Improvements in GORE-TEX® Vascular Graft Performance by Carmeda® BioActive Surface Heparin Immobilization

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Objectives: a performance improvement in small-diameter bypass grafts remains a clinical objective. The purpose of the present investigation was to evaluate the potential of enhancing the thromboresistance of ePTFE grafts using a bioactive heparinized graft luminal surface in a canine model.

Material and Methods: this study investigated the utility of heparin immobilization onto expanded polytetrafluoroethylene using Carmeda® BioActive Surface technology (CBAS-ePTFE) as a means of improving vascular graft thromboresistance. Graft luminal surfaces were covered uniformly with the stably bound, end-point immobilized heparin.

Results: acute canine (5 greyhounds) interposition experiments comparing CBAS-ePTFE grafts to control ePTFE grafts showed that CBAS-ePTFE grafts remained patent and had significantly greater thrombus-free luminal surface ($p < 0.05$). In a chronic canine (16 greyhounds) interposition experiment, significantly improved patency ($p < 0.05$) was observed with CBAS-ePTFE grafts compared to controls. Long-term in vivo heparin bioactivity was demonstrated on CBAS-ePTFE grafts explanted between 1 and 12 weeks. On all CBAS-ePTFE grafts, heparin activity levels ranged from 15–25 pmol/cm^2 and did not differ significantly ($p > 0.05$).

Discussion: these results support the conclusion that a stable, CBAS-ePTFE surface provides improved thromboresistance and improved patency in canine interposition models. Maintenance of heparin catalytic activity on the graft surface in vivo likely contributes to this outcome and holds promise for the utility of this graft surface for clinical applications.

Key Words: ePTFE; Heparin; Vascular graft; Patency; Thromboresistance.

Introduction

Improved patency of synthetic vascular grafts has been a sought-after clinical goal since their introduction nearly four decades ago. Expanded polytetrafluoroethylene (ePTFE) vascular grafts have demonstrated a long history of good clinical performance as vascular conduits. While ePTFE grafts show patency performance comparable to saphenous vein in above-knee clinical applications,[1–4] there continues to be room for performance improvement in grafts used for ≥6-mm diameter applications. In small-diameter applications (<6 mm diameter) such as below-knee bypass, however, all prosthetic vascular graft materials are less than desirable in terms of performance relative to autologous vein. Much effort has gone into development of prosthetic small-diameter grafts; but, due to the complex interactions of blood-biomaterial contact surfaces, a clinically acceptable small-diameter graft has yet to be developed.[5] Bypass graft failure generally occurs as a consequence of thrombus deposition on the graft luminal surface, anastomotic intimal hyperplasia, or progression of vascular disease.[5] Although anastomotic hyperplasia and disease progression are important factors, reducing the propensity for acute thrombotic failure by improving luminal-surface thromboresistance has significant potential for improving clinical performance of small-diameter grafts.

One potential strategy for reducing thrombogenicity of prosthetic materials is to bind heparin to the surface.[6] Heparin is a polysaccharide anticoagulant with a long history of clinical use.[7] Consequently, a number of heparin coating technologies have been developed based upon different heparin binding modalities.[6,8–11] Some of the ideal functional characteristics of a heparinized graft include uniform heparinization, retention of heparin on the graft surface, and maintenance of heparin bioactivity. One of the most clinically successful heparin technologies has

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been the Carmeda® BioActive Surface (CBAS).[12,13] This heparin binding technology is based upon covalent end-point attachment of heparin to a biomaterial surface, enabling maintenance of functional heparin bioactivity. CBAS immobilization has been shown to lower platelet deposition, decrease the inflammatory response including complement activation in oxygenator membranes and extracorporeal circuits,[14–16] and to reduce thrombogenicity in heart-assist devices[17] and coronary stents.[18–21]

The purpose of the present investigation was to evaluate the potential of enhancing the thromboresistance of GORE-TEX® Vascular Grafts using CBAS technology to provide a bioactive heparinized graft luminal surface. We investigated the hypothesis that stable CBAS heparin immobilization on an ePTFE vascular graft provides improved thromboresistance relative to untreated ePTFE control grafts.

