Predictability of the relative patency of small caliber vascular prostheses

by

David Bernard Padget

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TABLE OF CONTENTS

LIST OF SYMBOLS

π	pi (3.14 ...)
^{o}	degree
θ	theta (represents the value of an angle)
cos	cosine
γ	gamma
γ_S	surface tension of a solid
γ_L	surface tension of a liquid
γ_{SL}	solid-liquid interfacial surface tension
γ_c	critical surface tension
cm	centimeter, 10^{-2} meter
mm	millimeter, 10^{-3} meter
μm	micrometer, 10^{-6} meter
cc	cubic centimeter
$\mathring{A}$	angstrom, 10^{-10} meter
ml	milliliter, 10^{-3} liter
μl	microliter, 10^{-6} liter
g	gram (mass)
mg	milligram, 10^{-3} gram
kg	kilogram, 10^{3} grams
ft.	foot
EDTA	edetic acid
EGDM	ethylene glycol dimethacrylate monomer
EMA	ethyl methacrylate monomer
HEMA	2-hydroxyethyl methacrylate monomer

MMA	methyl methacrylate monomer
PEMA	polyethyl methacrylate
PHEMA	polyhydroxyethyl methacrylate
PMMA	polymethyl methacrylate
PTFE	polytetrafluoroethylene
TEGDA	tetraethyleneglycol diacrylate
^{60}Co	the element cobalt with atomic weight of sixty
Ca^{++}	calcium ion
HF	hydrofluoric acid
H_2O	water
H_2O_2	hydrogen peroxide
OH	hydroxide
Na	the element sodium
sec.	second
hrs	hours
ACT	activated coagulation time
ADP	adenosine diphosphate
LS	percent of luminal stenosis
RBCs	red blood cells
SEM	scanning electron microscopy
TFS	percent of thrombus free surface
TTV	thrombotic threshold velocity
TVO	percent of total volume occlusion
USCI	U.S. Catheters and Instruments
WBCs	white blood cells

I.D.	inside diameter
%	percent
ave.	average
Wt.	weight
conc.	concentration
r	correlation coefficient
t_p	probability of t-score
ν	degrees of freedom

INTRODUCTION

Sauvage and co-workers in 1974 stated, "the majority of deaths in the Western World are caused by arterial impairment to distal tissues." Since the most successful method of increasing distal arterial flow is the bypass graft, an enormous market is available for a satisfactory arterial prosthesis. For the past twenty years, large caliber fabric prostheses, especially Dacron®, have provided good results in bypass or replacement applications at high flow conditions. Abdominal aorta, aorta-iliac and aortofemoral reconstructions have shown the best results with Dacron® fabrics (Edwards, 1978).

Presently, fabric materials have shown little success with medium and small caliber (7 mm diameter or less) applications. Replacement of small caliber (5 mm diameter or less) arteries below the knee, such as tibial, popliteal and peroneal, has resulted in minimal success (DeBakey, 1979). Coronary bypass applications have shown similar results.

The state-of-the-art for small caliber arterial replacement or bypass applications, the use of autogenous veins (primarily saphenous), has demonstrated superior patency over fabric, polymeric or heterographic prostheses (Andrew and Lewis, 1976). Even though autogenous applications have demonstrated the greatest success, an accelerated effort to develop and fabricate a suitable hemocompatible material presently exists. Autogenous veins for arterial replacement are far from perfect due to the following: reduction of venous return from removal location, increased chance of hemorrhage or infection and occasional vein un-

suitability due to disease or abnormal development. Therefore, an off-the-shelf prosthesis for low flow, small diameter applications is desirable.

With the interest in developing an off-the-shelf prosthesis that could be used in a small caliber arterial application, an evaluation procedure to assess degree of performance is manditory.

LITERATURE REVIEW

Researchers studying small caliber vascular prostheses must consider several main topics. These include surface parameters of the blood-material interface, factors of thrombus formation or activation and the method of evaluating performance.

Small Caliber Prostheses

There are two approaches to the design of small caliber vascular prostheses. The first considers the development of a nonpervious material capable of resisting thrombus activation and immune responses (the complement system of immune responses). With a thrombus resistant material, development of an endothelial neointima (complete healing) is unnecessary. Two graft materials have been utilized in the development of a thromboresistant material. These include immobilized enzymes on polymer substrates and cultured endothelial seeding on Dacron® graft material.

The second approach involves the development of a material that allows cellular ingrowth and the formation of an endothelial neointima, i.e., complete healing (Sauvage, et al., 1974). A number of graft materials have been tested to achieve complete healing and biocompatibility. Such materials include Dacron® (polyethylene terephathalate) knit and woven fabrics, expanded polytetrafluoroethylene (expanded Teflon®, Gore-tex®), and Hydron® coated or impregnated materials. Others include replamineform grafts, human umbilical cord vein allografts, foreign body tissue and bovine arterial heterografts.

Immobilized enzymes

Deactivating enzymes like heparin, immobilized on Dacron® and polymer substrates, theoretically appear attractive as vascular substitutes (Hersh, et al., 1972; Kaetsu, et al., 1980). Kaetsu and associates extended the activity of immobilized enzymes on a 1:1 copolymer of 2-hydroxyethyl methacrylate (HEMA) and tetraethyleneglycol diacrylate (TEGDA) using radiation-induced polymerization at low temperatures (- 78°C). There are insufficient data to assess the significance of this recently developed immobilizing process. Developing a way to prolong the activity of immobilized enzymes should receive further study.

Endothelial seeded Dacron®

Cultured endothelial seeding on Dacron® grafts has shown significant promise and deserves future investigation. Mansfield, et al. (1975) indicated that endothelial cells prevented thrombus formation rather than thinning or organizing existing thrombus. The difficulty in fabricating a dependable endothelial neointima is the permanent bonding, without initiating cell lysis, of endothelial cells to the Dacron® fabric. Eskin, et al. (1978) reported that results were better with endothelial cells supported by tightly knit Dacron® fabrics rather than by loose knits and velours. They attributed these observations to the inability of endothelial cells to bridge spaces greater than 20-30 μm. Precultured endothelial cell linings may find application in a variety of cardiovascular prostheses (Eskin, et al., 1978).

