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Evaluation of the Thrombogenecity of Microvascular prosthesis by in vivo Microscopy

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Expanded polytetrafluoroethylene(ePTFE) grafts 4mm long and 1mm in diameter were implanted into the iliac artery of 100-150g male rats using standard microvascular technique. Prior to clamp removal, the cremaster muscle was isolated as an island flap based on the iliac artery and observed using intravital fluorescene microscopy. Fields which contained a bifurcation of a first order arteriole(80-100 μm diameter) into second order arteriole(50-80 μm) were chosen for observation. Platelets were labeled in vivo with acridine red to visualize and quantify the aggregates. Images of microemboli were counted manually and the area was measured by computerized planimetry. Six control grafts were implanted with no further processing, six were irrigated with heparin, and six were coated with tridodecylmethylammonium chloride(TDMAC) and heparin. Most thrombi appeared within the first five minutes after implantation in all groups. The total number of emboli observed in the control group was 91 per animal, in the heparin irrigation group it was 84, and in the TDMAC-heparin group it was 22. The total thrombus area observed per animal was 137,660±29,467 μm² in the control group, 79,040±10,893 μm² in the heparin irrigation group, and 17,498±6,059 μm² in the TDMAC-heparin group($p<.01$ vs control or heparin irrigation group). With this results we could find that heparin irragation and TDMAC-heparin coating appear to reduce the number, size, and total amount of microemboli generated by ePTFE graft implantation and apparent thromboresistant property of TDMAC-heparin coating may have widespread application in many clinical and research areas and this experimental model can be used for evaluation of other graft matrials.

Key Words : *ePTFE, Thrombogenecity, Cremaster, Graft.*

INTRODUCTION

Surface induced thrombogenesis limits the use of vascular prostheses. Even when patent, downstream microemboli may adversely effect micro-

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vascular perfusion. In addition, in vivo tests of thrombogenicity may not correlate well in vivo performance of biomaterials. Thus, the purposes of this study were to develop a model for quantifying the microemboli which are produced by microvascular prosthses and to test the hypothesis that heparin bonding reduces microemboli formation.

MATERIALS AND METHODS

General Animal Preparation

Male CD virus antibody free rats (Dominion Labs, Dublin, VA) weighing 100-150 gm were anesthetized with intraperitoneal sodium penthotal (Nembutal, Abbott Labs, Chicago, IL) of 60 mg/kg body weight with supplemental doses as required. The entire lower abdomen and scrotal area were shaved. The rats were placed on a heating pad and the rectal temperature was maintained at 37± 1℃.

Anatomy and Dissection

The cremaster muscle was isolated on its vascular pedicle as same manner previously described (Acland et al., 1989) and is shown in Fig. 1. A long incision from the anterior superior iliac spine to the upper portion of the scrotum was made, and the lower portion of the external oblique muscle was exposed and incised to expose the iliac artery. The hypogastric artery and all small branches of the iliac artery were ligated with 8-0 nylon or cauterized with bipolar coagulater except the pudic-epigastric artery and the superior pudendal artery. Then a loop was formed around the common femoral artery. The right iliac artery was then transected and a 4mm long and 1mm in diameter ePTFE interpositional graft was implanted in the arterial gap(Fig. 1). Prior to release of the clamp, the scrotum was incised and separated from the cremaster muscle. The dorsl avascular area of the cremaster muscle was then cut and separated from the testicle. The rat was then placed on a clear plastic stage with warm water circulating under the rat torso as well as the cremaster muscle(Fig. 1). Before releasing the clamp, the ipsilateral femoral artery was ligated with 6-0 silk.

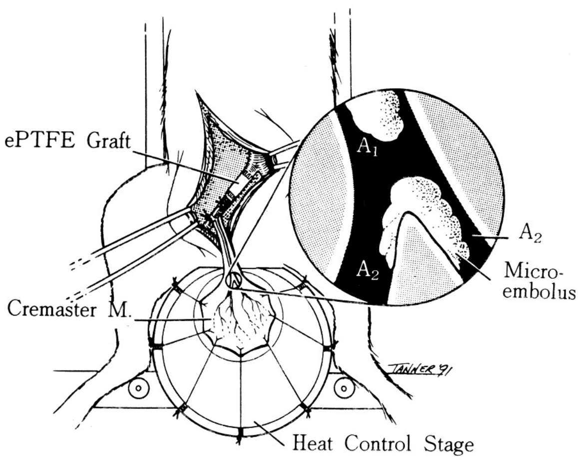


Fig. 1. An ePTFE graft was inserted in the hypogastric artery and downstream microemboli were evaluated on isolated cremaster muscle.

