Article

Six years of clinical follow-up with endothelial cell–seeded small-diameter vascular grafts during coronary bypass surgery

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Abstract

This clinical study was performed to investigate the patency rate of endothelial cell–seeded small-diameter expanded polytetrafluoroethylene grafts during coronary artery bypass surgery. Between September 1995 and December 1998, 14 patients (median age: 71 years, range: 61–79 years) received 21 endothelial cell–seeded small-diameter grafts. In all, 43% of the performed implantations were reoperations. Endothelial cells were harvested from a forearm vein, cultured and characterized in the laboratory until a sufficient number was available. After in vitro seeding, the grafts were allowed to mature for another 10 days, prior to implantation. Graft patency was investigated with angiography, angioscopy, and intravascular ultrasonography during follow-up. Cumulative data represented 58 patients' years and was 100% complete. The seeded autologous vascular endothelial cell density was $1.05 \times 10^5 \pm 0.12 \times 10^5$ cells/cm^2 with a cell viability of 95.5 ± 1.5%. Operative mortality was 7.1% (one patient). Patency rate at discharge was 95.2%, and at a mean follow-up of 27 months was 90.5%. The proven patency rate at up to 72 months was at least 50.0%, as five patients refused angiographic evaluation. None of these five patients suffered from angina pectoris and so the best scenario would have shown a patency rate of 85.7%. Angioscopy and intravascular ultrasonography showed absence of atheroma or stenosis in the investigated patent grafts. Autologous vascular endothelial cell seeding improves patency rate of small-caliber expanded polytetrafluoroethylene grafts in patients without suitable autologous graft material.

Keywords

Polytetrafluoroethylene (PTFE) grafts, endothelial cells, in vitro seeding, alternative bypass material

Introduction

Each year more than 500,000 coronary bypass operations are performed in the United States.[1] As the number of patients undergoing reoperations with insufficient autologous bypass material increases,[2] several alternative graft materials are under investigation.[3,4] This clinical feasibility study was performed to investigate whether in vitro autologous vascular endothelial cell (AVEC) will be able to improve the patency rate of small-diameter expanded polytetrafluoroethylene (ePTFE) grafts during coronary bypass surgery.

Patients and methods

After institutional review board approval and informed consent were obtained, the first implantation of a seeded ePTFE graft was performed on 5 September 1995. The grafts used to seed were two 4-mm Mediflex vascular grafts (Medino GmbH, Gehrden, Germany) and nineteen 4-mm Gore-Tex vascular grafts (WL Gores & Associates, Inc., Flagstaff, AZ, USA), commercially available vascular prostheses.

Patient selection

Suitable candidates to implant a seeded ePTFE graft are patients with relatively stabile angina who can undergo

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elective coronary bypass surgery 4–6 weeks after diagnostic workup. Patient selection criteria were insufficient saphenous vein material because of varicose veins, deep vein thrombosis, and previous use for peripheral or coronary bypass surgery. If autologous grafts, for example, radial artery, right and/or left internal mammary artery, and vena saphena parva, were available, these grafts were used to perform complete coronary artery revascularization.

Since no previous clinical studies were performed with 4-mm seeded ePTFE grafts during coronary bypass surgery, target coronary arteries were always the most severally diseased arteries, showing the most restricted run off. These grafts were used to perform a complete revascularization.

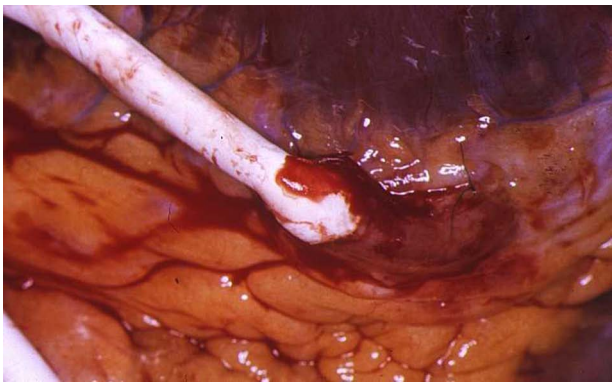

Figure 1. An autologous vascular endothelial cell–seeded 4-mm ePTFE graft showing the distal anastomosis at the coronary artery. ePTFE: expanded polytetrafluoroethylene.

