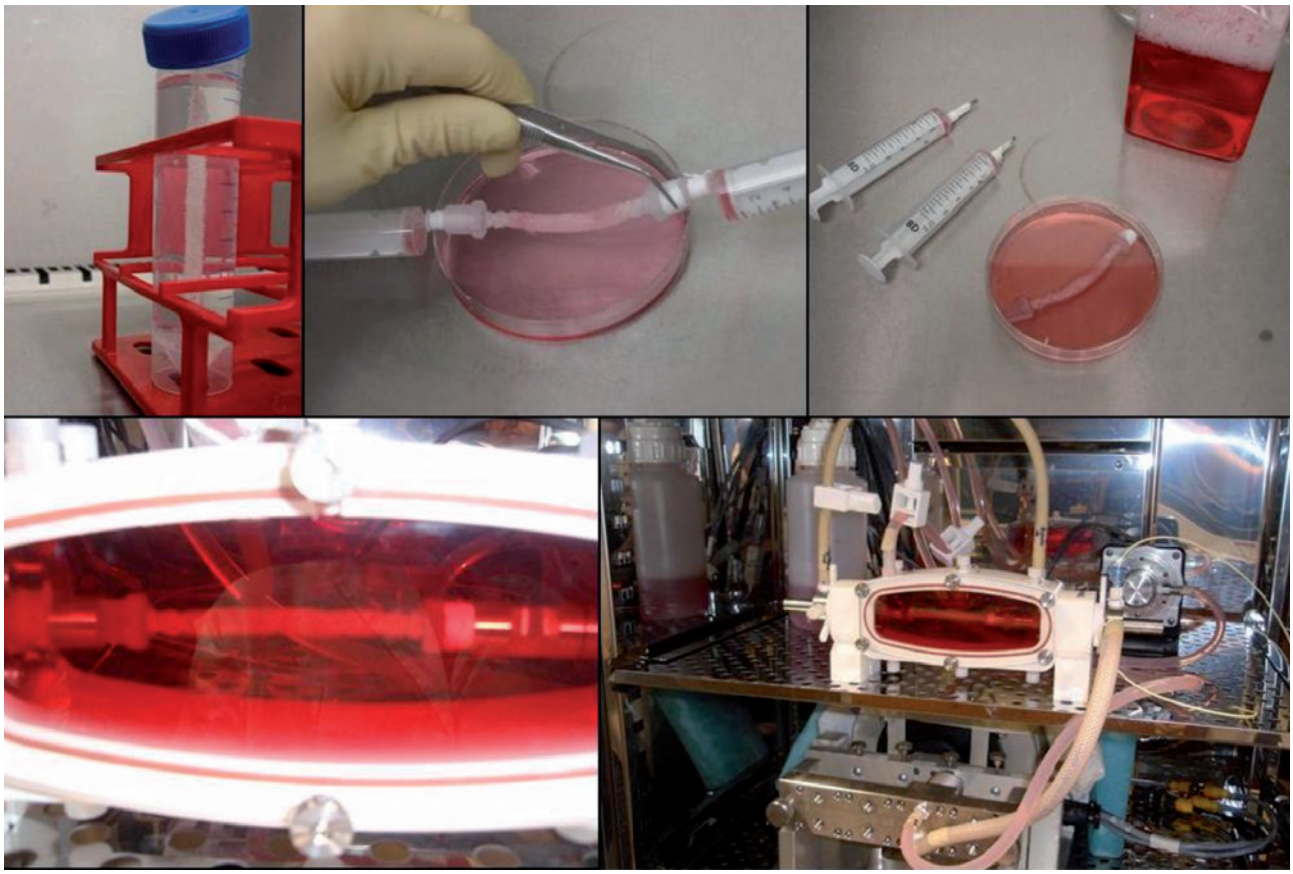

Figure 2. Overview of vascular tissue engineering. From left to right and top to bottom: tubular PTMC scaffold, SMC seeding, SMC-containing construct, construct in flow chamber, pulsatile flow bioreactor.

are investigating the endothelialization of the luminal surface of the constructs. Since extracellular matrix proteins will be synthesized by the SMCs during dynamic culturing, it is anticipated that the luminal surface of the grafts is a good substrate for EC seeding. After endothelialization, the performance of the grafts will be tested in a porcine carotid artery model. In addition to primary SMCs and ECs, we are also investigating the use of mesenchymal stem cells from adipose tissue. These cells can be obtained relatively easy in large quantities, and can be efficiently differentiated into SMCs. Endothelial progenitor cells from peripheral blood may be a suitable cell source for autologous ECs.

Acknowledgements

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