Platelet Labeling

To visualize and quantify microemboli, platelets were labeled with acridine reddimethyl [6-(methylamino)-3H-xanthen-3-yliden] ammonium chloride (Pfaltz and Bauer, Waterbury, CT). A working solution was prepared by the method of Burger and Salzman(Berger and Salzman, 1974) by dissolving 50 mg of the acridine red in 0.5 ml ethanol and diluting to a total of 10ml with saline, resulting in a final concenturation of 5mg/ml at a pH of 3.3. The solution was then filtered through a filter of 0.22 μm pore. A fresh solution was made each day.

Heparin irrgation

In the heparin irrigating group, the prostheses were dippened in 1 : 10,000IU heparin solution for 5 minutes before implantation and were irrigated with this solution during implantation.

TDMAC-Heparin Coating

The six TDMAC-heparin grafts wers prepared by immersion in a solution of 25mg/ml tridodecylmethylammonium chloride(TDMAC) in ethanol for 30 minute and air dried overnight. Grafts were washed in distilled water and then immersed in a 10.000IU/ml heparin solution and again air dried. Finally. grafts were immersed in heparinized saline for at least 10 minutes prior to implantation.

Intravital Microscopy

Observation were made with a Zeiss ACM

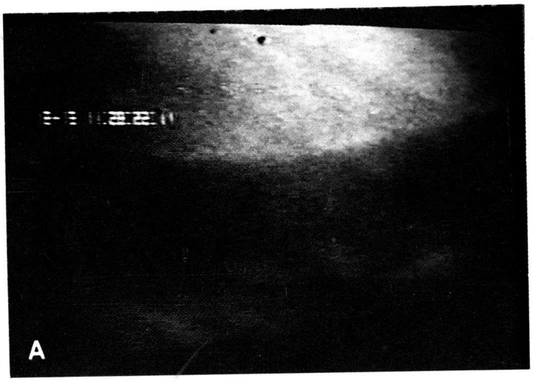
fluorescence microscope using a 75 watt Xenon arc lamp. A Zeiss FT 520-560 excitation filter and LP 590 barrier filter was used. Image were recorded with a Cohu 4410 silicon intensified target camera and Toshiba DX-7 videocassette recorder and viewed on a Panasonic WV-5410 video monitor. Time accurate to 0.01 second was encoded on the recorded image using a For-A VTG-33 videotimer. The diameter of each vessel was measured using a videocaliper(Microcirculation Research Institute, Texas A & M University, College Station, TX).

Image Analysis

Videotapes were replayed on the videocassette recorder. The number of microemboli passing a reference point on the vessel was counted with each one minute interval for 60 minutes. Fields containing emboli were digitized(PCVision+frame grabber) and the sizes of the embolus were determined using computerized planimetry with the image analysis program JAVA(Jandel Scientific, CA).

RESULTS

In all implant groups, there was immediate return of blood flow upon release of microvascular clamps. Fluorescent emboli were observed moving with the blood stream. The microemboli appeared to be most clearly visible at the bifurcation of first order(A_1) to second arteriols(A_2)(Fig. 2). Typically, large emboli were arriving at the distal wall of the bifurcation. The number and size of the emboli situated proximal to the were measured. Most microemboli appeared within the first five minutes in all groups, althougth some appeared as late as 20 minutes(Fig. 3).



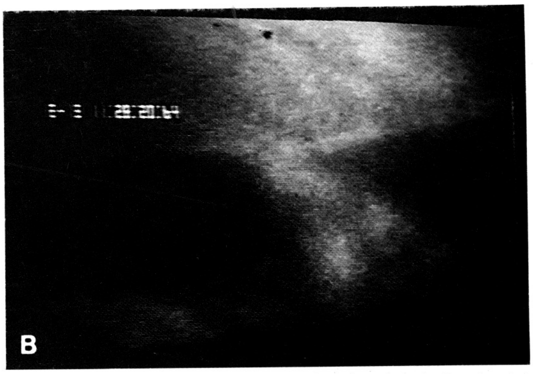


Fig. 2. The microemboli appeared to be most clearly visible at the bifurcation of first order to second arteriolcs in the cremaster muscle
A : Bifurcating area B : microembolus in Bifurcation

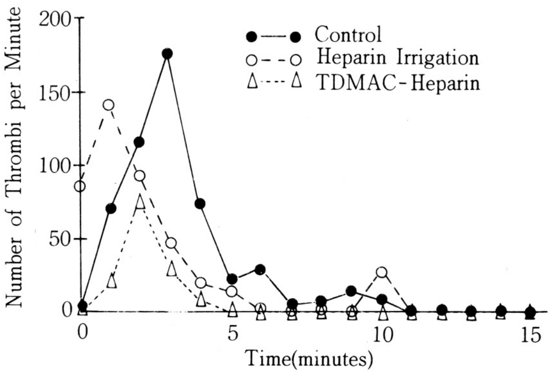


Fig. 3. Total number of thrombi per minute

An ANOVA(Analysis of Varience) was performed with stastistic program the SAS, to analyze the signigicance of difference. The total number of microemboli observed per animal in the control group was 9±136(mean±SE), with heparin irrigation 84±24 and with TDMAC-heparin coating 22±47. The mean area of each embolus was 1057±87 μm^2 for control, 940±37 μm^2 for heparin irrigated, and was 808±68 μm^2 for TDMAC-heparin coated grafts. The total embolus area observed per animal was 137,600 μm^2 in the control group, 79,040 μm^2 in the heparin irrigated group and 17,498 μm^2 in the TDMAC-heparin group(Fig. 4).