Graft preparation

Autologous vascular endothelial cell harvesting. A piece of left or right forearm vein was harvested under local anesthesia. Proximal and distal intubation of the vein was performed and the side branches were ligated. Care was taken during manipulation of the vein to prevent endothelial cell loss. The vein was carefully flushed, using Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co., St Louis, MO, USA), with 20 mmol/mL L-glutamine (Sigma Chemical Co.) and antibiotics (penicillin 100 U/mL, streptomycin 100 µg/mL; Sigma Chemical Co.). The vein was immediately transported to the tissue laboratory where AVECs could be harvested. In addition, 200 mL of blood was drawn from each patient.

Endothelial cell culturing. At the tissue laboratory, the vein was again carefully flushed with a modified DMEM solution. AVECs were harvested by the use of Collagenase II 0.1% (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA) for 10 min at 37°C. The solution of AVEC and Collagenase II was collected and temperature-controlled centrifuged at 500 *g* for 10 min. AVECs were then seeded in 25-cm^2 culture bottles (Falcon, Becton Dickinson Labware, NJ, USA) with DMEM, 20 mmol/mL L-glutamine, 10 µg/nL basic fibroblast growth factor (Boehringer Ingelheim Pharmaceuticals, Inc.), 10% of autologous serum, 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified incubator (37°C, 5% CO_2 and 98% air saturation).

During the first 24 h, cell cultures were not manipulated to allow endothelial attachment to the culture bottles. AVEC growth and fibroblast contamination was evaluated by daily microscopical examination. After a confluent monolayer of AVEC was identified, passages were performed until two culture bottles of 75 cm^2 or two culture bottles of 175 cm^2, depending on the length of the seeded grafts needed during coronary bypass surgery, were obtained. The medium was changed every second day. A maximum of three passages were performed, to assure good endothelial cell quality. AVEC viability was controlled by the use of trypan blue solution (Sigma Chemical Co).

Endothelial cell seeding of the ePTFE grafts. Endothelial cell seeding has been previously described.[5] In brief, after having a sufficient number of AVEC available, an ePTFE graft was coated with Tissuecol Duo S (Immuno, Baxter, Unterschließheim, Germany) to increase the binding capacity of cells. The grafts were seeded by a sedimentation technique using a biostabilizer (Biegler Medizinelektronik GmbH, Mauerbach, Austria). It took 3 h to complete the seeding procedure.

Graft maturation. After the seeding was completed, the seeded ePTFE grafts were stored in a humidified incubator (37°C, 5% CO_2 and 98% air saturation) for another 10 days to improve the binding stability between endothelial cells to the fibrin-coated graft.

Quality control of the seeded ePTFE graft. To verify that cell cultures were free of contamination with interstitial cells, von Willebrand factor staining was performed. The bioreactor was built by a bio-nonreactive material and so AVEC could only be bound to the coated ePTFE grafts. Prior to implantation, sterility was proven for each of the seeded ePTFE grafts.

Implantation technique

Routine coronary bypass surgery was performed with normothermic cardiopulmonary bypass. Cardiac arrest was induced with intermittent application of Bretschneider cardioplegic solution. The endothelial cell–seeded ePTFE grafts were sutured using a no-touch technique. The distal anastomoses were sutured end to end, using a running 7-0 Prolene (Figure 1) (Ethicon, Inc., Somerville, NJ, USA). The proximal anastomoses were performed with a running 5-0 Prolene suture line (Figure 2). The chest was closed in