Dacron® grafts

Since 1952, when Voorhees and associates demonstrated the usefulness of fabrics as vascular materials, Dacron® has proven superior in mechanical and compatible properties. Variations in knits, weaves, fiber caliber, velours, porosity and crimps have been tested to maximize cellular ingrowth and compatibility (Sawyer, et al., 1979; Sauvage, et al., 1976; Guidoin, et al., 1977). Fabrics require a preclotted fibrin surface to prevent hemorrhage. The preclotted fibrin surface is thrombogenic because of activated thrombin trapped within the fibrin mesh (Sauvage, et al., 1976). A four-step preclotting procedure that neutralizes thrombin with heparin has improved the thromboresistance of Dacron® preclotted fabrics (Yates, et al., 1978). Dacron® grafts are most successful as large artery aneurysm reconstructions (DeBakey, 1979). A 4 mm I.D. noncrimped polypropylene supported filamentous velour knitted Dacron® graft shows promise as a low flow arterial replacement when saphenous autografts are not available (Kenny, et al., 1980; Sauvage, et al., 1979).

Expanded Teflon®

Polytetrafluoroethylene has been reported to be the most chemically inert and hydrophobic polymeric material known (Campbell, et al., 1979). Expanded microporous polytetrafluoroethylene (Gore-tex®) has been suggested as the best small arterial substitute for femoropopliteal, distal popliteal or tibial bypasses (Campbell, et al., 1979). Microporous Gore-tex® was referred to as an ideally suited small arterial replacement by Hiratzka and Wright (1978). Despite the enthusiasm shown for

Gore-tex® as an arterial replacement, there are several undesirable characteristics. Early occlusion has been reported by several surgeons (Hiratzka and Wright, 1978; Selman, et al., 1980; Veith, et al., 1980). Explanations for early occlusion include atherogenesis (Selman, et al., 1980) and anastomotic geometry and flow factors caused by local vessel injury (Veith, et al., 1980). In addition, increased incidence of wound edema has been reported by Hollier, et al. (1980). Data concerning long-term patency for Gore-tex® are not yet available.

Hydron® impregnated materials

Lester R. Sauvage and associates in 1974 suggested use of hydrogel on Dacron® fabrics to decrease blood permeability and enhance cellular ingrowth as a means of improving thromboresistance. Knoll (1980) fabricated a 4mm hydrogel impregnated Dacron® prosthesis with morphologically different outer and luminal surfaces. The luminal surface had microvoids less than 1 μm, for greater blood compatibility, and an outer surface with microvoids between 5-15 μm for better tissue ingrowth. Implantation for twenty-one days in the canine carotid artery resulted in 80% patency rates, 30-40 μm of exterior tissue ingrowth and the development of an endothelial-like neointima. The above prosthesis used 20% HEMA/2% EGDM copolymer formulations with varied solvent ratios of methanol and water. Ratner and Hoffman (1975) reported that changing the methanol/water ratio allows variation of the morphology of polymer systems. Using scanning electron microscopy (SEM) for microstructural studies, Knoll observed the control of morphology by varying methanol/water ratios.

Other prostheses

Replamineform grafts of Bioelectric Polyurethane (BEP) and Silastic® using spines of the sea urchin H. trigonarious, were reported by Hiratzka, et al. (1979). These vascular prostheses contain pore diameters between 40 to 45 μm and demonstrate good tissue ingrowth as well as possible endothelial development. Additional data must be obtained before assessing future contributions of replamineform grafts to vascular reconstruction.

The use of human umbilical cord vein allografts has been limited because of degenerative changes in the allograft wall. The allograft veins that have been unsuccessful were fresh or fresh-frozen (Mindich, et al., 1977). Mindich and associates (1977) demonstrated improved results with ethanol-dialdehyde starch treated human umbilical cords. This treatment produces a thin walled, immunologically inactive, and mechanically stronger conduit. No long-term patency data are available.

Heterografts of bovine arteries demonstrate aneurysmal degeneration, early occlusion and infection (Dale and Lewis, 1976; Hollier, et al., 1980). Bovine arterial heterografts are presently inferior as arterial replacements. Some success with heterologous foreign body reactive tissue grafts of less than 5 mm was reported by Schoen, et al. (1979). Schoen and associates reported 50% patency when implanting grafts for two to three weeks in the canine carotid and femoral arteries. Further investigation of foreign body reactive tissue grafts has not been reported.

Surface Parameters

The surface parameters of interest are texture (micro and macro), hydrophilicity and blood-solid interfacial energy. In addition, when considering a material for vascular use, one must examine the immunological response, structural strength, flexibility and chemical stability. The latter parameters were not included in this study.

Texture

The texture of a surface has a significant effect on flow parameters. Micro and macro imperfections or voids can cause blood stagnation points that may initiate platelet and leukocyte deposition and possibly lead to aggregation and eventual thrombus formation (Cumming, 1980). Large surface imperfections in contact with flowing blood, may cause the formation of a localized wedge-shaped thrombus downstream from the imperfection (Herzlinger and Cumming, 1980). The relationship of flow to thrombus formation is primarily a shear effect. Shear has greater influence on fibrin and red cell deposition than on platelets. Therefore, one finds "red thrombi" (red blood cells trapped in a fibrin mesh) predominately in venous or slow flow regions while "white thrombi" (platelets) are found in arteries or areas with high flow conditions (Schultz, et al., 1980).

Hydrophilicity

A polymer's degree of hydrophilicity is dependent on the number of electrophilic groups available on the monomer molecule and density of the cross-linking network. These two considerations affect the polymer's

ability to imbibe water (Holly and Refojo, 1976). Holly and Refojo refer to gels with large water contents as materials that increase in hydrophobicity with increasing water content. Structured water at the polymer-blood interface, rather than total water content, has been postulated to affect the blood compatibility of a material (Andrade, et al., 1973; Jhon and Andrade, 1973; Garcia, et al., 1980; Hoffman, 1975).

