SURGERY FOR ACQUIRED CARDIOVASCULAR DISEASE

CLINICAL EXPERIENCE WITH AUTOLOGOUS ENDOTHELIAL CELL-SEEDED POLYTETRAFLUOROETHYLENE CORONARY ARTERY BYPASS GRAFTS

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Objective: Autologous endothelial cell seeding was used to improve the patency of 4-mm polytetrafluoroethylene vascular prostheses.

Methods: Since 1995, 14 patients with coronary artery disease received 21 autologous endothelial cell-seeded polytetrafluoroethylene vascular bypass grafts for coronary artery revascularization. The polytetrafluoroethylene grafts were seeded with the endothelial cells in a multiple step procedure, including cell culture techniques before coronary bypass operation. With the use of extracorporeal circulation and cardioplegic arrest, a bypass operation was performed by means of conventional surgical techniques.

Results: After a mean postoperative follow-up of 27.7 months (range, 7.5-48 months), the graft patency rate is 90.5%. Follow-up angiograms of the aorta-coronary polytetrafluoroethylene bypass grafts showed patent bypasses in all cases except two. Angiograms of all 19 patent endothelial cell-seeded polytetrafluoroethylene bypass grafts showed a smooth luminal borderline without stenotic regions. The percutaneous transluminal angioscopic evaluation showed a glossy white and smooth endoluminal graft surface without any fibrin, platelet, or erythrocyte deposits. Intravascular ultrasonographic examinations confirmed the results.

Conclusion: Patency of autologous endothelial cell-seeded 4-mm polytetrafluoroethylene vascular prostheses as coronary artery bypass grafts was much better than that of unseeded polytetrafluoroethylene grafts. Further evaluations and a larger population of patients will prove whether the encouraging patency will last. (J Thorac Cardiovasc Surg 2000;120:134-41)

Patients with symptomatic, multiple-vessel coronary artery disease often need bypass operations for coronary revascularization. Because of previous throm-

bophlebitis, severe varicosities, or prior coronary bypass operations, an increasing number of patients requiring coronary bypass operations do not have sufficient bypass material of their own for coronary revascularization. It is estimated that up to 20% of all patients requiring coronary bypass fall into this category.¹ If complete arterial coronary artery revascularization can be achieved by using the internal thoracic artery (ITA), the radial artery as a free graft, or the gastroepiploic artery, this is the method of first choice, despite the increased incidence for some relevant complications (eg, postoperative ischemia of the hand after using the radial artery for coronary bypass is reported).^{2,3} Our intention was to increase the biocompatibility of commercially available expanded 4-mm polytetrafluoroethylene (PTFE) vascular grafts by seeding the luminal wall of the graft with vital autologous endothe-

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Table I. Patients receiving autologous EC-seeded PTFE grafts

Postthrombotic syndrome	n = 5
Severe varicosis	n = 9
ITA already used	n = 3
Redo CABG operation	n = 6
Age >60 y	n = 14

CABG, Coronary artery bypass graft.

lial cells (ECs) from cell cultures, preparing a physiologic luminal graft surface by the patient's own vital EC monolayer.^{4,5}

Patients and methods

Patient selection. Patients with stable angina pectoris suitable for elective coronary artery bypass operations were chosen who had hemodynamically relevant coronary artery stenosis and in whom complete coronary revascularization with the ITA and saphenous vein was not possible. The patients were over 60 years of age. The study protocol was approved by the local ethics committee of the Humboldt University, and informed documented consent was obtained from all patients before the bypass procedure.

Graft preparation

Harvest of the autologous ECs. After achievement of local anesthesia, a 5- to 10-cm long segment of a cutaneous vein (eg, forearm or neck) of the patient was removed atraumatically, occluding all side branches with 5-0 ligatures. Simultaneously, 200 mL of the patient's blood was taken to prepare approximately 100 mL of serum.