Materials and Methods

Graft materials

Control grafts were commercially available ePTFE grafts (GORE-TEX® Vascular Grafts, W. L. Gore & Associates, Inc., Flagstaff, AZ, U.S.A.). Test grafts were the same ePTFE grafts treated with immobilized heparin, using the proprietary Carmeda BioActive Surface technology, and are referred to as CBAS-ePTFE grafts. This CBAS heparin immobilization produces a surface microstructure having stable, covalently bound heparin. The covalent end-point linkages maintain the catalytic bioactivity of the antithrombin (AT) sites of the bound heparin.[13] Uniformity of luminal surface heparinization was assessed with toluidine blue staining. The graft microstructures were assessed by Scanning Electron Microscopy (SEM).

Acute in vivo interposition

This study was conducted to compare the relative thromboresistance of CBAS-ePTFE grafts to that of control ePTFE grafts. Relative thromboresistances were assessed by comparing percentages of thrombus-free luminal surface in an acute canine carotid artery interposition model that shows a propensity for luminal thrombus build-up, often resulting in occlusion of control ePTFE grafts. Thrombotic propensity was enhanced by using small-diameter (3 mm × 4 cm) grafts without antiplatelet or antithrombotic agents. The hypothesis tested was that the bioactive surface of CBAS-ePTFE grafts would inhibit thrombus

accumulation thereby yielding greater thrombus-free luminal surface compared to untreated control ePTFE grafts. Five pairs of grafts implanted in five adult greyhounds were evaluated. This study, and all subsequent *in vivo* studies, were reviewed and approved by the Animal Care and Use Committee in accordance with the Animal Welfare Act.

CBAS-ePTFE and control ePTFE grafts were randomized to the left or right carotid arteries and interposed via end-to-end anastomoses. Blood flow then was restored for 2 h. At the end of the flow period, grafts were excised and rinsed carefully with saline to remove residual blood. Each then was carefully opened longitudinally and photographed. Percent thrombus-free luminal surfaces (%TFS) were computed from luminal-surface thrombus areas and total luminal surface areas measured from digital images of the photographs using Bioquant software (BQ TCW95 V2.50.4; R&M Biometrics, Inc., Nashville, TN, U.S.A.).

Chronic in vivo patency

Chronic *in vivo* performance studies were conducted in a small-diameter (3 mm × 3 cm) canine carotid interposition model to compare the chronic patency performance of CBAS-ePTFE grafts to that of control ePTFE grafts. The hypothesis that CBAS-ePTFE grafts would have improved patency performance compared to control ePTFE grafts was tested.

The experiment included 16 greyhounds, each of which received bilateral end-to-end carotid interpositions, one CBAS-ePTFE and one control ePTFE. To model clinical pharmacology, daily aspirin (325 mg) and dipyridamole (50 mg) were begun two days prior to implant and continued throughout the duration of the study. Prior to cross clamping, animals received intraoperative heparin (100 IU/kg) which was not reversed. Weekly duplex ultrasound was used to assess *in vivo* graft patency. Patencies of graft pairs retrieved at postoperative intervals up to 180 days were compared.

In vivo heparin function

This study was conducted to assess *in vivo* duration of heparin bioactivity on CBAS-ePTFE grafts. The hypothesis that heparin bioactivity would persist under chronic *in vivo* exposure to blood flow was tested in a canine model. Twenty grafts (15 CBAS-ePTFE and 5 control ePTFE) were implanted in ten adult female greyhounds (BW > 28 kg). Each animal received bilateral, side-to-side, 6 mm × 12 cm