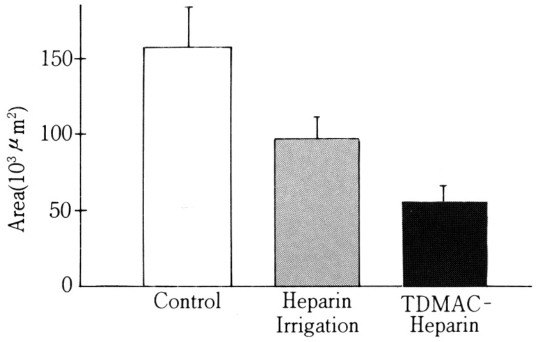


Fig. 4. Total area of thrombi per rat

DISCUSSION

The high occlusion rate of small diameter prosthetic grafts limits their usefulness. Excluding surgical techniques, the main reason for occlusion of microvascular prosthesis is graft-induced thrombosis and microemboli(Berger and Salzman, 1974 ; Callow, 1982a, b ; Tangelder et al., 1982). This phenomenon is related to graft-induced platelet consumption and the release of platelet-specific proteins after the placement of synthetic grafts(Hanson et al., 1985).

Multiple experimental trials have been performed to reduce the thrombogenicity of grafts. Various agents have been tried including aspirin(Allen et al., 1984), aspirin plus dipyridamole(Oblath et al., 1978 ; Hancock et al., 1980), heparin(Barry et al., 1981), ibuprofen(Kaye et al., 1984 ; Claus et al., 1982). and prostacyclin(Callow, 1982a, b). Endothelial cell growth factor(Herring et al., 1979 ; Schmidt et al., 1985) and changing of the physical properties of the graft material(Seeger and Kligman, 1988 ; Madras et al., 1980 ; Demas et al., 1988) have not as yet shown consistent and clinically reliable results.

A heparinized solution is often used to irrigate blood from the vessel lumen in many microvascular surgical procedures.

The present study suggests that intraluminal heparin irrigation may reduce the adherence of platelets and the formation of surface thrombi on a microvascular grafts. However, the technique of bonding TDMAC-heparin on the intraluminal surface of the graft(Talgelder et al., 1982) appears to be significantly better than heparin irrigation.

The cremaster muscle has a history of use for studies of the microcirculation(Acland et al., 1989 ; Anderson et al., 1988 ; Baez, 1973 ; Grant, 1966). The vascular supply which includes the small arteries that lie between the cremaster and the aorta were clarified(Meininger et al., 1987). The cremaster muscle is supplied mostly by the pubic-epigastric artery and occasionally by the superior pudendal artery. These arteries arise behind the inguinal ligament from the external iliac artery and run within the abdominal muscle parallel to the inguinal ligament. Usually they give off several branches in the perineal area before dividing into their terminal branches, the external spermatic arteries.

The isolated muscle technique described by Acland et al. allows continuous monitoring of microcirculatory blood flow of the cremaster muscle by intravital microscopy after implanting a 4mm long $\times$ 1mm diameter Gore-tex ePTFE interpositional graft into the iliac artery. The thrombogenicity of ePTFE grafts can be readily quantified using this model.

One noteworthy finding in this study was the appearance time of microemboli. In most cases a shower of microemboli appeared 60 to 90 seconds after release of the microvascular clamp and ceased within 6 minutes. In a very few cases, the thrombi were still apparent as late as 20 minutes.

Histologic studies showed the patency of all grafts. This was expected since the length of the implants was relatively short(4mm) and the duration of the study was very short. Other studies with longer graft length and duration have demonstrated uniformly low patency rates of untreated ePTFE(Esquivel and Blasdell, 1986).

Microvascular prostheses generate platelet aggregatates which diturb the microcirculatory downstream flow. The amount of the aggregates can be quantified using intravital microscopy of the cremaster muscle. Heparin irrigation and TDMAC-heparin coaption appear to reduce the number, size, and total amount of microemboli generated by the ePTFE. The apparent thrombo-resistant property of TDMAC-heparin coating may have widespread application in many clinical and research areas. Other modifications of ePTFE thrombogenicity may be easily evaluated by this model.

