Tissue Engineering for Blood Vessels

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Abstract: The artificial vascular grafts currently in clinical use still have problems with respect to late calcification, restenosis, antithrombotic property, durability, biocompatibility, and safety, and no ideal artificial vascular graft has been developed to date. In view of this, we have been continuing basic experimental studies and their clinical applications in an attempt to develop tissue-engineered regenerative blood vessels. Tissue-Engineered Vascular Grafts (TEVG) autologous cells have no graft rejection potential, and as they are comprised of viable autologous cells, a longer sustained durability can be expected. Eventually, no foreign matter remains, and the lumen becomes completely endothelized. Therefore, no long-term anticoagulant therapy is required post-implantation, and the implanted graft, being autologous tissue, may have higher growth potential: Such artificial grafts are considered ideal blood vessels. We will continue to seek methods that enable less cumbersome treatments that are less stressful for patients in the future.

Key words: Tissue engineering; Blood vessel; Autogenic cell implantation

Introduction

Cardiac surgery has spread explosively throughout the world since J. H. Gibbon succeeded in performing open heart surgery using a pump-oxygenator in 1953. With the Westernization of lifestyles in Japan, open heart surgery for valvular diseases or congenital heart diseases, bypass surgery for ischemic heart diseases and operations on greater blood vessels have been increasing rapidly. In the field of cardiovascular surgery, revascularization using artificial vascular grafts has been undertaken to treat congenital defects or hypoplasia and to correct acquired vascular stenosis or occlusion due to atherosclerosis.

Especially in cases of low-pressure or small-caliber artificial vascular graft implantation, however, anticoagulant therapy is required and reoperations are inevitable due to late calcification, stenosis, and the non-growth nature of the graft. As many as some 80 different types of artificial vascular grafts are currently in clinical use in this country, but there is no ideal artificial vascular graft developed/available to date. There is great need for development of vascu-
lar prostheses with higher biocompatibility such as greater antithrombotic property, durability, and safety.

In view of this, we have been continuing basic experimental studies aimed at development and clinical application of TEVG as ideal artificial vascular grafts. We make it a rule to use “autologous cells” for preparation of regenerative blood vessels to ward off potential graft rejection. Viable autologous cells exist within the tissue regenerated. Therefore, a longer sustained durability can be expected, and eventually, no foreign matter remains, and the lumen becomes completely covered by endothelial cells. Thus, no long-term anticoagulant therapy is required post-implantation, and the implanted device, being autologous tissue, may have a higher growth potential.

This article describes the method of regenerative vascular graft preparation we perform with tissue engineering techniques and the present status of its clinical application.

Basic Research and Results

Basic experiments designed to construct a large-caliber blood vessel from peripheral vascular cells and to determine whether the constructed vasculature might withstand surgical grafting at its acute phase and continue to function satisfactorily in the long term were conducted on large animal models (pulmonary artery: sheep; and inferior vena cava: dog).1,2)

1. TEVG construction (i. cell collection, ii. mass cell production, and iii. seeding on polymer graft)

An approximately 2-cm segment of the femoral artery was obtained from an animal and trypsinized to prepare isolated vascular cell cultures using the simple explant technique. The resultant mixed cell cultures were grown over a period of about 6–8 weeks for mass production of cells. A concentrated cell suspension was made up from the mass culture about one week prior to implantation, and then seeded on a biodegradable polymer conduit. The biodegradable polymer is hydrolyzed non-enzymatically and has been verified in many clinical studies to be safe not only early after implantation, but its degradation products in vivo have been shown to be safe as well. As the rates of biodegradation vary with the types of polymer, the polymer graft was designed to set a degradation/absorption period of 6–8 weeks by combining a plurality of different polymers. The cells disseminated on the polymer carrier continued to divide and proliferate to form a three-dimensional confluent growth during incubation, preceding its implantation.

2. TEVG implantation procedure

Implantation in animals was performed between Days 7 and 10 after cell seeding. A conduit prepared from vascular wall cells was implanted in the same animal from which the cells had been collected (autografting). Extracorporeal circulation was established with the animal placed under general anesthesia, and TEVG was implanted at the main pulmonary artery in sheep. Replacement of the inferior vena cava by TEVG was carried out under general anesthesia.

Tissue engineering for the cardiovascular system provides a condition favoring the seeded cells: the implanted cells/structure can be in direct contact with intravascular blood and can thus be supplied with oxygen, nutrients, and humoral factors from just after the implantation. Therefore, the cells on the structure implanted further differentiate, enabling reconstruction of the tissues.

3. Late (follow-up) evaluation

The diameter of each artificial vascular implant was evaluated at 10–36 weeks after the implantation. In every case, the implanted polymer was completely absorbed and the TEVG presented features similar to intact vascular tissues (Fig. 1). These implants were subjected to histological, biochemical and biodynamic assessments.
The tissue collagen content tended to increase progressively with time, and this suggested a tissue remodeling in vivo. There was also an increase in tensile strength with time as assessed by the biodynamic test. An immunohistological study with the factor VIII and anti-α-smooth muscle actin verified that the vascular tissue prepared was covered with endothelial cells, and there was evidence of the presence of smooth muscle cells in the tunica media. Vascular diameter increased progressively with the growth of the recipient host.