The role of plasma protein deposition on hydrophilic/hydrophobic materials has initiated recent interest in blood-polymer interactions. Selective adsorption and denaturing of plasma proteins are the two major considerations. Proteinated surfaces are formed several seconds after blood-material contact. This forms an intermediate bridge layer for adhering platelets. Platelet membranes contain glycosyl transferase enzymes that become active when in contact with glycoproteins (Kim, et al., 1974). Albumin is the only major nonglycoprotein found in blood. Therefore, a material that selectively adsorbs a greater quantity of albumin compared to other glycoproteins (fibrinogen or Y-globulin) would be more thromboresistant (Nyilas, et al., 1977). Boffa, et al. (1977) reported the extent of albumin adsorption on several polymeric materials. Albumin adsorption on polymethyl methacrylate (PMMA) was considerably higher than on polyhydroxyethyl methacrylate (PHEMA), while Polytetrafluoroethylene (PTFE) did not indicate appreciable bonding. Hydrophilic PHEMA surfaces have greater affinity for fibrinogen than hydrophobic polyethyl methacrylate (PEMA) surfaces. Hydrophilic surfaces show a greater rate of desorption or exchange of proteins, within 2-3 weeks, compared with that of hydrophobic

materials (Weathersby, et al., 1977). Once a proteinaceous layer forms on a material in contact with blood, the proteins will be influenced by the characteristics of both liquid-protein and solid-protein interfaces. Hoffman (1974) attributed the greater compatibility of polar hydrogel surfaces to body fluids compared to nonpolar hydrogel surfaces to reduced protein denaturing or unfolding. Hoffman explained the denaturing as an attraction between the centrally located nonpolar hydrophobic sites of the protein molecule and a nonpolar surface material. Bruck (1977) emphasized the role of denatured proteins in the activation of plasma coagulation factors and blood elements, especially platelets.

Several investigators have improved blood compatibility with copolymer mixtures of hydrophilic-hydrophobic monomers resulting in microphase-separated domains (Garcia, et al., 1980; Nakashima, et al., 1977; Ratner, 1980; Ratner, et al., 1978). Nakashima, et al. (1977) attributed the compatibility of hydrophilic-hydrophobic microphase-separated domains to the structuring of water around hydrophobic sites and similarity with cellular membranes. Nakashima concluded that the distribution of hydrophilic-hydrophobic sites may be the most important factor for blood compatibility. Garcia, et al. (1980) stated that the hemocompatibility of hydrogels was sensitive to the hydrophilic-hydrophobic ratio and was independent of water content.

Interfacial energy

Surface energy is a function of a material's available surface electrophilic groups (mainly OH), adsorbed or absorbed ions and inter-

facial structured water. A relative measure of a solid's interfacial energy can be calculated from the contact angle (θ) formed between a drop of liquid with known surface tension and the surface of the material in question. The solid-liquid contact angle determines the wettability of the material (Holly and Refojo, 1976). Wettability is the extent of spreading of a liquid over a material. As the contact angle decreases for various materials, the wettability and interfacial energy increase. A true hydrophilic material is completely wettable as water spreads spontaneously over the surface forming a zero contact angle (Holly and Refojo, 1976). Lindsay, et al. (1980) reported a correlation between platelet adhesion and interfacial energy. As interfacial energy decreases so does platelet adhesion. Lindsay also mentioned a decrease in whole blood clotting time with decreasing interfacial energy. The results of platelet adhesion and whole blood clotting time are contradictory with respect to interfacial energy. Baier and Dutton (1969) reported the deposition of high energy proteins (primarily fibrinogen) on high energy surfaces exposed to blood. The high energy protein surfaces favored platelet adhesion and subsequent thrombus. This sequence of events resembles the exposure of blood to high surface energy collagen from damaged vessels. Andrade, et al. (1973) attributed the low interfacial energy (interfacial surface tension (γ_{SL}) between 1-3 dynes/cm) of a cell/medium to a carbohydrate rich cellular coating. The outer regions of the coat contain hydrated oligosaccharides and gel-like proteins that highly extend into the aqueous solution. Andrade referred to an implant/blood interfacial tension (γ_{SL}) of 5 dynes/cm or less as hemocompatible.

Critical surface tension (γ_c), in addition to interfacial energy, is another energy parameter that is used to predict hemocompatibility. When obtaining the critical surface tension of a material, one must plot the surface tension (γ_L), of several liquids, against the cosine of their solid/liquid contact angles and extrapolate to cosine $\theta = 1$. This procedure is referred to as a Zisman plot or format (Baier and Dutton, 1969). A solid's critical surface tension (γ_c) is equal to the difference between the surface tension (γ_S) of the solid and the solid/liquid interfacial surface tension, γ_{SL} (Owens and Wendt, 1969). Owens and Wendt (1969) reported energy values of $\theta = 101^o$, $\gamma_c = 24$ dynes/cm and $\theta = 108^o$, $\gamma_c = 18.5$ dynes/cm for Silastic® and Teflon®, respectively. Baier, as reported by Nyilas, et al. (1977) and Andrade, et al. (1973), suggests the inner surface of blood vessels consists primarily of methyl groups having a critical surface tension (γ_c) of 25 dynes/cm. Therefore, materials with critical surface tensions (γ_c) in the range of 20-30 dynes/cm would likely be hemocompatible.

Thrombus Formation and Activation

The sequence of events for blood coagulation appears to be the following: adsorption of plasma proteins, adhesion of platelets and leukocytes, activation of platelets followed by the release reaction (degranulation), recruitment of nearby platelets via released ADP and thromboxane A_2 and platelet aggregation on adherent platelets with eventual formation of thrombus (Lindsay, et al., 1980). There are three pathways that activate clotting mechanisms and possible thrombus

formation. These pathways include intrinsic, extrinsic and complementary systems. A flow chart of the intrinsic and extrinsic pathways is outlined in the Appendix.

Intrinsic pathway

Initiation of the intrinsic pathway usually occurs via blood trauma or exposure of blood to collagen. This initial impetus activates a cascade of factors and platelet phospholipids that eventually lead to activation of prothrombin to thrombin (Guyton, 1976). The protein enzyme thrombin strongly polymerizes the soluble protein fibrinogen to nonsoluble fibrin. Once polymerized, fibrin forms a fibrous sheet over the area of activation.

Extrinsic pathway

The initial activation of the extrinsic pathway occurs via tissue trauma outside blood vessels. Activation occurs when blood contacts proteolytic enzymes (tissue factor) and tissue phospholipids (from cell membranes) released from injured tissue (Guyton, 1976). Following blood activation, the extrinsic and intrinsic pathways proceed by means of identical mechanisms with eventual fibrin formation.

Complementary activation

The components of the complement system contain a group of serum proteins that mediate both immune and allergic reactions. Therefore, activation of the complement system against foreign cells provides an immune response. There are two modes of activation: classical and alternative. The classical pathway is activated primarily by immune

complexes and occasionally specific types of viruses. The alternative pathway is activated by plant and bacterial polysaccharides and lipopolysaccharides which are polymeric in nature (Lindsay, et al., 1980). Lindsay and coworkers recommend further study of the complement system as an important factor in coagulation processes.