aorto-iliac bypasses. Five received bilateral CBAS-ePTFE grafts and five received one CBAS-ePTFE graft and one contralateral, negative control ePTFE graft. Implant durations were 1, 2, 4, 8, and 12 weeks. Two dogs (grafts: $n_{cbas} = 3$; $n_{ctrl} = 1$) were implanted per time point. Beginning 48 h prior to surgery, each animal received aspirin (81 mg) bid which was continued, minus the day of surgery, through the duration of the experiment. Prior to cross clamping, animals received intraoperative heparin (100 IU/kg) which was not reversed. At explant, two representative 2 cm segments from each graft were rinsed in isotonic saline, cleared of endogenous heparin in buffered 0.6 M borate in 0.01 M NaCl (pH = 9), and rinsed again with deionized H_2O. Samples then were assayed for luminal-surface heparin activity measured as capacity to bind AT and expressed as AT bound per unit surface area (pmol/cm²) as described.[22,23]

Statistical analyses

Percent thrombus-free surface of acute *in vivo* interposition grafts were compared with a paired Student's *t*-test.[24] Chronic *in vivo* patencies were evaluated with Kaplan–Meier life table analysis and compared with a log-rank test.[25] One-way analysis of variance (ANOVA) was used to compare mean heparin activities on postexplant graft samples from the *in vivo* heparin function study.[24]

Results

Heparin uniformity and microstructure

Uniform CBAS heparin immobilization on the ePTFE microstructure is illustrated by the stained CBAS-ePTFE graft in Figure 1. The similarly treated control ePTFE graft segment in Figure 1 is unstained. The SEM surface appearance of the CBAS-ePTFE shows the typical ePTFE node-fibril microstructure (Fig. 2), thereby confirming that the ePTFE microarchitecture is unaffected by the heparin immobilization. The mechanical properties of the CBAS-immobilized grafts (data not shown) also were comparable to previously published data on the control ePTFE grafts.[26]

Acute interposition performance

In the five graft pairs, three control ePTFE grafts occluded while all CBAS-ePTFE grafts remained patent. In general, control ePTFE grafts exhibited
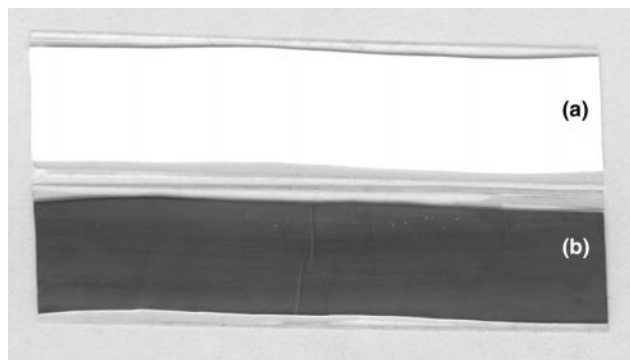

Fig. 1. Heparin coating uniformity demonstrated by toluidine blue staining. (a) Control ePTFE graft showing no affinity for the toluidine blue stain. (b) CBAS-ePTFE graft showing uniformly strong affinity for the stain.
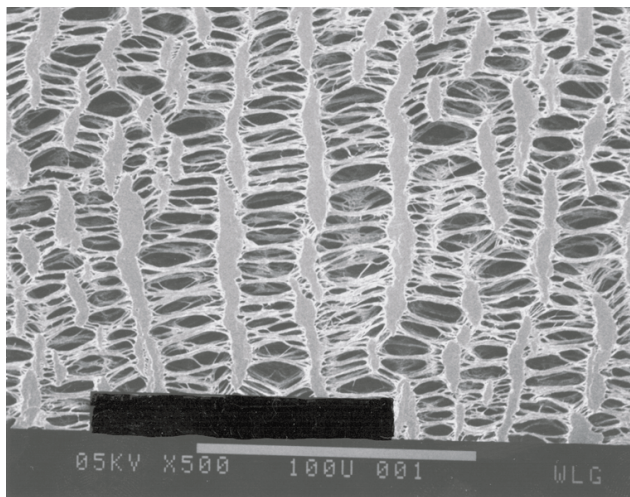

Fig. 2. Scanning electron micrograph of a CBAS-ePTFE graft luminal surface showing typical node-fibril microstructure indicating that the CBAS-immobilization does not alter the ePTFE microstructure.

thrombus over substantial portions of their luminal surfaces while CBAS-ePTFE grafts exhibited almost no thrombus except for occasional, small accumulations located mostly at the anastomoses. Control ePTFE grafts also exhibited greater degrees of anastomotic thrombus than were observed on CBAS-ePTFE grafts.

