SLIDE FORUM 5

VASCULAR PROSTHESIS/NEW DESIGNS AND MATERIALS

Microporous Small Diameter PVDF-TrFE Vascular Grafts Fabricated by a Spray Phase Inversion Technique

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Microporous prostheses of 1.5 mm internal diameter were fabricated with a polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE)$_n$ co-polymer by the spray phase inversion technique. Some of the grafts were made piezoelectric by poling under a high electrical field. Overall, 24 poled grafts (P) and 24 unpoled grafts (UP) (15–22 mm in length) were implanted in the infrarenal aorta of 48 adult rats. Patency rates in P were 100% (8/8) at 2 days, 100% (8/8) at 2 weeks, 75% (6/8) at 6 months, and 92% total (22 of 24). Patency rates in UP were 100% (8/8) at 2 days, 63% (5/8) at 2 weeks, 100% (8/8) at 6 months, and 88% total (21 of 24). Thus there was no significant difference in patency between the two types of grafts. Both showed similar macroscopic and microscopic findings. At 2 days, fibrin deposition was somewhat heavier on the poled grafts, but no difference in surface platelet deposition could be detected. Endothelialization was observed from both anastomoses at 2 weeks and was almost complete at 6 months. The excellent biocompatibility of PVDF-TrFE and the microporous structure of the grafts were probably the dominant factors in success with these grafts. Although piezoelectric activity in excised cleaned poled prostheses remained significantly higher than that in the control UP, the charges developed may have been too small to exert a biologic effect, either because of insufficient dipole orientation or inadequate mechanical deformation. *ASAIO Journal* 1992; 38: M201–M206.

Microporous polyurethane vascular prostheses fabricated by the spray phase inversion technique have good patency and excellent endothelialization in the rat aorta replacement model.[1] Grafts with a negative electric charge on the luminal surface may have a lower incidence of early thrombosis than neutral materials.[2,3] Moreover, some articles suggest that the electrical field generated by a graft when deformed by arterial flow pulsations increases the repopulation rate of endothelial cells on the luminal surface of the graft.[4] Thus, the hypothesis that a microporous wall structure and a negatively charged surface in contact with blood may potentiate each other and enhance graft patency and healing was evaluated by combining a known piezoelectric polymer material with a fabrication technique that produced a microporous wall structure.

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Materials and Methods

Graft Fabrication

Tubular prostheses (1.5 mm internal diameter and 250 μm wall thickness) were prepared with a 70% polyvinylidene fluoride and 30% trifluoroethylene (PVDF-TrFE) co-polymer by the spray phase inversion technique.[1,5] To freeze the molecular dipoles and create a permanent dipole moment in the material, some of the tubes were exposed to a high intensity electric field with a corona poling device **(Figure 1)** that established a high voltage drop across the wall of the graft. The charge developed by mechanical deformation of the piezoelectric material was evaluated with a laser interoferometer to measure the deformation and an impinger amplifier oscilloscope arrangement to measure the charge **(Figure 2)**.

The microstructure of the material was characterized by scanning electron microscopy (SEM, Hitachi, S-2700). The hydraulic permeability (HP) of the grafts was measured by collecting, during the first minute, the water volume passing through the graft wall at 120 mmHg.

The grafts were sterilized with ethylene oxide and stored in the dry state until implantation.

Implantation

Overall, 24 poled grafts and 24 unpoled grafts (15–22 mm in length) were implanted end-to-end by the same surgeon

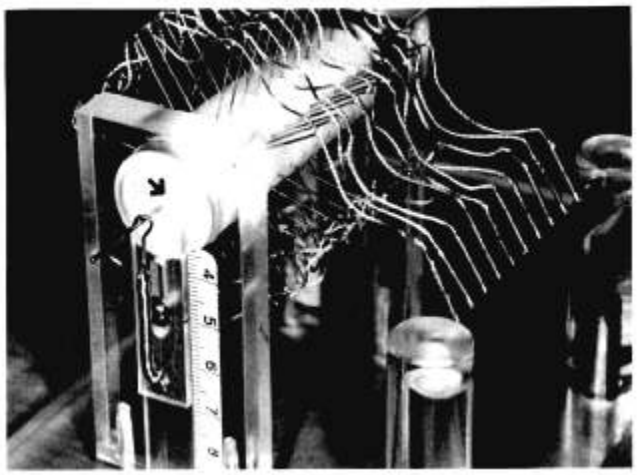

Figure 1. Corona poling device: a PVDF-TrFE graft (*arrow*) is placed in the center of the column of the device.