**Cases of Clinical Application**

Cell collection from humans began with the approval of the Tokyo Women’s Medical University Ethics Committee in April 1999. Clinical applications began upon obtaining fully informed consent from patients/their family members. The first case was of a 4-year-old child who received a regenerative vascular implantation for reconstruction of the pulmonary artery in May 2000, with satisfactory results.3,4)

This method is used at present only in cases where it is obvious that correction with conventional techniques will result in a poor outcome, as no satisfactory biomaterials are currently available in cardiac surgery. We plan to extend clinical applications through accumulation of experience in clinical cases. The method of clinical application is described in detail below.

1. **Preparation of grafts with tissue engineering techniques**
   (1) **Collection of venous segment and cell culture**
   A 3 cm segment of the great saphenous vein
was cut from the patient. It was then placed in a dish for culture in a clean bench, cut into 1 to 2-mm pieces with a knife, and incubated with an added culture medium in a 5% CO₂–95% air atmosphere at 37°C. The medium was changed at intervals of 2–3 days. The tissue fragments began to grow about 10 days after the start of incubation, and continued to proliferate to form confluent growths in the dish about 2 weeks later. The growths were then trypsinized and transferred to culture flasks for further incubation. The cells grew confluent in the flasks in about 4 weeks. When examined by immuno-staining at this stage, the cell population that had grown was found to be a mixture of about 10% endothelial cells, about 20–30% smooth muscle cells and about 60–70% fibroblasts.

(2) **Seeding on polymer graft**

The cell sheets grown in flasks were trypsinized and prepared into a cell suspension, which was then centrifuged to obtain 1 to 2 ml of a concentrated cell suspension, discarding the supernatant. This suspension was spread for seeding on a bioabsorbable polymer graft system to complete a graft. The graft was cultured by incubation for about one week and subsequently used in a surgical operation. Scanning electron microscopic observation of the graft surface showed that the seeded cells adhered to the polymer surface, entering polymer inter-space. The polymer graft was comprised of spongy polycaprolactone-polylactic acid high
polymer reinforced with polyglycolic acid fibers.

2. Case 1: A 4-year-old girl

The patient had previously undergone Fontan operation (right atrium-pulmonary artery anastomosis), but occlusion of the right inferior pulmonary artery occurred postoperatively. In view of the child’s QOL, we judged that her condition would be indicated for pulmonary artery reconstruction using TEVG. It took about 3 months from vascular cell collection until graft implantation.

Operative findings: Upon approach through a midsternal incision, a cardiopulmonary bypass was established. The right pulmonary artery was completely occluded at the site of the entrance to the middle and lower lobe branches, forming a cord about 1 cm in length. The uniform segment of the pulmonary artery was then resected, an incision was made in the anterior aspect of the pulmonary artery wall, and while securing the arterial lumen, the posterior wall was directly anastomosed. The incised anterior aspect was patched up with a piece that had previously been cut out of a tissue-engineered tubular graft to complete the pulmonary artery reconstruction (Fig. 2).

Postoperative radiographic examination: The operation produced improvement in blood flow to the middle and lower lobes of the right lung. On examination by selective angiography of the pulmonary arteries, the wall of the TEVG was uniformly even and the graft was satisfactorily patent with no particularly significant stenosis (Fig. 2).

3. Case 2: A 2-year-old male infant

The infant had received a palliative operation at infancy with the diagnosis of asplenia, atrial ventricular septal defect, common atrium, bilateral discontinuous central pulmonary artery, partial perfusion abnormality of the pulmonary vein, and bilateral superior vena cava. The patient was admitted to the hospital for reconstruction of the left and right pulmonary artery continuity and total cavopulmonary connection (TCPC; the superior vena cava and inferior vena cava are respectively anastomosed directly to the pulmonary artery).

Operative findings: TEVG, 17 mm in diameter, was prepared and used to bridge the inferior vena cava and the pulmonary artery.

Postoperative radiographic examination: The postoperative course was uneventful, and postoperative angiographic examination verified a satisfactory patency of the TEVG (Fig. 3).
Current Problems and Future Development

The vascular tissue we constructed and used with the tissue engineering technique is applicable, but only under medium or lower blood pressure such as the pulmonary artery, and its use is limited in corrections of blood vessels bearing higher systemic blood pressure. This is because the durability of the graft to stress by systemic blood pressure after disappearance of the polymer is questionable. It would be possible to use the TEVG even within the blood pressure of greater vessels provided absorbability of the polymer is improved or the strength of the graft is augmented by early introduction of stromal (interstitial) protein after in vitro seeding.

Bioreactors functioning to create the pulsating circulation of the culture medium have been put to practical use to permit in vitro “conditioning” of the cell/polymer structure under more physiological conditions. We have been pursuing studies to seek methods of TEVG preparation that are less cumbersome and less stressful on children, taking note of marrow stem cells and peripheral blood stem cells as the source of autograft cells.

Development of engineering techniques to produce flexible, elastic biodegradable polymers with long-term absorption periods as well as polymers capable of sustained release of cytokines is considered essential to bring about advances in tissue engineering for the cardiovascular system.

REFERENCES