The removed segment of the vein was immediately rinsed with Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical Co, St Louis, Mo) to wash out all remaining blood particles. The lumen of the vein was filled with 0.2% collagenase P (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn) to remove the ECs, both ends of the vein being occluded with a stopcock. An incubation for 30 minutes followed under cell culture conditions (37°C/5% CO₂/saturated humidified air). The luminal liquid with the suspended ECs was then collected and centrifuged at 500g for 10 minutes. The pellet containing the ECs was resuspended in DMEM plus 20% autologous patient serum.

EC culture. The resuspended pellet of the ECs was seeded in cell culture flasks (12.5 cm²) in DMEM plus 20% autologous serum enriched with 5 ng/mL human recombinant basic fibroblast growth factor (hbFGF) carrier free (Boehringer).^{6,7} Under cell culture conditions, the ECs were cultured until reaching confluency, changing the culture medium every 48 hours. The confluent cell layer was tested for homogeneity of the ECs by means of anti-factor VIII-conjugated monoclonal antibodies (MAB) (Sigma, Fig 1), a specific test for ECs to exclude a contamination of the cell culture by fibroblasts. The vitality of the ECs was tested with trypan blue (Sigma). After 2 passages of subculturing (time period, 4-6 weeks), enough

Fig 1. Positive immunohistochemical staining for anti-factor VIII of human ECs (cephalic vein) from a subconfluent monolayer in cell culture (dots). (Indirect peroxidase staining, counterstaining with Mayer's acid hemalum; original magnification 200×.)

ECs could be achieved to cover the luminal surface of a 20-cm long PTFE graft that was 4 mm in diameter, with a confluent EC monolayer (seeding density, 6×10^4 EC/cm²).

Precoating of the PTFE graft with a fibrin matrix. Commercially available sterile expanded PTFE vascular grafts of 4 mm in diameter*† were cut into 20-cm long segments. The luminal surface of the graft was coated with modified two-component fibrin glue (Tissucol; Immuno, Olivos, Buenos Aires, Brazil), including 5 ng/mL hbFGF (Boehringer). Fibrin and bFGF both support EC adhesion, spreading, migration, and proliferation.⁸⁻¹¹ Finally, the luminal surface of the graft was smoothed by pulling through an inflated balloon of a small Fogarty catheter to remove excessive fibrin glue.

EC seeding of the PTFE graft. ECs of confluent cultures were harvested with the use of 0.2% collagenase P and counted and diluted with DMEM to the final volume of the PTFE graft. The EC suspension was filled into the fibrin-precoated PTFE graft, occluding both ends with stopcocks. Under cell culture conditions, the fibrin-precoated PTFE graft was placed into a special seeding device (Endostrabilisator; Biegler Medizintechnik, Mauerbach, Austria). As the graft was slowly rotated stepwise around its longitudinal axis, the ECs adhered to the fibrin-coated luminal PTFE graft wall within 3 hours, with gravity used for the sedimentation of the ECs. The adherence of the ECs to the luminal wall of the graft was controlled by counting the cell number of the eluate of the PTFE graft after finishing the seeding procedure and by electron microscopy of specimens of the graft.^{12,13} After each step of the coating procedure, 3 swabs were taken to exclude bacterial or fungal contamination.

*Gore-Tex vascular graft, registered trademark of W. L. Gore & Associates, Inc, Flagstaff, Ariz.

†Mediflex vascular graft; registered trademark of Medino GmbH, Gehrden, Germany.

Fig 2. Scanning electron microscopy of the luminal surface of an EC-seeded, fibrin matrix-precoated PTFE graft. ECs show the typical polygonal pattern. (Original magnification 1260 $\times$.)

Graft maturation. After the adherence of the ECs to the luminal graft surface, the graft was stored for 8 to 10 days in DMEM plus 20% autologous patient serum in an incubator under cell culture conditions to allow the maturation of the links between the fibrin-precoated graft luminal surface and the ECs.¹⁴

PTFE graft implantation. A routine coronary artery bypass procedure was performed with normothermic cardiopulmonary bypass and intermittent antegrade application of Bretschneider cardioplegic solution for cardioplegic arrest. The EC-seeded PTFE grafts were implanted by performing an end-to-side anastomosis with a running suture (7-0 Prolene; Ethicon, Inc, Somerville, NJ) between the coronary artery and the PTFE graft. The central bypass anastomoses of the PTFE grafts were created to the ascending aorta in an end-to-side fashion with a running suture (6-0 Prolene).