in the infrarenal aorta of 48 male Sprague-Dawley rats weighing 300–400 g. Pentobarbital sodium intraperitoneal anesthesia and standard microsurgical techniques were used. Two portions of the aorta around the level of the left renal vein for the proximal anastomosis, and proximal to the bifurcation of the aorta for the distal anastomosis, were independently dissected. The longest possible graft allowable by anatomic consideration was implanted in each case. The bypassed segment of the native aorta was ligated, divided on both ends, and left behind the implanted graft. Eight to nine individual 10-0 nylon sutures were used for each anastomosis. No antithrombogenic agents were administered pre- or postoperatively.

Retrieval

Specimens were excised at 2 days, 2 weeks, and 6 months after implantation. With the animal under deep intraperitoneal pentobarbital anesthesia, the entire vascular tree was perfused through the left ventricle and drained from the right atrium, first with 300–400 ml of a heparinized saline, and then with 150–200 ml of a modified Karnovsky's fixative (3% paraformaldehyde with 2.5% glutaraldehyde in phosphate-buffered saline). Thereafter, the specimen was resected together with surrounding tissues and margins of the native aorta. The graft was opened longitudinally, carefully examined, and photographed.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Institute of Laboratory Animal Resources" published by the National Institute of Health (NIH Publication No. 86-23, revised 1985).

Preparation of Specimens

For light microscopic examination, the specimen was embedded in resin (Historesin, Reichert-Jung Optische Werke AG, Wien, Austria), sectioned with a microtome (model 2050 Supercut, Reichert-Jung Optische Werke AG, Wien, Austria), and stained with hematoxylin and eosin.

The samples for SEM were dehydrated in graded alcohols (50–100%), critical point dried with CO_2, sputter coated with gold and palladium, and examined with a Hitachi Joel S-2700 microscope.

Evaluation of Piezoelectric Activity

To determine whether the piezoelectric properties of the material were still extant after implantation periods of 2 days and 2 weeks, a comparison was made between the original material from which each individual graft was made and the material retrieved from the animal after exposure to electrically conductive fluids. In such cases, the animals were perfused transcardially with heparinized saline only. Immediately after graft explanation, trypsin and sodium hypochlorite solutions were injected through the lumen and sprayed over the outer surface to remove the proteinaceous layer from the material. Then the tubes were freeze dried under a low vacuum overnight. The charge developed by mechanical deformation was characterized with the same equipment as previously described.

Statistical Analysis

The Fisher test was used to determine the significance of differences in patency between the P and UP groups. Differences were considered significant if the p value was < 0.05. Analysis of co-variance was applied to determine the significance of remaining piezoelectricity in the implanted P.

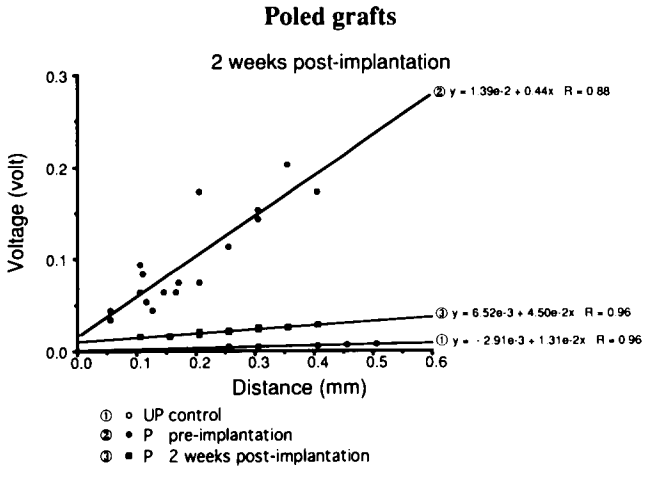

Figure 2. Effects of implantation on piezoelectric properties of the graft material. The voltage developed upon a standardized mechanical deformation in the poled graft explanted after 2 weeks is considerably below that measured with nonimplanted poled material but remains significantly higher than in the control UP ($p < 0.001$). Ordinate: voltage difference between the negatively charged internal surface and the positively charged external surface. Abscissa: actual linear deformation produced by the impinger.