Luminal thrombus-free surface was significantly greater ($p < 0.05$) on the CBAS-ePTFE grafts (%TFS = 91.8 ± 13.2) than on the contralateral control ePTFE grafts (%TFS = 33.2 ± 32.4) (Fig. 3). A representative graft pair illustrating the difference in thromboresistance is shown in Figure 4.

Chronic patency performance

Data from all implanted grafts were analyzed including those from one animal that did not receive
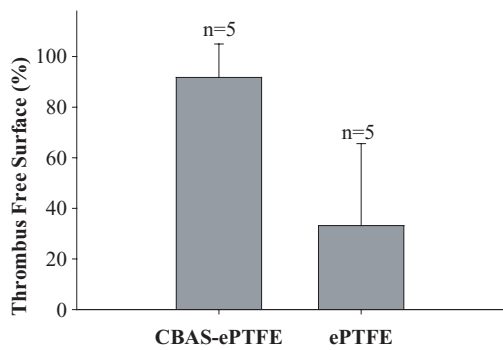
Fig. 3. Mean percent thrombus-free surface of 3 mm-diameter CBAS-ePTFE and control ePTFE grafts implanted acutely (2 h) as paired bilateral carotid artery interpositions in the canine model. Error bars = ±1 SD.
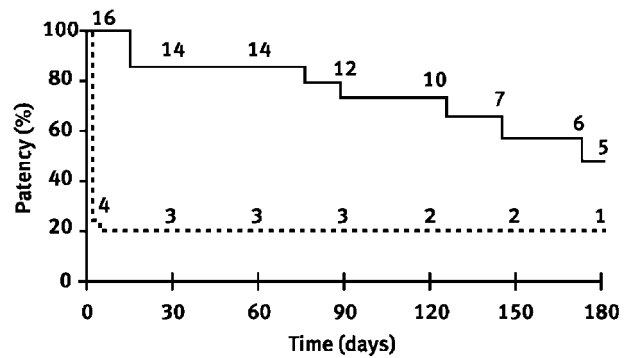

Fig. 5. Kaplan–Meier summary graph of patencies of 3 mm CBAS-ePTFE and control ePTFE grafts from canine carotid artery interpositions. CBAS-ePTFE (—); control ePTFE (----). Numbers above the lines indicate grafts remaining at risk.
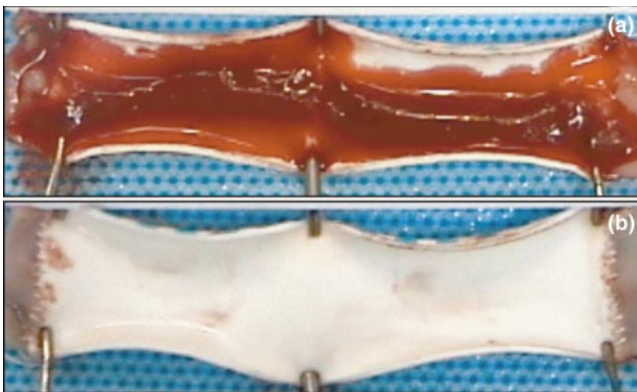

Fig. 4. Enhanced thromboresistance of a 3 mm-diameter CBAS-ePTFE graft compared to a contralateral control ePTFE graft from an acute (2 h) bilateral canine carotid artery interposition model. (a) Control ePTFE graft showing thrombus accumulation along the graft length. (b) CBAS-ePTFE graft showing minimal thrombus accumulation.