Follow-up conditions. Long-term anticoagulation with dicumarol derivatives was not given to any of the patients postoperatively. The patients received only 100 mg of acetylsalicylic acid per day orally, which was routinely given to all patients undergoing coronary artery bypass. Every patient with an EC-seeded PTFE graft as coronary bypass was checked by selective angiography, angioscopy (Baxter angioscope $\varnothing$ 1.5 mm, tip $\varnothing$ 1.0 mm; Baxter Healthcare Corp, Irvine, Calif) and intravascular ultrasonography¹⁵ 6 and 12 months postoperatively and then annually.

Results

Since September 1995, 14 patients (12 men and 2 women) between 61 and 79 years of age (median, 74 years) with severe symptomatic coronary artery disease received 21 autologous EC-seeded 4-mm expanded PTFE vascular bypass grafts for coronary artery revascularization. EC-seeded PTFE grafts were used in

Fig 3. Transmission electron microscopy of a transverse cut through the wall of an EC-seeded PTFE graft. ECs with a nucleus (n) are connected by tight junctions in the intercellular space (s) and cover the luminal surface of the fibrin-precoated PTFE graft (black border), completely separating the lumen (i) from the PTFE surface. The magnitude of the fibrin coating (f) is distorted by the preparation of the specimen for microscopy. (Original magnification 4000 $\times$.)

patients who lacked suitable autologous bypass material for coronary revascularization and were older than 60 years (Table I). Successful endoluminal seeding of 4-mm PTFE vascular prostheses with a confluent monolayer of ECs was demonstrated by means of electron microscopy. The scanning electron microscopy of a luminal EC-seeded 4-mm PTFE graft 10 days after EC seeding shows a confluent monolayer of ECs (Fig 2). Transmission electron microscopy of an EC-seeded PTFE prosthesis demonstrates linked ECs in monolayer formation resting on a fibrin matrix (Fig 3). The demographic data of the patients and the performed surgical procedures are listed in Table II. The 14 patients received 34 coronary artery bypass grafts. Six patients had previous bypass operations with occluded veins or ITA grafts. In 1 patient the aortic valve was replaced as well because of severe aortic stenosis. For revascularization of the left anterior descending artery (LAD), in 10 patients the left ITA was used. Twenty-one aorta-coronary bypasses were performed with the EC-seeded PTFE grafts to revascularize the LAD in 1 patient, the first diagonal branch in 3 patients, the ramus intermedius in 1 patient, the first marginal

Table II. Demographics, performed procedures, and outcomes of the patients who received EC-seeded PTFE grafts for coronary artery bypass