Evaluation of Performance

The standard procedure for evaluating arterial graft performance compares the length of time and percent of patency achieved during implantation studies. Therefore, graft evaluation results in two levels of performance, patent or nonpatent.

Sauvage, et al. (1979) proposed a method of evaluation that allowed a graded comparison of graft materials. This procedure included a six-hour exposure of each prosthesis to blood at reduced flow rates of 25, 50, 75 and 100 cc/minute within the canine carotid artery. The method of evaluation included an assessment of percent of thrombus free surface (TFS) and thrombotic threshold velocity (TTV), defined as the velocity (cm/sec) of blood that would produce a 50% TFS rating. The following five 4 mm arterial grafts were evaluated: three Dacron®, an expanded Teflon® (Gore-tex®) and a preserved umbilical vein Meadox Biograft®). The gradation of performance from satisfactory to unsatisfactory was two noncrimped Dacron® grafts, Gore-tex®, crimped Dacron® and Meadox Biograft®, respectively. A later publication by Kenny, et al. (1980), of the same research team, used this method of evaluation to compare a noncrimped polypropylene-

supported filamentous velour knitted Dacron® graft with Gore-tex®. Comparable results were obtained for both grafts, yet the Dacron® graft showed slight superiority.

STATEMENT OF THE PROBLEM

Nature

The vascular prosthesis industry evaluates various graft designs in an attempt to model parameters that affect thromboresistance and patency. Factors relevant to thromboresistance include surface morphology, hydrophilicity, surface energy and flow conditions. Mechanical properties, material chemistry and surgical techniques should also be considered.

With nonstandardized procedures and evaluation criteria, a comparison of relevant parameters among researchers is nonconclusive and frequently contradictory. Development of universally accepted testing procedures and evaluations is desirable.

Approach to Problem

Sauvage and associates at the Providence Medical Center in Seattle, Washington are presently extending preliminary work published in 1979 that rates five prostheses by percent of thrombus free surface (TFS). TFS for six 4 mm arterial grafts were evaluated at four controlled flow rates (25, 50, 75 and 100 cc/minute) for six hours in the canine carotid artery. The TFS rating was used to predict each graft's ability to maintain long-term patency. Sauvage observed better predictability of long-term patency using TFS ratings than information obtained from seven-day implantation studies. This observation was attributed to the gradation of the TFS values compared to the biphasic patent/nonpatent information obtained from implant studies. A graph relating TFS to

flow rate showed an area of interest that included a flow rate of 50 cc/minute.

This study will incorporate a procedure and method of analysis similar to Sauvage and co-workers. Six different types of grafts will be tested by implanting a six centimeter section of each graft in the canine carotid artery for six hours at a reduced flow of 50 cc/minute. The assessment of performance will utilize the percent of thrombus free surface (TFS), percent of luminal stenosis and percent of total volume occlusion. The thromboresistant character of three surface parameters will be compared. These comparisons will include preclotted fibrin to hydrogel impregnated Dacron® to expanded Teflon® (Gore-tex®), smooth (less porous) to rough (porous) and variations of hydrophilicity (HEMA-EGDM to HEMA-MMA to expanded Teflon®). Additional information of interest will include: scanning electron micrographs (SEM) of surface morphology, light micrographs of interfacial thrombus, contact angles, critical surface tension (γ_c) and water imbibement data.

Significance of Research

The results of this investigation will provide additional information on the importance of surface texture, hydrophilic/hydrophobic character, blood-solid interfacial energy and water content. This information may lead to improvements in thromboresistant materials as well as a better understanding of the influence of such parameters

as microstructure, hydrophilicity, interfacial energy and water imbibement.

MATERIALS AND METHODS

General Description

All hydrogel grafts were fabricated using the procedure outlined by Knoll (1980). All other grafts were prepared for utilization using the procedures recommended by the manufacturers.

The experimental design shown in Figure 1 was employed. Grafts (4 mm in diameter and 6 cm long) were implanted, using simple continuous sutures, bilaterally in canine carotid arteries and maintained for six-hours at a flow rate of 50 cc/minute. The rate of flow was controlled using a loop of umbilical tape and monitored by means of a cuff electromagnetic flow meter. Mean, maximum (systolic) and minimum (diastolic) flow rates were recorded during the six-hour blood-prosthesis interaction. A platelet count and blood coagulation time were taken 1-2 hours following the initial flow measurement in order to assess animal variation. At least four trials were obtained for each prosthesis using both right and left carotid arteries.

The samples were analyzed for surface morphology, percent of thrombus free surface, histology of interfacial thrombus and percent of luminal stenosis. Each formulation was used to obtain water imbibement and contact angle information.

Materials

Fabrication of hydrogel grafts

The USCI® Sauvage™ filamentous straight vascular crimped Dacron® graft material (Lot 10K94073) was obtained from U.S. Catheters and

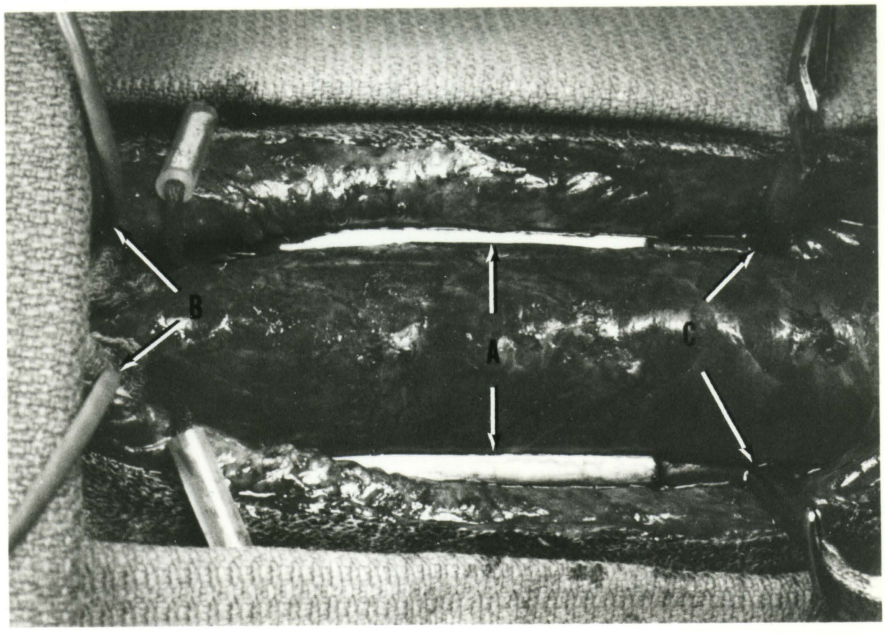

Figure 1. Experimental design showing placement of grafts (A), flow meters (B) and stenoses (C)