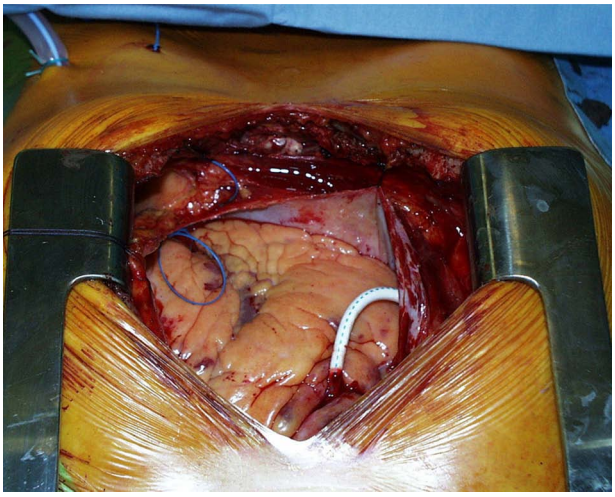

Figure 2. An overview of the operative site using an autologous vascular endothelial cell–seeded 4-mm ePTFE graft during coronary bypass grafting.
ePTFE: expanded polytetrafluoroethylene.

a standard way. Anticoagulation was performed with unfractionated heparin for only 3 days. Afterward, 100 mg of aspirin was given daily.

Follow-up

Patient follow-up was performed with angiography, intravascular ultrasonography (40 MHz intravascular ultrasound (IVUS) catheter; Atlantis, Boston Scientific, Natick, MA, USA) and angioscopy (Baxter angioscope 1.5 mm diameter, tip 1.0 mm diameter; Baxter Healthcare Corporation, Irvine, CA, USA) to evaluate patency rate of the seeded ePTFE graft during follow-up.

Results

Cumulative data collected represented 58 patients' years. Follow-up was 100% completed, but some patients refused angiography. The reason of refusing angiography in these five patients was the perceived risk of complications due to catheterization. All patients who refused graft evaluation are currently in New York Heart Association (NYHA) classification I. During the observation period, none of these five patients suffered from angina pectoris or used nitroglycerin. Additional examinations such as stress electrocardiogram (ECG) or isotope studies were also refused by these patients.

Endothelial cell seeding of the ePTFE grafts

The mean rate of re-endothelialization was $1.03 \times 10^5 \pm 0.12 \times 10^5$ cells/cm^2 (Figure 3), with an effective seeding percentage of 90.1 ± 6.7% (Figure 4), and endothelial cell viability was 95.5 ± 1.5% by trypan blue staining in a

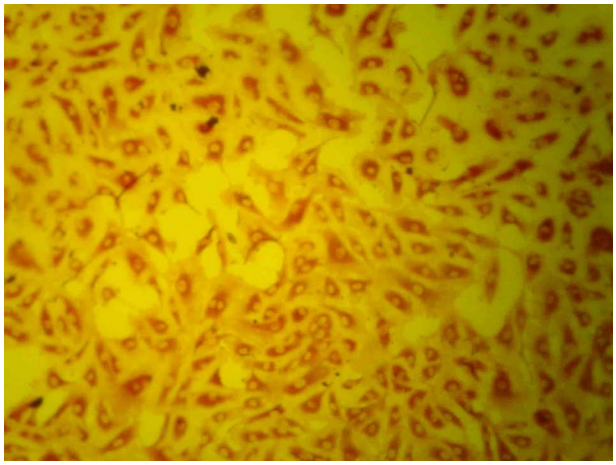

Figure 3. A representative monoculture of anti-Factor VIII–stained positive cells.

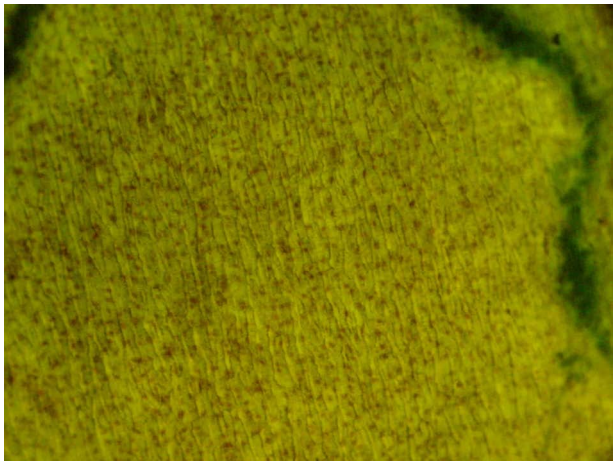

Figure 4. A representative Giemsa-stained sample of a seeded ePTFE graft showing an effective in vitro endothelial cell seeding.
ePTFE: expanded polytetrafluoroethylene.