Patient No. (sex, age[y])	Diagnosis	Operation	Bypasses/ additional procedures	EC-seeded 4-mm PTFE graft	Follow-up
1 (M, 71)	3-vessel CAD, postthrombotic syndrome	CABG II	LITA → LAD	M1 (PTFE) [†]	Patent at 48 mo
2 (M, 69)	2-vessel CAD, postthrombotic syndrome	CABG II	LITA → LAD	RCA (PTFE) [‡]	Asymptomatic occlusion after 20.5 mo
3 (F, 73)	3-vessel CAD, varicosis	CABG III	LITA → LAD, SVG → RIM	RCX (PTFE) [‡]	Patent at 45.0 mo
4 (M, 78)	3-vessel CAD, postthrombotic syndrome	CABG III	LITA → LAD	M1-RCA, horseshoe jump (PTFE) [‡]	Patent at 37.0 mo
5 (M, 64)	3-vessel CAD, varicosis	CABG III	LITA → LAD	RIM (PTFE) [†]	Patent at 34.0 mo
6 (M, 62)	3-vessel CAD, varicosis	CABG II	LITA → LAD, SVG → M1	Distal RCA (PTFE) [‡]	Patent at 34.0 mo
7 (M, 71)	Redo 2-vessel CAD, aortic stenosis	Redo CABG II, AVR	29-mm SJM Toronto SPV xenograft [*]	LAD-RCA, horseshoe jump (PTFE) [‡]	Patent at 29.5 mo
8 (M, 66)	Redo 2-vessel CAD, CABG III (1992)	Redo CABG II		D1, M1; single (PTFE) [‡]	Patent at 29.5 mo
9 (M, 61)	Redo 2-vessel CAD, CABG II (1992) occluded	Redo CABG II		M1, distal RCA; single (PTFE) [‡]	Multiorgan failure 4 wk postop
10 (M, 72)	Redo 3-vessel CAD, CABG III (1985) occluded	Redo CABG II	LITA → LAD	M1 (PTFE) [‡]	Patent at 23.0 mo
11 (F, 73)	3-vessel CAD, varicosis	CABG III	LITA → LAD, free RITA → RCX	RCA (PTFE) [‡]	Patent at 18.0 mo
12 (M, 64)	Redo 3-vessel CAD, CABG II (1991)	Redo CABG III	LITA → LAD	D1, distal RCA, single (PTFE) [‡]	Patent at 17.0 mo
13 (M, 75)	Redo 2-vessel CAD, CABG I (1986)	Redo CABG II		RCX-RCA, horseshoe jump (PTFE) [‡]	Patent at 10.0 mo
14 (M, 79)	3-vessel CAD, varicosis	CABG III	LITA → LAD	RCA, D1 single (PTFE) [‡]	Patent at 7.5 mo

AVR, Aortic valve replacement; CABG, coronary artery bypass graft; CAD, coronary artery disease; D1, first diagonal branch; LITA, left internal thoracic artery; M1, first marginal branch; RCX, right circumflex; RIM, ramus intermedius; RITA, right internal thoracic artery; SVG, saphenous vein graft.

[†]St Jude Medical, Inc, St Paul, Minn.

[‡]Mediflex vascular graft; Medino GmbH, Gehrden, Germany.

^{*}Gore-Tex vascular graft; W. L. Gore & Associates, Inc, Flagstaff, Ariz.

branch of the circumflex artery in 5 patients, the circumflex artery itself in 2 patients, and the right coronary artery (RCA) in 9 patients. In 2 patients an additional saphenous vein graft was used to revascularize the ramus intermedius and the first marginal branch. In 12 of the 14 patients, the postoperative recovery was free of complications. One patient receiving a redo procedure with the left ITA to the LAD and an EC-seeded PTFE bypass to the RCA died 4 weeks postoperatively of multiorgan failure after pneumonia. One patient had a perioperative myocardial infarction caused by the immediate failure and occlusion of the EC-seeded PTFE graft to the distal RCA because of poor runoff and possible contamination of the EC culture with fibroblasts, as proven by a re-evaluation of the cell culture. All other patients are still alive and out of the hospital. They are free of angina pectoris, and none had a

myocardial infarction. Between 7.5 and 48 months (median, 29.5 months) after the bypass operation, 19 (90.5%) of 21 EC-seeded PTFE grafts for coronary artery bypass in 14 patients were patent. In patient 2 the EC-seeded PTFE graft bypassing the RCA is asymptotically occluded, which was verified by means of routine coronary reangiography 20.5 months after the operation. Selective angiography of the EC-seeded PTFE grafts demonstrates a smooth luminal border of the grafts without stenotic regions (Fig 4). Selective angioscopic evaluation of the EC-seeded PTFE prostheses of the patients shows a glossy white lumen of the graft with a smooth luminal surface without any fibrin, platelet, or erythrocyte deposits on the luminal wall (Fig 5). By means of intravascular ultrasound (Fig 6), the smooth unobstructed luminal surface of the grafts could be confirmed.

Fig 4. Selective angiography of autologous EC-seeded 4-mm PTFE grafts as coronary bypass. **A**, Twelve months postoperatively (patient 1); **B**, 45 months postoperatively (patient 1); **C**, 24 months postoperatively (patient 7).