Instruments (USCI®), a division of C. R. Bard, Inc., Billerica, Massachusetts. USCI® Sauvage™ noncrimped supported Dacron® (Lot 61-2083) was received as a prototype from Sauvage and associates, Providence Medical Center, Seattle, Washington. Gore-tex® graft material (Lot 6334) was obtained from W. L. Gore and Associates, Inc., Medical Products Division, 1505 North Fourth Street, Flagstaff, Arizona.

The monomer 2-hydroxyethyl methacrylate (Lot B889F9) was acquired from Alcolac, Inc., 3440 Fairfield Road, Baltimore, Maryland. The cross-linking monomer ethylene glycol dimethacrylate (Lot 1-2-14) was obtained from Monomer-Polymer and Dajac Lab, Inc., 36 Terry Drive, Trevose, Pennsylvania. Methyl methacrylate (Lot 041557) was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin.

All other chemicals used were reagent grade. Glass distilled water was used for all solutions. Soft glass rodding and tubing were utilized as well.

Sample preparation and implantation

Vacuum tubes containing 12 milligrams of siliceous earth (Vacutainer #3865), used for activated coagulation times (ACT), were obtained from Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, New Jersey. Vacuum tubes (Vacutainer #3206Q) containing six milligrams of EDTA (Na_2) or Disodium edetate, an anti-coagulant, were obtained from Becton-Dickinson, a Division of Becton, Dickinson and Co., Rutherford, New Jersey. Heparin sodium, sodium pentobarbital and Sleepaway® were obtained from Fort Dodge Laboratories,

Inc., Fort Dodge, Iowa. Blood dilutions for platelet counts were performed using 1.98 ml Unopette reservoirs (Lot 9C5971Q) and 20 µl capillary pipettes (Lot 9B722) from Becton-Dickinson, Rutherford, New Jersey. A Spencer "Bright Line®" hemacytometer (Cat. 1492) made by AO Instrument Company, Buffalo, New York, was also utilized for platelet determinations. 5-0 silk suture material (K870-H) was obtained from Ethicon, Inc., Somerville, New Jersey.

Analysis of samples

All chemicals used were of reagent grade and all solutions were mixed with glass distilled water.

Instruments used for SEM analysis were the following: JEOL-U3 SEM, Japanese Electron Optics, Tokyo, Japan; Polaron SEM coating unit (E5100) and Polaron critical point dryer (E 3000), Polaron Instruments, Inc., Warrington, Pennsylvania. The colloidal silver medium was obtained from SPI Supplies, Division of Structure Probe, Inc., West Chester, Pennsylvania.

Fabrication of Hydrogel Grafts

Both hydrogel formulations (HEMA cross-linked by EGDM or MMA) were fabricated using the procedure outlined by Knoll (1980). The procedure required the preparation of a glass support apparatus and the utilization of a two-stage polymerization process.

Preparation of glass support apparatus

Soft five millimeter glass rods and nine millimeter glass tubes were cut ten centimeters long and etched with hydrofluoric acid (HF) to achieve a desired texture and diameter. A rough or smooth texture was produced by varying the HF concentration. To obtain a rough texture a 49% HF solution was used and a 17% HF solution was used for smooth surface preparation. Three different types of glass rods were fabricated, two having rough surfaces with diameters of 4.9 $\pm$ 0.02 mm and 4.4 $\pm$ 0.03 mm and one having a smooth texture with diameter of 4.4 $\pm$ 0.02 mm. All glass tubes were rough etched with inside diameters of 4.90 $\pm$ 0.03 mm.

Two-stage polymerization process

USCI® Sauvage™ crimped filamentous velour knitted Dacron® tubes (4 mm) were cut into 3.5 cm sections and stretched over 4.9 mm rough etched glass rods. Each Dacron®-glass apparatus was gently introduced into a rough etched 9 mm glass tube. The assembly was placed in a pyrex culture tube (16 x 125 mm) containing 16 cc of a nitrogen degassed monomer-solvent solution (20% HEMA/2% EGDM/19% methanol, 20% HEMA/2% MMA/15% methanol or 20% HEMA/2% MMA/20% methanol). Trapped air within the Dacron® fabric was removed by applying a partial vacuum to the culture tube assembly. The solution was replaced with a fresh aliquot to assure proper monomer/solvent ratios. The assembly was exposed to ^{60}Co radiation (0.25 Mrads for HEMA-EGDM and 0.50 Mrads for HEMA-MMA) in order to initiate polymerization. The glass assembly was removed by shattering the culture tube and peeling away the bulk

polymer. The 4.9 mm glass rod was removed from the Dacron®-polymer matrix and replaced by a 4.4 mm glass rod. The Dacron®-polymer matrix within the glass tube was then placed for twelve hours in a culture tube (16 x 125 mm) containing an ethanol-water solution (1:1 for HEMA-EGDM and 1:9 for HEMA-MMA).

The second-stage polymerization required the insertion of a 4.4 mm glass rod (rough for 20% HEMA/2% EGDM/19% methanol and 20% HEMA/2% MMA/15% methanol and smooth for 20% HEMA/2% EGDM/39% methanol and 20% HEMA/2% MMA/20% methanol) into the lumen of the first stage polymer-Dacron® tube. The nonetched 5 mm end of the 4.4 mm glass rod allowed a uniform polymerization by centering the rod inside the Dacron®-polymer tube. The polymerization and retrieval of the newly formed graft followed the same procedure outlined earlier with the addition of the cracking and removal of the glass tube. Twenty-four hours prior to utilization, the grafts were removed from the ethanol-water solution and placed in physiological saline containing 110 units of heparin per cubic centimeter of solution.