Fig. 6. Mean heparin activities (pmol/cm^2) measured as AT binding on replicate samples from CBAS-ePTFE and control ePTFE grafts explanted from canines at 1, 2, 4, 8, and 12 weeks. Error bars = ±1 SD.

antiplatelet medications. After 3 days, 13 of 16 control grafts had failed whereas all 16 CBAS-ePTFE grafts were patent. At 180 days, patency results for CBAS-ePTFE grafts were: five patent; four censored patent; and seven failed. Patency results for control ePTFE grafts were: one patent; two censored patent; and 13 failed. Compared to controls, CBAS-ePTFE grafts showed significantly improved patency ($p < 0.05$). The patency results are summarized graphically in Figure 5.

In vivo *heparin function*

Measurable heparin activity was detected on all explanted CBAS-ePTFE grafts at each time point examined out to 12 weeks. Mean activities on explanted CBAS-ePTFE graft samples ranged from
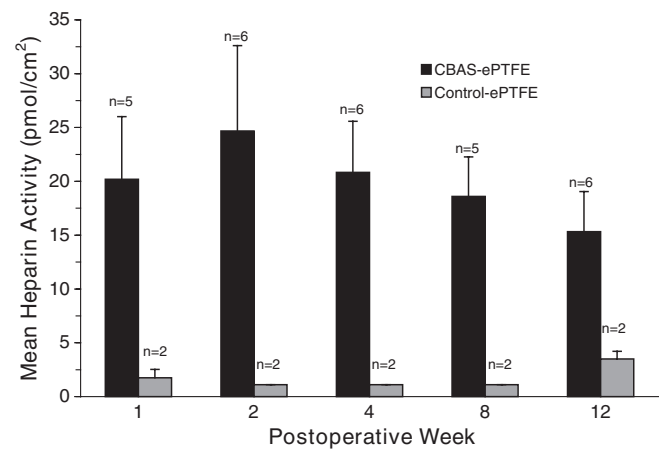
24.7 ± 7.9 pmol/cm^2 measured at 2 weeks to 15.3 ± 3.7 pmol/cm^2 measured after 12 weeks (Fig. 6). Differences between mean heparin activities of explanted grafts at the various time points were nonsignificant ($p > 0.05$). Mean heparin activities on control ePTFE grafts consistently were near or below the lower detection limit of the assay (Fig. 6).

Discussion

Early graft thrombosis has remained an obstacle in the development of a clinically acceptable, small-diameter prosthetic vascular graft (<6 mm diameter). Particularly in low-flow, high-resistance situations, no synthetic graft has shown sufficient resistance to thrombus accumulation to enable routine adoption in more demanding clinical applications such as below-knee bypass. In below-knee applications,

synthetic vascular grafts are generally recognized to have one-year patencies of around 60%.[4] Approaches to this clinical problem have included heparin bonding to biomaterial surfaces. Heparinization of ePTFE has been reported to inactivate thrombin; however, early sheep model testing did not indicate any difference from untreated control grafts.[27] Another heparin modification technique using TDMAC (tridodecyl-methylammonium) has been shown to reduce shedding of microemboli from an ePTFE graft.[28] Recently, a heparin-bonded Dacron graft has been reported to have 70% femoropopliteal patency at 1 year.[29] Collectively, these studies suggest the promise of a small-diameter graft alternative based upon heparinization technologies.

Important considerations of heparinization technologies are the bonding method and the long-term function of the heparin. The experimental CBAS-ePTFE graft has the following key properties: uniform heparinization as shown in Figure 1; and sustained *in vivo* heparin bioactivity (Fig. 6). Previous attempts to heparinize ePTFE did not incorporate these parameters which could explain the mixed *in vivo* results obtained to date.[27,30]

The findings from the present study demonstrate the improved thromboresistance of the immobilized heparin surface compared to an unmodified ePTFE graft *in vivo* in canine carotid artery models. This improvement was demonstrated both in acute and chronic time frames. The greater thrombus-free surface (Fig. 3) and obviously less thrombus accumulation (Fig. 4) observed on 100% of the CBAS-ePTFE grafts in the acute comparisons indicate that the early blood interactions are affected by the presence of bioactive heparin.