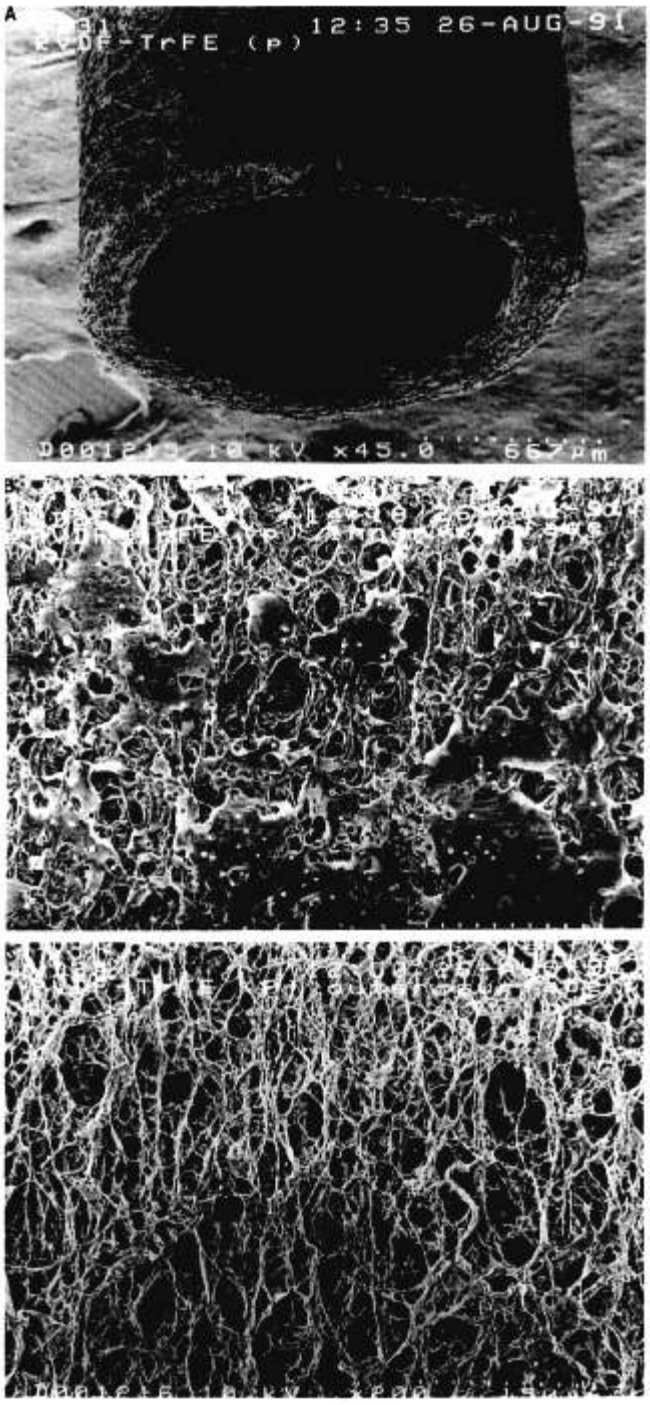

Figure 3. Scanning electron micrograph of a PVDF-TrFE graft: (A) global picture (original magnification ×45); (B) inner surface with a juxtaposition of membranous and microporous areas (original magnification ×200); (C) filamentous external surface (original magnification ×200).

Results

Our use of SEM revealed a patchy porous luminal surface (a mosaic of membranous and microporous areas) with voids measuring 10–60 μm, a 250 μm thick graft wall, and an entirely filamentous external graft surface with a pore size of 10–60 μm **(Figure 3)**. The HP averaged 41 ± 8 (mean ± standard deviation) ml/min/cm^2.

Patency rates in P were 100% (8/8) at 2 days, 100% (8/8) at 2 weeks, 75% (6/8) at 6 months, and 92% total (22 of 24). Patency rates in UP were 100% (8/8) at 2 days, 63% (5/8) at 2 weeks, 100% (8/8) at 6 months, and 88% total (21 of 24) **(Table 1)**. There was no statistical difference between the two groups at any time.