Sample Preparation and Implantation

Sample preparation

USCI® Sauvage™ noncrimped supported Dacron® prostheses were preclotted prior to cutdown using the procedure outlined by the manufacturer. This procedure includes the following four-step sequence proposed by Yates, et al. (1978):

Step 1 — Administer 6 cc of blood through the lumen, limit exposure time to 2-3 minutes and place graft in a clean

pan until blood clots.

Step 2 — Inject 6 cc of blood through lumen, limit exposure time to 20-30 seconds and remove any excess blood by passing a four French Fogarty catheter through the lumen.

Step 3 — Follow the procedure outlined in Step 2 but limit exposure time to 10-15 seconds.

Step 4 — Clamp one end of graft, add 4,000 units of heparin to 10 cc of blood and inject blood, to allow expansion of prosthesis, until blood no longer oozes. Inspect lumen for smoothness.

The prosthesis was then placed in heparinized saline (110 units/cc) until implanted. All hydrogel and Gore-tex® prostheses were placed in heparinized saline (110 units/cc) at least twelve hours before utilization.

Implantation procedure

Adult dogs weighing between 20-25 kg each were obtained through Laboratory Animal Resources, Iowa State University. The dogs were anesthetized by an intravenous injection of sodium pentobarbital (1 cc/2.3 kg body weight). Proper anesthesia was maintained by giving 0.5 cc injections as needed. The anesthetized animal was placed in a supine position with neck extended to enhance exposure of desired location. All surgical procedures were performed by the author using nonaseptic techniques.

A lactated-Ringers and dextrose solution (25 cc lactate-Ringers solution (conc.) and 25 g of dextrose in 500 cc of solution) was given

intravenously to maintain stability during the lengthy surgical procedure.

A midline incision was made through the subcutaneous tissue of the neck. Blunt separation of tissues was used to reduce hemorrhage. The sternocleidomastoid muscle and trachea were retracted laterally with blunt tip Gelpi self-retaining retractors. The carotid sheath containing the common carotid artery, vagus nerve and internal jugular vein, that lies immediately adjacent to the trachea, was exposed. A ten centimeter section of the common carotid artery was separated from the sheath. The retractors were then removed and placed to allow exposure of the remaining carotid artery using the same procedure. The diameter of each artery was then determined by wrapping four turns of suture material around the artery and dividing the suture length by 4π. The proper cuff electromagnetic flow meter size was determined by choosing the flow meter diameter that would result in a 25% constriction of the artery.

After the flow meter reached a steady state (pen deflection of 3-4 cm), the artery was occluded with umbilical tape ligatures at both exposed ends and transected approximately 6 cm cranial to the flow meter. The flow meter was then calibrated by ligating one end of a 4 mm diameter, 2 ft. Silastic® tube inside the transected artery, releasing the ligature just caudal to the Silastic® tube and collecting 20 cc of blood in a 50 ml graduated cylinder. A one centimeter deflection, controlled by a screw clamp, was maintained throughout the procedure. A pen deflection of one centimeter was approximately equivalent to a 50 cc/minute flow rate. A four centimeter section of artery, containing the Silastic® tube, was excised after tightening the umbilical tape ligature. The tunica adventitia was removed approximately five millimeters from the transected

ends by pulling the adventitia over the ends and transecting the overlap. The six centimeter prosthesis of interest was positioned by three 5-0 silk guide sutures inserted 120° apart (O'Brian, 1977). This triangulation technique enables the posterior arterial wall to fall away from the anterior wall to reduce the possibility of catching the posterior wall with a suture. A simple continuous suture pattern (5-6 sutures) was used between each pair of guide sutures to assure a proper bilateral attachment. The ends of the guide sutures were used to flatten and curl the edges of the artery and prosthesis in order to avoid the exposure of blood to the thrombogenic tunica media or adventitia of the artery wall. This procedure was repeated until a continuous vessel was obtained. All tissues were kept moist with saline to prevent drying. Gauze patches (2 x 2 inches) were cuffed around the anastomoses as a means of accelerating the formation of an impervious suture line. The distal stenosis was released first to permit the escape of trapped air.

After a few seconds of blood exposure, the proximal stenosis was released, allowing the blood to flow freely. The best results were obtained when blood was pulsated through the graft by a series of periodic occlusions. A different type of prosthesis was implanted in the remaining carotid artery using this procedure. Grafts differing in composition and/or morphology were implanted concurrently to reduce variations resulting from dissimilar blood parameters. After both grafts were functioning under normal flow conditions, a partial stenosis was achieved using umbilical tape placed distal (in order to avoid undesirable turbulence) to each prosthesis. The flow rate for both prostheses was maintained at 50 cc/minute for six hours.

After 1-2 hours of flow measurements, an activated coagulation time (ACT) and platelet count were taken. The activated coagulation time (ACT) was determined for each animal by withdrawing two milliliters of venous blood into a vacuum glass tube containing twelve milligrams of purified siliceous earth (Vacutainer #6522). The drawn blood specimen was incubated at approximately 37°C and gently tilted until the first unmistakable clot appeared. An additional two milliliters of venous blood were withdrawn into a vacuum glass tube containing six milligrams of EDTA (Na_2) (Vacutainer #3206Q) and used for counting platelets. Twenty microliters of blood were withdrawn with a capillary pipette and diluted by means of a Unopette® reservoir (1.98 ml). A small quantity of diluted blood was transferred to the hemacytometer and directly counted using the method of Rees and Ecker (Davidsohn and Henry, 1969). Values obtained for number of platelets were multiplied by one thousand.

Baseline adjustments at zero flow were taken every 30-45 minutes. After six hours of blood exposure at a flow rate of 50 cc/minute, both grafts were removed and rinsed with heparinized saline (1.5 units/cc). The grafts were then fixed in 10% formaldehyde-buffer solution and kept for further analysis.

Sample Analysis

Each prosthesis was transected one centimeter inside each anastomosis. A two millimeter section was transected from each one centimeter segment and measured photographically for extent of luminal occlusion. The four centimeter section was cut longitudinally and pinned on a cork

board to expose the lumen. Patent samples were tested for percent of thrombus free surface (TFS) by observation through a gridded dissecting microscope (Nikon). A histological characterization of the thrombus-prosthesis interface was obtained for each type of graft. Each polymer formulation was tested for surface morphology (including fibrin surface) using SEM techniques, solid-liquid contact angle, critical surface tension (γ_c = dynes/cm) and water imbibement.