The evaluation of chronic graft patency as a measure of performance clearly demonstrated that a CBAS-ePTFE graft surface improves patency compared to untreated control ePTFE grafts in the canine carotid interposition model. The patency difference that was apparent early in the postimplant period, and was sustained out to the 6-month end point (Fig. 5), likely was attributable to inhibition of thrombus formation conferred by the immobilized heparin.

It is recognized clinically that bypass graft failure may be caused by thrombotic processes, anastomotic hyperplasia, and/or disease progression. CBAS-ePTFE appears especially to affect surface thrombotic processes such that a greater proportion of CBAS-ePTFE grafts remain patent during the initial high-risk period for thrombotic failure. Consequently, though secondary failure modalities such as anastomotic hyperplasia and disease progression still may affect graft performance, the long-term patency rates

may be influenced positively by reduced thrombus accumulation in the first several months of implant. While there is no conclusive evidence regarding the influence of CBAS-ePTFE on anastomotic hyperplasia, it seems likely that sustained heparin activity may reduce thrombus accumulation, thereby decreasing the scaffold available for both midgraft and anastomotic remodeling events. Additional studies will be necessary to investigate this hypothesis.

The observed modulation of initial thrombus accumulation is consistent with reports of reduced platelet deposition on other CBAS-immobilized devices, including stents and oxygenator membranes.[12,15,31] Baboon *ex vivo* shunt testing of CBAS-treated coronary stents has shown a significant decrease in platelet deposition on heparinized stents compared to untreated control stents.[31] Although the highly negative charge associated with heparin may possibly explain this phenomenon, the decrease was correlated specifically with the immobilized high AT-affinity heparin fraction. In addition, significantly reduced platelet deposition also was correlated with heparin activity as low as $7\,pmol/cm^2$.[31] In the present study, under sustained exposure to blood constituents, heparin activities persisted *in vivo* for 3 months at levels between 15 and $25\,pmol/cm^2$, well above the activity shown to reduce platelet deposition on stents. Sustained AT binding is consistent with previous studies demonstrating maintenance of heparin activity for 4 months in porcine aortae,[32] and for upwards of 2 months clinically on the Berlin Heart Assist Device.[19] The improved performance of CBAS-ePTFE may derive from both the stability of the heparin surface and its sustained potential to bind AT *in vivo* (Fig. 6).

The precise mechanism at work on the CBAS-ePTFE graft cannot fully be explained by the present study. It seems likely though, that long-term maintenance of active heparin on the ePTFE surface influences not only the extrinsic, platelet-mediated pathways, but also the intrinsic coagulation cascade. CBAS-immobilized heparin previously has been shown to block Factor XII initiation of the coagulation cascade,[33] catalyze the rate of inactivation of thrombin by antithrombin effectively blocking conversion of fibrinogen to fibrin,[3] and improve resistance of synthetic surfaces to platelet deposition.[34] Furthermore, the heparin surface also is known to influence initial protein deposition on various biomaterials;[35] thus, it also is likely that the heparin-modified ePTFE surface is more hemocompatible due to its influence on protein adsorption during initial blood contacting events.[31]

Long-term clinical experience with CBAS-coated coronary stents has demonstrated that use of these stents effectively minimizes subacute thrombosis,[20]

reduces major adverse cardiac events compared to primary PTCA,[19] and can result in improved sustained patency.[21] The results from the present study are consistent with the hypotheses that CBAS-ePTFE is more thromboresistant than untreated control ePTFE and a CBAS-ePTFE surface improves short-term and long-term graft patency in the canine arterial interposition model. Further, the data illustrating sustained heparin activity on the CBAS-ePTFE graft surface *in vivo* support the inference that improved thromboresistance results from stable CBAS heparin immobilization. Given these findings and the extensive clinical experience to date with CBAS-treated medical devices, CBAS-ePTFE appears to hold promise for improved prosthetic graft performance in vascular reconstructions.