Both types of prostheses showed similar macroscopic and microscopic findings. After 2 days, fibrin, platelets, and erythrocytes were attached to the luminal surface, but there was no thick mural thrombus. The biologic material deposition was somewhat heavier on the poled grafts, but attached platelet counts were not significantly different. Numerous leukocytes had infiltrated the graft wall **(Figures 4 and 5)**. After 2 weeks, endothelialization was observed from both anastomoses in both groups. Leukocytes had almost disappeared, and fibroblasts were recognizable in the walls of both materials. Giant cells were occasionally seen on the external surface of both types of grafts. After 6 months, endothelialization was almost complete. Fibroblasts were less numerous, and collagen fibers had been deposited in the voids of the material. A few giant cells persisted around the graft outer surface **(Figures 6 and 7)**. The graft material appeared intact, and no dilatation or aneurysmal deformation was found.

The piezoelectric activity of the excised cleaned P was less than that of the control pre-implanted poled graft, but it remained significantly higher than in the control UP specimens ($p < 0.001$, **Figure 2**).

Discussion

Pulsed electric charges developed by piezoelectric vascular grafts may have the double advantage of discouraging early graft thrombosis related to platelet adhesion[2,3] and accelerating the development of a stable mature nonthrombogenic neointima.[4] The combination of piezoelectric properties and a microporous wall structure may enhance these effects.

Polyvinylidene fluoride, $(CH_2—CF_2)_n$, has the interesting property of being inherently polar, or piezoelectric, because of the arrangement of its fluorine and hydrogen atoms. When it directly crystallizes from the liquid form, it goes into the alpha form, which is not piezoelectric.[6,7] However, upon application of a mechanical stretching force, followed by exposure to a strong electrical field, as can be achieved by

Table 1. Patency of Poled and Unpoled PVDF-TrFE Grafts

	2 Days	2 Weeks	6 Months	Total
Poled grafts	100% (8/8)	100% (8/8)	75% (6/8)	92% (22/24)
Unpoled grafts	100% (8/8)	63% (5/8)	100% (8/8)	88% (21/24)

There was no statistically significant difference between the two groups at any retrieval time.