Discussion

This is the first clinical report about autologous EC-seeded 4-mm PTFE vascular grafts used for coronary artery bypass grafting. In a mean of 27.7 months (range, 7.5-48 months) after the operation, 90.5% (19/21) of the autologous EC-seeded 4-mm PTFE grafts are patent and show an improved patency compared with the unseeded PTFE prostheses with patency rates of 59% to 64.3% after 12 months.^{16,17} To determine whether long-term patency of autologous EC-seeded PTFE grafts surpasses the patency of saphenous vein grafts will require a larger number of patients and

a prolonged period of follow-up investigations. After 10 years, the patency of saphenous vein grafts used as coronary bypass conduits declines to 60% to 70%.^{18,19} It is encouraging that the good patency of the EC-seeded PTFE grafts is achieved without anticoagulation of the patients postoperatively. All patients received only an antiplatelet medication with 100 mg of acetylsalicylic acid per day. Selective angiographic, angioscopic, and intravascular ultrasonographic evaluations of the patients up to 48 months postoperatively showed a very smooth and glossy luminal graft surface, which implies indirectly a very biocompatible graft surface naturally

Fig 5. Selective intravital angioscopy of autologous EC-seeded 4-mm PTFE grafts as coronary bypass grafts (Baxter angioscope with 1-mm tip). **A**, Twelve months postoperatively (patient 1); **B**, 24 months postoperatively (patient 7).

Fig 6. Intravital intravascular ultrasonography of autologous EC-seeded 4-mm PTFE grafts as coronary bypass grafts. **A**, Forty-five months postoperatively (patient 1); **B**, 18 months postoperatively (patient 10). A small arteriosclerotic plaque is marked by the *arrow*.

provided by autologous ECs, as demonstrated in Figs 5 and 6. In vitro flow experiments with endoluminal seeded 4-mm PTFE grafts with indium- ^{111}In -oxin labeled vital human ECs revealed only a washout of ECs between 10% and 15% in the first minute after the installation of a physiologic pulsatile flow. Dependent

on different flow conditions, 69% to 83% of the vital seeded ECs adhered permanently to the luminal graft wall,^{20,21} supporting the hypothesis that the ECs adhere and can survive in the long term. An endoluminal vital confluent monolayer of autologous ECs reduces the thrombogenicity of the PTFE graft and increases the

patency of 4-mm PTFE vascular grafts for coronary artery bypass compared with unseeded grafts.^{16,17} Similar results were published by Zilla and colleagues²² in 1994. They reported that EC-seeded 6-mm PTFE vascular prostheses have a much better patency rate than unseeded PTFE grafts for femoropopliteal reconstruction in peripheral vascular occlusive disease with a follow-up of more than 3 years. In one case Deutsch and colleagues²³ could demonstrate 41 months after the implantation of an autologous EC-seeded 6-mm PTFE graft for revascularization of the lower limb that a vital confluent EC monolayer still covered the lumen of the PTFE prosthesis, which indicates that in vitro vital EC seeding of PTFE grafts leads to a stable luminal EC lining.

The ITA is undoubtedly the coronary bypass graft of first choice, with a patency of over 90% after 10 years.^{18,19} The gastroepiploic artery is also superior, with a patency rate of 94% 24 to 60 months after the operation.²⁴ The inferior epigastric artery, with a patency of 90% after 12 months,²⁵ and the radial artery, used as a free graft with 88% patency as a coronary artery bypass graft 12 months after the operation,²⁵ seem of equal quality compared with EC-seeded PTFE. The cephalic vein, with 47% patency after 54 months' follow-up,²⁶ shows a much poorer patency rate when compared with the autologous EC-seeded PTFE coronary bypass graft. Even worse are the patencies of cryopreserved homologous saphenous vein grafts (41% after 7 months)²⁷ and the bovine ITA (14% 1 year after the operation).²⁸

Conclusions

Autologous EC seeding increases the biocompatibility of 4-mm PTFE coronary artery bypass grafts. Autologous EC-seeded 4-mm PTFE bypasses are an alternative as coronary artery bypass for patients with insufficient saphenous vein graft bypass material in elective coronary artery bypass operations who are not candidates for complete arterial revascularization.

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