Percent of thrombus free surface

The central four centimeters of each patent prosthesis was pinned to a cork board, to expose the lumen, and observed with a gridded dissecting microscope (Nikon). An average of forty-four squares for each prosthesis was assigned a value of 0, 25, 50, 75, or 100% thrombus covered. All thrombus percentages were totaled, divided by forty-four and subtracted from one-hundred to obtain percent of thrombus free surface (TFS).

Percent of luminal stenosis

A two millimeter cross-section was obtained from each of the two one centimeter segments that were transected from each patent graft. Each two millimeter section was photographed, printed, and analyzed for percent of luminal stenosis with the aid of a planimeter.

Histology of interfacial thrombus

Microscopic observations of thrombus were achieved with the use of light microscopy. A sample from each prosthetic type was imbedded in epoxy resin, sectioned and stained with toluidine blue and azure II.

Photographs were taken of each representative thrombus formation.

Surface morphology

Scanning electron microscopy (SEM) techniques were used to observe the surface morphology of each prosthetic material. A five millimeter segment from each type of graft was cut longitudinally and dehydrated by a series of acetone rinses (30, 60, 75, 90, 100 and 100%). The samples were then critical point dried with liquid carbon dioxide in a Polaron model E3000 drier. The critical point dried samples were mounted on carbon stubs using a colloidal silver medium adhesive and sputter coated with 300 Å of gold using a Polaron Instruments SEM coating unit (E5100). Microscopic observations were made by the use of a JEOL-U3 scanning electron microscope. Photographs were prepared at a 50 second per frame scan speed using Polaroid type 55 film.

Solid-liquid contact angles

Hydrogel samples were obtained by cutting flat bulk polymer sheets with the aid of a razor blade. Water (H_2O), glycerin, pyridine, formamide and hydrogen peroxide (H_2O_2) were the liquids used in this study. The solid samples were placed on a level platform, photographed with the aid of close-up sleeves and prints used for contact angle analysis.

Water imbibement

Twelve, one centimeter long, samples (three for each formulation) of four millimeter USCI® Sauvage™ crimped filamentous Dacron® were weighed and placed in a vacuum desiccator. After obtaining two

weights within 0.001 g, the samples were placed in a glass mold apparatus and polymerized with ^{60}Co radiation under normal fabrication conditions. The samples were placed in an ethanol and water solution for twenty-four hours to leach out any unpolymerized monomer. They were then transferred to distilled water for twenty-four hours and weighed after gentle blotting to remove surface water. The weight of the Dacron® and polymer were found by dehydrating the samples in incremented acetone solutions of 30, 60, 75, 90, 100 and 100%, placing them in a desiccator and weighing them periodically until no further weight changes occured.

RESULTS AND DISCUSSION

Results

Table I contains surgical and evaluation data for each individual prosthesis examined. Table II summarizes the overall hemocompatible performance of each type of prosthesis. Tables III and IV provide information about each material's physical and chemical characteristics that may or may not prove significant in reference to thrombogenicity.

When considering overall performance, the USCI® Sauvage™ non-crimped supported Dacron® and 20% HEMA/2% MMA/20% methanol impregnated Dacron® prostheses demonstrated superior thromboresistance when compared with other test grafts. The overall level of hemocompatibility resulted in the following descending order: USCI® Sauvage™ noncrimped supported Dacron®, 20% HEMA/2% MMA/20% methanol impregnated Dacron®, Gore-tex®, 20% HEMA/2% MMA/15% methanol impregnated Dacron®, 20% HEMA/2% EGDM/39% methanol impregnated Dacron® and 20% HEMA/2% EGDM/19% methanol impregnated Dacron®. The top two grafts had similar values as well as the third and fourth. The level of hemocompatibility utilized the following criteria: percent of thrombus free surface, percent of luminal stenosis and percent of total volume occlusion. The degree of thromboresistance may reflect one or more of the surface characteristics listed in Tables III and IV. The parameters of interest are histology of interfacial thrombus, surface morphology, water imbibement, solid-liquid contact angles and critical surface tension (γ_c).

Table I. Complete surgical and analytical data

Length of patency (hours)	Sample number	Prosthesis or formulation	Flow meter size (mm)	Flow rate[a] (cc/minute) Diastolic	Flow rate[a] (cc/minute) Systolic	Activated clotting time (seconds)	Platelet count	% luminal stenosis Ends	% luminal stenosis Av.	% TFS
		USCI®								
5.4	2s	Sauvage™	3.0	24	118	92	263,000	—	100	0
6	3s	supported	2.5	38	88	90	269,000	40/19	29	52
6	4s	noncrimped	3.5	19	144	90	278,000	14/13	13	43
6	5s	Dacron®	3.0	39	77	75	225,000	11/ 8	9	55
2.5	20	20% HEMA	2.5	19	91	96	334,000	—	100	0
6	21	2% MMA	3.0	24	108	96	377,000	27/ 8	18	60
1	22	20% methanol	2.5	43	80	83	228,000	—	100	0
6	26	58% water	2.5	35	76	96	205,000	26/26	26	43
6	27	—	3.5	24	107	86	136,000	23/18	20	55
6	23	20% HEMA	2.5	38	73	96	377,000	60/17	39	33
4.1	24	2% MMA	2.5	32	95	104	125,000	—	100	0
6	28	15% methanol	3.0	6	131	96	205,000	59/33	46	36
6	29	63% water	3.0	29	96	86	136,000	22/22	22	12
6	1g	Gore-tex®,	3.0	26	105	90	313,500	24/18	21	34
6	2g	expanded	2.5	35	80	90	313,500	62/22	42	12
0	3g	PTFE	2.5	—	—	97	279,500	—	100	0
5.2	4g	—	3.0	33	61	97	279,500	—	100	0
0.7	3	20% HEMA	3.0	39	69	90	269,000	—	100	0
6	4	2% EGDM	2.5	34	93	96	422,500	82/37	60	16
0	5	39% methanol	3.0	—	—	86	194,500	—	100	0
6	7	39% water	3.0	41	92	90	278,000	69/40	54	24
0.6	8	—	3.0	25	94	96	334,000	—	100	0
0	12	—	3.0	—	—	83	228,000	—	100	0
2.6	13	—	3.0	37	81	104	125,000	—	100	0

[a]The mean flow rate was maintained at 50 cc/minute.