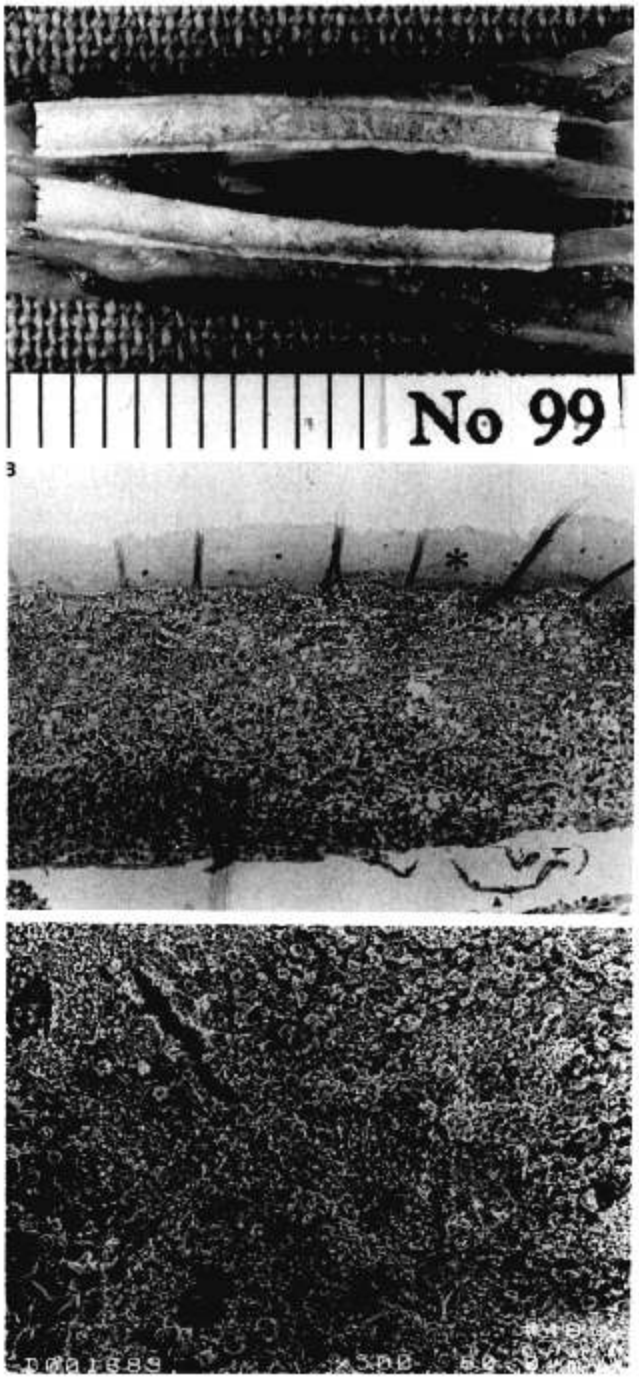

Figure 4. Poled PVDF-TrFE graft retrieved 2 days after implantation: (A) tiny red thrombi and fibrin layers; (B) in a cross-section of the graft wall, a fibrin layer* formed on the luminal surface (H&E, original magnification ×50); (C) scanning electron micrograph shows fibrin, platelets, and erythrocytes on the luminal surface (original magnification ×500).

corona poling, the fluorine atoms align themselves into the beta phase on the opposite side of the hydrogen atoms. Thus, the resulting all-trans configuration yields a polar material that can develop charges upon deformation of the material.[8] Because polyvinylidene fluoride must be stretched to convert the alpha form into the beta form, it does not lend

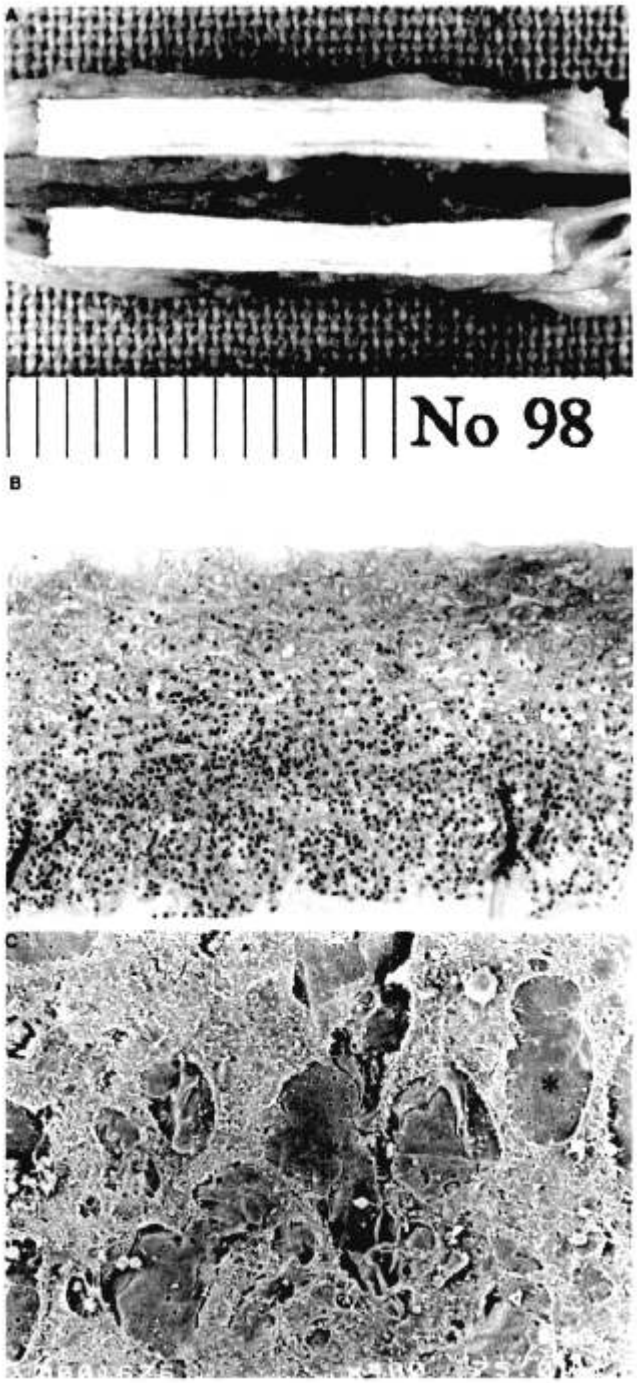

Figure 5. Unpoled PVDF-TrFE graft retrieved 2 days after implantation: (A) occasional tiny red thrombi and fibrin layers (B) in a cross-section of the graft wall, a thin fibrin layer is observed on the luminal surface, and numerous white blood cells have infiltrated within and outside the graft wall (H&E, original magnification ×50); (C) scanning electron micrograph reveals fibrin, platelets, and bare areas* on the luminal surface (original magnification ×400).