Neubauer chamber. The effective seeding percentage was calculated of the cells which were given into the bioreactor, made from a bio-inherent material, and therefore, AVEC could only bind to the coated ePTFE grafts.

Quality control of the seeded ePTFE graft

Sterility was proven in all samples, prior to implantation. In vitro endothelial cell seeding efficiency was documented by calculation as previously described as well as through electron microscopy of specimens of the grafts.

Follow-up

Between September 1995 and December 1998, 14 patients with a mean age of 69.9 ± 5.7 years, (median 71 years; range 61–79 years) received AVEC-seeded 4-mm-diameter ePTFE

Table 1. Patient characteristics.

Patient	Gender, M/F	Age, years	LVEF, %	Vein status	Target vessel ePTFE	Operation
1	M	71	44	SE	M1	CABG
2	M	69	47	TF	RCA	CABG
3	F	73	83	V	RCX	CABG
4	F	75	51	TF	RCX	CABG
5	M	78	48	V	M1-RCA	CABG
6	M	64	44	V	RIM	CABG
7	M	62	78	V	RIVP	CABG
8	M	71	61	TF	M1	Re-CABG
9	M	71	52	V	LAD-RCA	Re-CABG
10	M	67	62	SE	D1-M1	Re-CABG
11	M	61	63	V	M1-RIVP	Re-CABG
12	F	73	46	V	RCA	CABG
13	M	64	65	V	D1	Re-CABG
14	M	75	58	SE	RCA-RCX	Re-CABG

LVEF: left ventricular ejection fraction, SE: saphenectomy, V: varicosis, TF: tromboflebitis, M1: first marginal, RCA: right coronary artery, RCX: ramus circumflexus, RIM: ramus intermedius, RIVP: ramus intervantricularis posterior, LAD: left anterior descending coronary artery, CABG: coronary-aorta bypass grafting, Re-CABG: redo-coronary-aortic bypass grafting; ePTFE: expanded polytetrafluoroethylene.

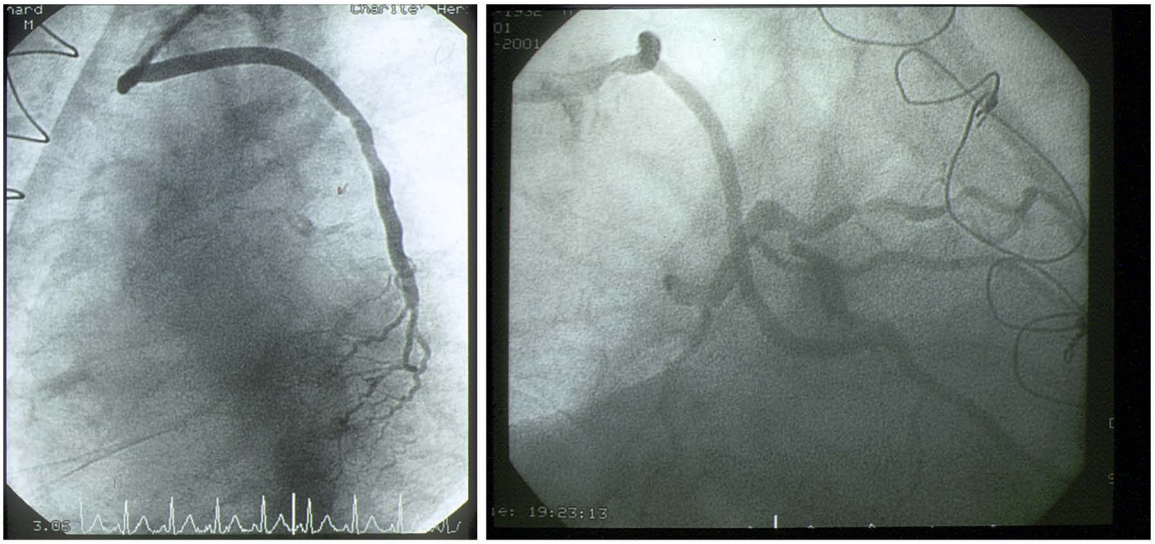

Figure 5. Angiography of an autologous vascular endothelial cell–seeded 4-mm ePTFE graft as aorto-coronary bypass graft in (a) a patient 45 months postoperatively and (b) 60 months postoperatively in another patient.
ePTFE: expanded polytetrafluoroethylene.