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REFERENCES

Acland RD, Anderson G, Siemionow M, McCabe S . *Direct in vivo observations of embolic events in the microcirculation distal to a small-vessel anastomosis. Plast Reconstr Surg 1987 ; 84 : 280-7.*

Allen BT, Sparks RE, Welch MJ . *Reduction of platelet deposition on vascular grafts using an antiplatelet graft coating technique. J Surg Res 1984 ; 36 : 80-8.*

Anderson GL, Acland RD, Sieminonow M, McCabe SJ . *Vascular isolation of the rat cremaster muscle. Microvasc Res 1988 ; 36 : 56-65.*

Baez S . *An open cremaster muscle preparation for the study of blood vessels by in vivo microscopy. Microvasc Res 1973 ; 5 : 384-8.*

Barry KJ. Scott RM, Keough EM . *Heparin and small caliber polytetrafluoroethylene grafts in the carotid arteries of rats. J Microsurg 1981 ; 3 : 72-6.*

Berger S. Salzman EW . *Thromboembolic complications of prosthetic devices. In : Spaet TH, ed. Progress in hemostasis and thrombosis, vol 2. New York : Grune & Straton, 1974 ; 273-309.*

Callow AD . *Currunt status of vascular grafts. Surg Clin North Am 1982 ; 62 : 501-17(a).*

Callow AD . *Platelet-arterial synthetic graft interaction and its modification. Arch Surg 1982 ; 117 : 1447-54(b).*

Claus PL, Gloviczki PG, Hollier LH, Kaye MP . *Patency of polytetrafluoroethylene microarterial prostheses improved by ibuprofen. Am J Surg 1982 ; 1454 : 180-5.*

Clagett GP, Burkel WE, Sharefkin JB . *Platelet reactivity in vivo in dogs with arterial prostheses seeded with endothelial cells. Circulation 1984 ; 69 : 632-40.*

Demas CP, Vann RD, Ritter EF, Sepka RS, Klitzman B, Barwick WJ . *Decreased thrombogenicity of vascular prostheses following gas denucleation by hydrostatic pressure. thrombogenicity of vascular prostheses following gas denucleation by hydrostatic pressure. Plast Reconstr Surg 1988 ; 82 : 1042-53.*

Esquivel CO, Blasdell FW . *Why small caliber grafts fail ; a review of the clinical and experimental experience and the signficance of the interaction of blood at the interface. J Surg Res 1986 ; 41 : 1-9.*

Grant RT . *Direct observation of skeletal muscle blood vessels (rat cremaster). J Physiol 1964 ; 172 : 123-9.*

Grant RT . *The effect of denervation on skeletal muscle blood vessel (rat cremaster). J Anat 1966 ; 100 : 305-12.*

Hancock JB, Forshaw PL, Kaye MP . *Gore-tex(polytetrafluoroethylene) in canine coronary artery bypass. J Thorac Surg 1980 ; 80 : 94-9.*

Hanson SR, Harker LA, Bjornesson TD . *Effect of platelet modifying drugs on srterial thromboembolism on baboons. J Clin Invest 1985 ; 75 : 1591-93.*

Herring MB, Dilley R, Jersild RA, Boxer L, Gardner A, Glover J . *Seeding arterial prostheses with vascular endothelium. Ann Surg 1979 ; 190 : 84-93.*

Kaye MP, Gloviczki PG, Dewanjee MK, Claus PL, Lovaas ME . *Ibuprofen in experimental vascular surgery. Am J Med 1984 ; 77 : 95-101.*

Madras PN, Ward CA, Johnson WR . *Enhanced thromboregistance of surfaces by denucleation. Trans Am Soc Artif Int Organs 1980 ; 26 : 153-7.*

Meininger GA, Fehr KL, Yates MB . *Anatomic and hemodynamic characteristics of the blood vessels feeding the cremaster skeletal muscle in the rat. Microvasc Res 1987 ; 33 : 81-7.*

Oblath RW, Buckley FO, Green RM, Schwartz SI, Dewese JA . *Prevention of platelet aggregation and adherence to prosthetic vascular grafts by aspirin and dipyridamole. Surg 1978 ; 84 : 37-43.*

Schmidt SP, Hunter TJ, Falkow LJ, Evancho MM, Sharp WV . *Effects of antiplatelet agents in combination with endothelial cell seeding on small-diameter vascular graft performance in the canine carotid artery model. J Vasc Surg 1985 ; 2 : 898-902.*

Seeger JM, Klingman N . *Improved in vivo endothelialization of prosthetic grafts by surface modification with fibronectin. J Vasc Surg 1988 ; 8 : 476-82.*

Tangelder GJ, Slaaf DW, Reneman RS . *Flurescent labeling of blood platelets in vivo. Thrombosis Res 1982 ; 28 : 803-8.*