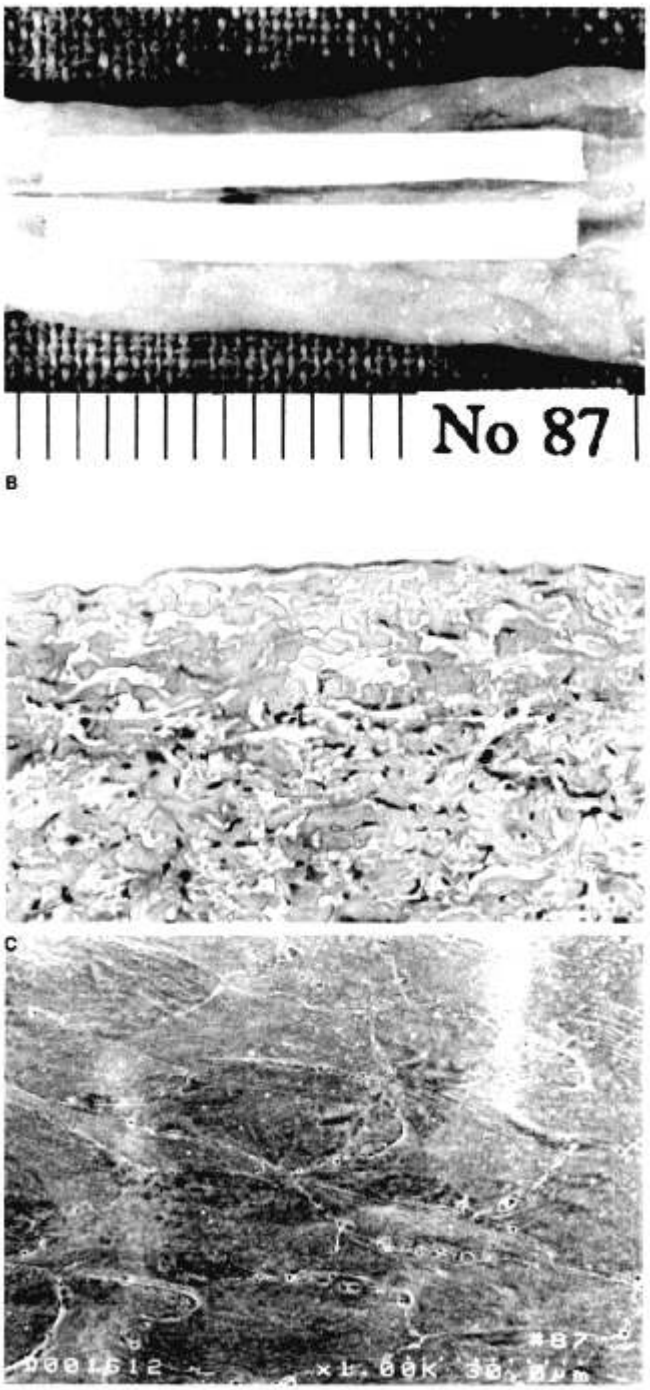

Figure 6. Poled PVDF-TrFE grafts at 6 months after implantation: (A) the graft luminal surface is macroscopically clean, with no visible red thrombi; (B) a cross-section of the graft wall shows well organized neointima and endothelial lining in the middle portion of the graft (H&E, original magnification ×100); (C) a scanning electron micrograph displays endothelial-like cells in the middle portion of the luminal surface (original magnification ×1000).

itself to the fabrication of a microporous structure by the spray phase inversion technique. However, a co-polymer of PVDF-TrFE might be used because it crystallizes directly into the polar phase of the all-trans configuration.[9] We were able to fabricate microporous piezoelectric tubes with this co-polymer, and these were the grafts we tested in this study. Both