Acknowledgements

References

1 VEITH FJ. Femoral-popliteal-tibial occlusive disease. In: More WS, ed. *Vascular Surgery – A Comprehensive Review.* Philadelphia: W. B. Saunders Company, 1993; **27**: 465–489.

2 VEITH FJ, GUPTA SK, ASCER E *et al.* Six-year prospective multi-center randomized comparison of autologous saphenous vein and expanded polytetrafluoroethylene grafts in infrainguinal arterial reconstructions. *J Vasc Surg* 1986; **3**: 104–114.

3 QUINONES-BALDRICH WJ, PREGO AA, UCELAY-GOMEZ R *et al.* Long-term results of infrainguinal revascularization with poly-tetrafluoroethylene: A ten-year experience. *J Vasc Surg* 1992; **16**: 209–217.

4 CONSENSUS DOCUMENT. Chronic Critical Leg Ischaemia. *Eur J Vasc Surg* 1992; **6**(Suppl. A): 1–28.

5 MONETA, GL, PORTER JM. Arterial substitutes in peripheral vascular surgery: a review. *J Long-Term Eff Med Implants* 1995; **5**: 47–67.

6 LARM O, LARSSON R, OLSSON P. Surface-immobilized heparin. In: Lane DA, Lindahl U, Arnold E, eds. *Heparin.* London, 1988; 597–608.

7 HIRSH J, ANAND SS, HALPERIN JL, FUSTER V. AHA Scientific Statement. Guide to anticoagulant therapy: heparin. *Circulation* 2001; **103**: 2994–3018.

8 LI-CHIEN HSU. Heparin-coating of bypass circuits. *Perfusion* 1991; **6**: 209–219.

9 ANDERSON AB, CLAPPER DL. Coatings for blood-contacting devices. *Med Plas Biomaterials* 1998; **5**: 18–26.

10 AL-LAMEE K, TAKTAK Y. New methods of surface modification and covalent attachment of heparin. *Med Device Technology* 1998; 24–27.

11 KIM SW, JACOBS H. Design of nonthrombogenic polymer surfaces for blood-contacting medical devices. *Blood Purif* 1996; **14**: 357–372.

12 MATHEVE C. Clinical evidence of improved biocompatibility using heparin-coated surfaces. *Perfusion* 1996; **11**: 264–269.

13 RIESENFELD J, OLSSON P, SANCHEZ J, MOLLNES TE. Surface modification with functionally active heparin. *Med Device Technology* 1995; **6**: 24–31.

14 BOZDAYI M, BOROWIEC J, NILSSON L *et al.* Effects of heparin coating of cardiopulmonary bypass circuits on *in vitro* oxygen free radical production during coronary bypass surgery. *Artif Organs* 1996; **20**: 1008–1016.

15 FUKUTOMI M, KOBAYASHI S, NIWAYA K *et al.* Changes in platelet, granulocyte, and complement activation during cardiopulmonary bypass using heparin-coated equipment. *Artif Organs* 1996; **20**: 767–776.

16 BOROWIEC J, THELIN S, BAGGE L *et al.* Heparin-coated circuits reduce activation of granulocytes during cardiopulmonary bypass. *J Thoracic Cardiovasc Surg* 1992; **104**: 642–647.

17 KAUFMANN F, HENNIG E, LOEBE M *et al.* Improving the anti-thrombogenity of artificial surfaces through heparin coating – clinical experience with the pneumatic extracorporeal Berlin Heart assist device. *Cardiovasc Engineering* 1996; **1**: 40–44.