grafts (Table 1). Patients received in total 34 coronary bypass grafts: 21 seeded ePTFE grafts, 10 left internal mammary arteries, 1 right internal mammary artery, and 2 saphenous vein grafts. One patient received an additional aortic valve replacement; 42.9% ($n = 6$) of the performed implantations were reoperations. Thirteen patients showed no early postoperative complications. One patient died early postoperative because of multiorgan failure. This patient showed one closed and one open graft at autopsy. The seeded ePTFE graft at posterior descending coronary artery was closed and the ePTFE graft bypassing the obtuse marginal was shown to be open. Two late deaths were seen, one patient died at 34 months (after re-bypass operation this patient died) and one died at 37 months postoperatively because of noncardiac death (at autopsy both grafts were open).

At discharge, 20 of 21 (95.2%) seeded ePTFE grafts were patent. At a mean follow-up time of 27 months (range 7.5–34 months), 19 of 21 (90.5%) seeded ePTFE grafts were patent. One seeded ePTFE graft at a small-diameter first diagonal branch was occluded at this time; however,

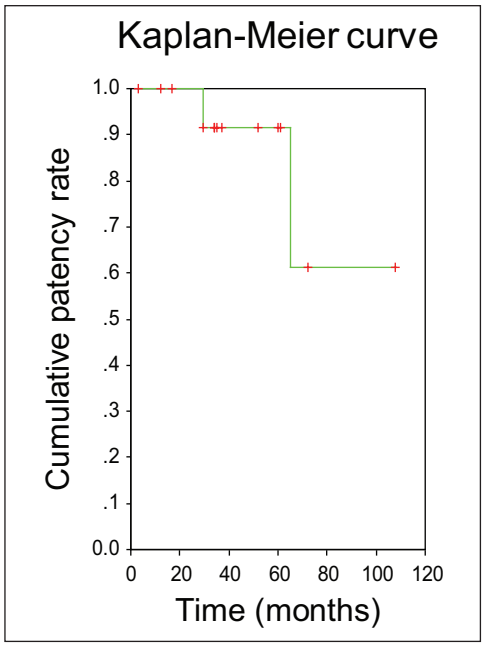

Figure 6. Kaplan–Meier curve estimated the patency rate of 21 autologous vascular endothelial cell–seeded ePTFE grafts for 14 patients.
ePTFE: expanded polytetrafluoroethylene.

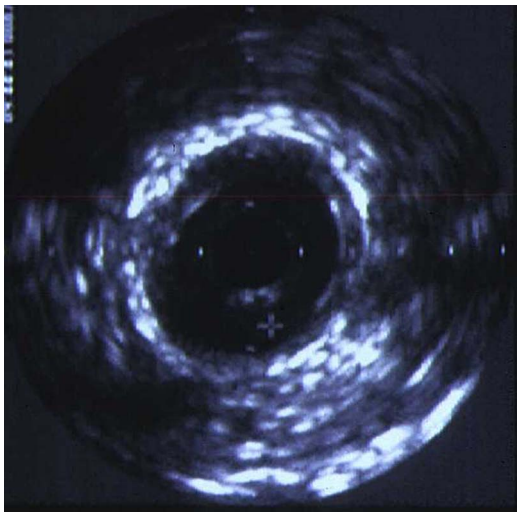

Figure 7. Intravascular ultrasonography of an autologous vascular endothelial cell–seeded 4-mm ePTFE graft as aorto-coronary bypass graft in a patient after 60 months of implantation.
ePTFE: expanded polytetrafluoroethylene.

the patient was in NYHA Class I and showed no sign of angina pectoris. This patient was not considered to be reoperated. At a mean follow-up of 60 months (range 34–72 months), there were another two grafts verified to be occluded at, respectively, 34 and 66 months postoperative; however, 17 of 21 (81.0%) of the seeded ePTFE grafts were patent at angiography (Figure 5(a) and (b)).