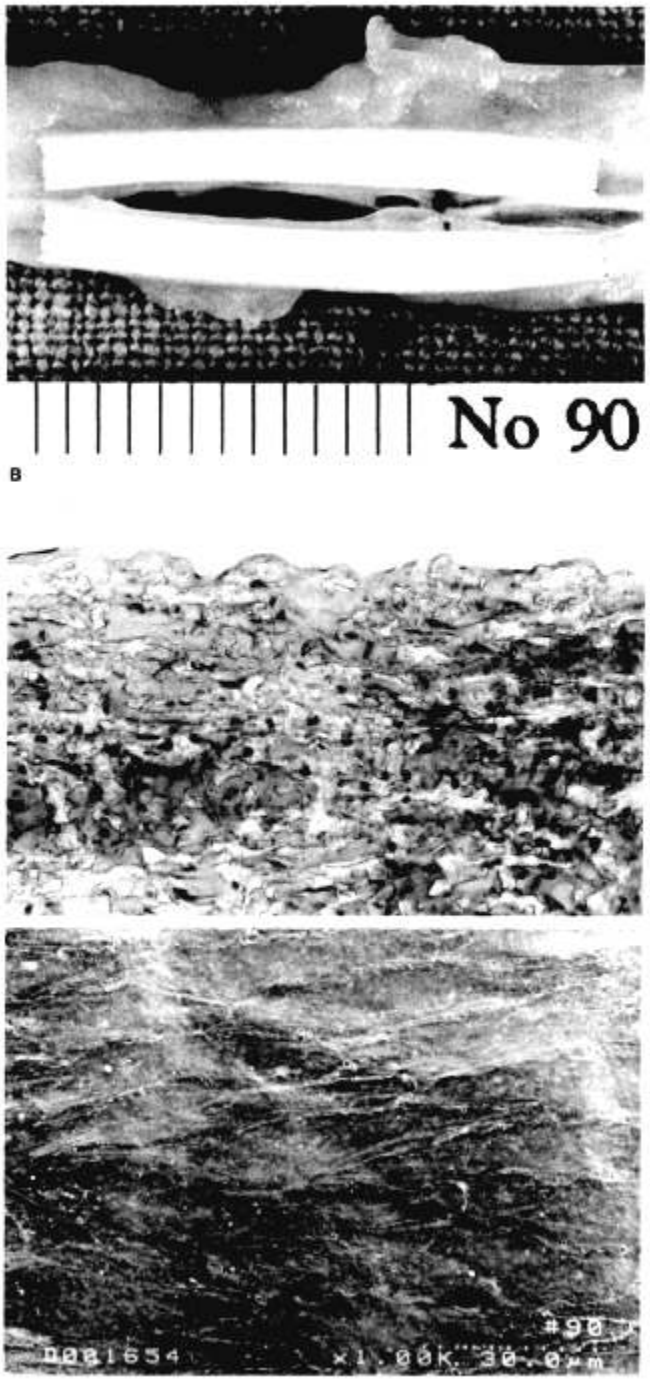

Figure 7. Unpoled PVDF-TrFE grafts at 6 months after implantation: (A) the graft luminal surface is macroscopically clean and shiny, with no visible red thrombi; (B) endothelialization is seen in the middle portion of the graft, with good tissue organization in the graft wall (H&E, original magnification ×100); (C) in a scanning electron micrograph, the middle portion of the graft luminal surface is covered with endothelial-like cells (original magnification ×1,000).

P and UP showed excellent patency and good graft healing in the rat infrarenal aorta replacement model, and there was almost no difference between the poled and unpoled material. Piezoelectric activity in the explanted P remained significantly higher than that in the control UP.

In terms of physical properties, both the PVDF-TrFE grafts and polyurethane grafts we previously reported were fabricated by the spray phase inversion technique. This method provided these different materials with a similar microporous structure; the HP of the graft types was almost the same.[1]

The spray phase inversion technique lends itself to the use of various materials when fabricating microporous structures of varying diameter, wall thickness, and porosity. For miniature vascular grafts, we have experimented with several types of polyurethane and polyurethane co-polymers of blends and obtained favorable results with vascular grafts of almost the same hydraulic permeability as reported here.[1] In the current study, the excellent biocompatibility of polyvinylidene fluoride,[10] the microporous structure, and the appropriate HP of the grafts were probably the dominant factors in the high patency rate achieved. The charges developed may have been too small to exert a biologic effect, either because of insufficient dipole orientation in the material or inadequate mechanical deformation of the graft wall. Further experiments will be necessary to optimize the mechanical and electrical properties of grafts made of PVDF-TrFE. It appears, however, that this co-polymer can help us in the investigation of the relationship of charged surfaces to thrombus formation and vascular healing.

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