18 SERRUYS PW, EMANUELSSON H, VAN DER GIESSEN W *et al.* Heparin-coated Palmaz-Schatz stents in human coronary arteries. *Circulation* 1996; **93**: 412–422.

19 SERRUYS PW, GRINES CL, STONE GW *et al.* Stent implantation in acute myocardial infarction using a heparin-coated stent: a pilot study as a preamble to a randomized trial comparing balloon angioplasty and stenting. *Int J Cardiovasc Intervent* 1998; **1**: 19–27.

20 SERRUYS PW, VAN HOUT B, BONNIER H *et al.* Randomised comparison of implantation of heparin-coated stents with balloon angioplasty in selected patients with coronary artery disease (Benestent II). *Lancet* 1998; **352**: 673–681.

21 DZAVIK V, CARERE RG, TEO KK *et al.* An open design, multi-centre, randomized trial of percutaneous transluminal coronary angioplasty versus stenting, with a heparin-coated stent, of totally occluded coronary arteries: rationale, trial design and baseline patient characteristics. *Can J Cardiol* 1998; **14**: 825–832.

22 LARSEN ML, ABILGAARD U, TEIEN AN, GJESDAL K. Assay of plasma heparin using thrombin and the chromogenic substrate H-D-Phe-Pip-Arg-pNA (S-2238). *Thromb Res* 1978; **13**: 285–288.

23 PASCHE B, ELGUE G, OLSSON P *et al.* A binding of antithrombin to immobilized heparin under varying flows conditions. *Artif Organs* 1991; **15**: 481–491.

24 SOKAL RR, RHOLF FJ. *Biometry,* 2nd edn. New York: W. H. Freeman and Co., 1981; v–xviii: 1–859.

25 LEE ET. *Statistical Methods for Survival Data Analysis,* 2nd edn. New York: John Wiley & Sons, Inc., 1992; vii–xii: 1–482.

26 BOYCE B. Physical characteristics of expanded polytetrafluoroethylene grafts. *Biol Synthet Vasc Prosthes* 1982; **33**: 553–561.

27 BERGQVIST D, JENSEN N, PERSSON NH. Heparinization of poly-tetrafluoroethylene (ePTFE) grafts. The effect on pseudointimal hyperplasia. *Int Angiology* 1988; **7**: 65–69.

28 RITTER EF, KIM YB, REISCHL HP *et al.* Heparin coating of vascular prostheses reduces thromboemboli. *Surg* 1997; **122**: 888–892.

29 DEVINE C, HONS BA, MCCOLLUM C. Heparin-bonded Dacron or polytetrafluoroethylene for femoropopliteal bypass grafting: a multicenter trial. *J Vasc Surg* 2001; **33**: 533–539.

30 BJORCK CG, BERGQVIST D, ESQUIVEL CO *et al.* Thrombin uptake and inhibition on heparinized polytetrafluoroethylene (PTFE) grafts and native sheep vessels. *Thromb Res* 1986; **42**: 605–612.

31 KOCSIS JF, LLANOS G, HOLMER E. Heparin-coated stents. *J Long-Term Eff Med Implants* 2000; **10**: 19–45.

32 ARNANDER C, BAGGER-SJOBACK D, FREBELIUS S *et al.* Long-term stability *in vivo* of a thromboresistant heparinized surface. *Biomaterials* 1987; **8**: 496–499.

33 MOLLNES TE, VIDEM V, CHRISTIANSEN D *et al.* Platelet compatibility of an artificial surface modified with functionally active heparin. *Thromb Haemost* 1999; **82**: 1132–1136.

34 SANCHEZ J, ELGUE G, RIESENFELD J, OLLSON P. Studies of adsorption, activation, and inhibition of factor XII on immobilized heparin. *Thromb Research* 1998; **89**: 41–50.

35 VROMAN L. The life of an artificial device in contact with blood: initial events and their effect on its final state. *Artif Device* 1988; **64**: 352–357.