One was an asymptomatic occlusion at the circumflex coronary artery and in one patient a symptomatic occlusion was shown. This patient was reoperated, receiving a radial artery to the right coronary artery. During the postoperative course the patient died because of multiorgan failure. At the latest follow-up with a mean of 87 months (range 53–108 months), five patients refused angiography; however, all these patients were in NYHA Class I, without suffering from angina pectoris. Patency rate of the seeded ePTFE graft was analyzed according to the Kaplan–Meier method (Figure 6). Angioscopy and intravascular ultrsonography showed no atheromas or stenosis, but a smooth and glossy white endoluminal graft in the investigated seeded ePTFE grafts (Figure 7).

Furthermore, one was able to see the influence of the different coronary arteries which were bypassed. After 60 months of follow-up, all the seeded ePTFE grafts to the right interventricular posterior artery were occluded. Also, the patency rate of the first diagonal was restricted as two grafts were occluded, and patency in one graft was unknown after up to 60 months of follow-up as this was not evaluated. Restriction to graft only the right coronary artery, the circumflex artery (including the intermedius and first marginal) and the left anterior descending artery, the patency rate would increase up to 60 months of follow-up to at least 69% (11 of 16 grafts) and in the best scenario 88% (14 of 16 grafts).

Discussion

Alternative grafts need to be investigated to allow complete revascularization in patients with insufficient, autologous graft material. An ideal artificial graft for coronary bypass surgery should have a small diameter, should be easy to implant, and have "off-the-shelf" availability. PTFE grafts have been investigated in the past showing low thrombogenicity in large-diameter grafts. It is, however, well known that small-diameter prostheses may induce thrombus formation because of platelet aggregation.[6] Platelet aggregation can lead to occlusion or induce small thrombi, which can cause embolization.[7] Decreasing thrombogenicity of PTFE grafts could theoretically be obtained by the use of a modified material called expanded PTFE. The improvement of antithrombogenicity of this material is explained by the increased porous hydrophobic polymer, which has a highly electronegative surface.[8]

However, the use of ePTFE grafts showed rather low patency rates compared to autologous graft material, and clearly further modifications are needed. Veith's multicenter study used 6-mm ePTFE graft in peripheral vascular bypass surgery and showed a patency rate of 38% compared to saphenous vein patency rates of 68% (up to 5 years of follow-up).[9]

In coronary bypass surgery, the use of 5-mm ePTFE grafts showed a patency rate ranging between 60% at 12

months[10] and 14% at 45 months follow-up.[11,12] The introduction of AVECs could theoretically improve graft patency, by neutralizing thrombin and thereby preventing thrombus formation. In human, no spontaneous endothelialization of graft material occurs, so vascular prostheses must be seeded in vitro.[13]

Meinhart et al.[14] performed autologous endothelial cell seeding at the inner surface of ePTFE grafts for infra inguinal bypass grafting. This study showed an improvement of the patency rate up to 83.7% after 4 years of follow-up. The diameter of the grafts was shown to be important in this study. By reducing the diameter of the graft with 1 mm from a 7-mm internal diameter to a 6-mm graft, the patency rate decreased from 83.7% to 64.4%.

We used 4-mm ePTFE grafts as an alternative conduit in patients without sufficient autologous graft material to perform aorto-coronary bypass surgery. These grafts were seeded in vitro with AVECs, resulting in an angiographic patency rate of at least of 50% at a maximum follow-up of 72 months. The patency rate would even increase to 85.7% if one would consider that the grafts of the five patients, refusing angiography, would be open. This is of course speculative, however realistic, as none of the patients were suffering from angina pectoris or using nitroglycerin.

It seems that even in the worse scenario endothelial cell seeding indeed can improve patency rates from 14% up to 50%. This 14% patency rate was achieved in 5-mm ePTFE grafts, and knowing the results of Meinhart et al.,[14] showing the influence of decreasing the diameter of the implanted grafts by 1 mm, these results are promising.

Next to this, one should also take into consideration that the seeded ePTFE grafts were always used to graft those coronary arteries with the smallest diameter and most restricted peripheral run off. Therefore, it is also not possible to compare grafting the left internal mammary artery grafted at the left descending artery with these results. Of course, we are also convinced that the mammary artery is the golden standard; the left descending artery in general has a best run off. Using these seeded ePTFE grafts in better coronary arteries could probably also lead to a higher patency rate.

The disadvantage of seeding ePTFE grafts with AVEC is that the patient needs to be able to wait at least 4–6 weeks until the graft is available for graft preparation. This implies that the grafts will not be available for emergency coronary bypass surgery.

It is important that a no-touch technique be employed during implantation of these grafts to avoid destruction of the seeded cell layer at the inner surface of the grafts.

Future studies should focus on decreasing the waiting time for the patient, using alternative cell sources which will be able to cultivate faster.

In conclusion, AVEC seeding seems to increase the patency rate of small-caliber ePTFE grafts in coronary bypass surgery; however, the methods will be needed to decrease the waiting for graft implantation.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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References

1. Kozak LJ, Hall MJ and Owings MF.National hospital discharge survey: 2000 annual summary with detailed diagnosis and procedure data. *Vital Health Stat* 13 2002; 153: 1–194.
2. Huynh T, Abraham G, Murray J, et al. Remodeling of an acellular collagen graft into a physiologically responsive neovessel. *Nat Biotechnol* 1999; 17: 1083–1086.
3. Weyland M, Kerber S, Schmid C, et al. Coronary artery bypass grafting with an expanded polytetrafluoroethylene graft. *Ann Thorac Surg* 1999; 76: 1240–1245.
4. Lamm P, Juchem G, Milz S, et al. Autologous endothelialized vein allograft: a solution in the search for small-caliber grafts in coronary artery bypass graft operation. *Circulation* 2001; 104(Suppl. 1): I108–I114.
5. Dohmen PM, da Costa F, Lopes SV, et al. The juvenile sheep model for evaluation of tissue engineered cardiovascular grafts. *LA Arch Cardiovasc Sci* 2003; 4: 45–53.
6. Salzman EW, Rosenberg RD, Smith MH, et al. Effect of heparin and heparin fractions on platelet aggregation. *J Clin Invest* 1980; 65: 64–73.
7. Allen BT, Mathias CJ, Sicard GA, et al. Platelet deposition on vascular grafts. The accuracy of in vivo quantitation and the significance of in vivo platelet reactivity. *Ann Surg* 1982; 203: 318–328.
8. Hanel KC, McCabe C, Abbott WM, et al. Current PTFE grafts: a biomechanical, scanning electron, and light microscopic evaluation. *Ann Surg* 1982; 195: 456–463.
9. Veith F, Gupta S, Ascer E, et al. Six-year prospective multicenter randomized comparison of autologous saphenous vein and expanded polytetrafluoroethylene grafts in infrainguinal arterial reconstruction. *J Vasc Surg* 1986; 3: 104–114.
10. Hehrlein FW, Schepper M, Loskot F, et al. The use of expanded polytetrafluoroethylene (PTFE) grafts for myocardial revascularization. *J Cardiovasc Surg (Torino)* 1984; 25: 549–553.
11. Sapsford RN, Oackley GD and Talbot S. Early and late patency of expanded polytetrafluoroethylene vascular grafts in aorta-coronary bypass. *J Thorac Cardiovasc Surg* 1981; 81: 860–864.
12. Tomizawa Y. Vascular prostheses for aortocoronary bypass grafting: a review. *Artif Organs* 1995; 19: 39–45.
13. Berger K, Sauvage LR, Rao AM, et al. Healing of arterial prostheses in man: its incompleteness. *Ann Surg* 1972; 175: 118–127.
14. Meinhart JG, Deutsch M, Fischlein T, et al. Clinical autologous in vitro endothelialization of 153 infrainguinal ePTFE grafts. *Ann Thorac Surg* 2001; 71: S327–S331.