Table I. Continued

Length of patency (hours)	Sample number	Prosthesis or formulation	Flow meter size (mm)	Flow rate (cc/minute)		Activated clotting time (seconds)	Platelet count	% luminal stenosis		% TFS
				Diastolic	Systolic			Ends	Av.	
1.4	2	20% HEMA	2.5	25	130	92	263,000	–	100	0
2.2	6	2% EGDM	3.0	30	94	96	422,500	–	100	0
0.7	9	19% methanol	2.5	38	65	86	194,500	–	100	0
1.3	10	59% water	3.5	34	102	75	225,000	–	100	0

Table II. Summary of performance of patent prostheses

Prosthesis or formulation	Number implanted	Number patent (6 hrs)	% luminal stenosis	% TFS	Volume of thrombus[a] (cc)	% of occluded volume[b]
USCI® Sauvage™ supported noncrimped Dacron®	4	3	17 ± 11	50 ± 6	0.043 ± 0.03	8.7 ± 6
20% HEMA 2% MMA 20% methanol 58% water	5	3	21 ± 4	53 ± 9	0.050 ± 0.02	10 ± 4
20% HEMA 2% MMA 15% methanol 63% water	4	3	36 ± 12	27 ± 13	0.13 ± 0.08	25 ± 16
Gore-tex®, expanded PTFE	4	2	31 ± 15	23 ± 16	0.12 ± 0.09	24 ± 18

[a] Volume of thrombus calculations: luminal stenosis x luminal area (0.13 cm^2) x length (4 cm) x (1 - TFS).

[b] Percent of occluded volume: $\frac{\text{volume of thrombus } (cm^3)}{\text{total luminal volume } (0.50\ cm^3)} \times 100.$

Table II. Continued

Prosthesis or formulation	Number implanted	Number patent (6 hrs)	% luminal stenosis	% TFS	Volume of thrombus[a] (cc)	% of occluded volume[b]
20% HEMA 2% EGDM 39% methanol 39% water	7	2	57 ± 4	20 ± 6	0.23 ± 0.03	45 ± 6
20% HEMA 2% EGDM 19% methanol 59% water	4	0	100	0	0.50	100

Table III. Summary of flow surface characteristics

Prosthesis or formulation	Imbibed water[a] (% by wt)	Void size (μm)		Histological analysis
		Macro	Micro	
USCI® Sauvage™ supported noncrimped Dacron®	–	–	–	Dense fibrin formation containing degranulated platelets
20% HEMA 2% MMA 20% methanol 58% water	84 ± 0.1	Smooth at 5,000X		Fibrin and loose RBCs
20% HEMA 2% MMA 15% methanol 63% water	83 ± 0.4	Flakey at 5,000X		Degranulated platelets with leukocytes covered by fibrin
Gore-tex®, expanded PTFE	0	9-14	0.5-1.5	Thick fibrin layer containing RBCs and leukocytes
20% HEMA 2% EGDM 39% methanol 39% water	59 ± 1	0.2-1	< 0.1	Leukocytes and RBCs covered by a thin fibrin layer

[a] % water imbibed calculation: $\frac{\text{Wt. } H_2O}{\text{Wt. } H_2O + \text{Wt. Polymer}} \times 100.$

Table III. Continued

Prosthesis or formulation	Imbibed water[a] (% by wt)	Void size (μm)		Histological analysis
		Macro	Micro	
20% HEMA 2% EGDM 19% methanol 59% water	75 ± 0.2	1-4	~ 0.2	Leukocytes, RBCs and a thin fibrin network

Table IV. Data from contact angle and critical surface tension determinations

Material	Liquid	Contact θ (degrees)	Cosine θ	Surface tension, γ_L [a] (dynes/cm)	γ_c (dynes/cm)
Silicone rubber	H_2O	101 ± 0	- 0.19 ± 0	72.8	26
	Glycerine	99 ± 1	- 0.16 ± 0.01	63.4	
	Pyridine	42 ± 0.5	0.74 ± 0.01	38.0	
	Formamide	91	- 0.017	58.2	
HEMA/MMA 20% methanol	H_2O	59 ± 3	0.52 ± 0.04	72.8	31
	Glycerine	60 ± 1	0.50 ± 0.01	63.4	
	Pyridine	20 ± 1	0.94 ± 0.01	38.0	
	Formamide	55	0.57	58.2	
HEMA/MMA 15% methanol	H_2O	70 ± 5	0.34 ± 0.08	72.8	35
	Glycerine	83 ± 1	0.12 ± 0.01	63.4	
	Pyridine	22 ± 1	0.93 ± 0.01	38.0	
	Formamide	42 ± 0	0.74 ± 0	58.2	
HEMA/EGDM 39% methanol	H_2O	41 ± 1	0.75 ± 0.01	72.8	54
	Glycerine	30 ± 4	0.87 ± 0.03	63.4	
	Formamide	13 ± 0	0.97 ± 0	58.2	
HEMA/EGDM 19% methanol	H_2O	34 ± 2	0.83 ± 0.02	72.8	46
	Glycerine	30 ± 4	0.87 ± 0.03	63.4	
	H_2O_2	40 ± 0	0.77 ± 0	76.1	
Gore-tex®	H_2O	134 ± 0.5	- 0.69 ± 0.01	72.8	28
	Glycerine	133 ± 4	- 0.68 ± 0.03	63.4	
	Pyridine	50 ± 1	0.64 ± 0.01	38.0	
	Formamide	102 ± 0	- 0.21 ± 0	58.2	

[a] Weast, ed. (1976).

Percent of thrombus free surface

The percent of thrombus free surface (TFS) for each graft is tabulated in Table I with the averages for the patent grafts of each type summarized in Table II. A graphic comparison of % TFS for each type of prosthesis is illustrated in Figure 2. The USCI® Sauvage™ noncrimped supported Dacron® preclotted grafts and the 20% HEMA/2% MMA/20% methanol impregnated Dacron® grafts showed superior thromboresistance. Both 20% HEMA/2% EGDM impregnated Dacron® graft formulations (19 and 39% methanol) showed inferior levels of thromboresistance. Figures 3-7 show the luminal surface of each patent graft. Notice the longitudinal streaming of thrombus across the lumen, possibly caused by proximal suture lines. Also, notice the cross-sectional thrombus formations on the hydrogel impregnated grafts, initiated by crimps in the Dacron® substrate.

Percent of luminal stenosis

The percent of luminal stenosis for each graft is tabulated in Table I with the average for each patent graft summarized in Table II. Each type of graft is graphically represented in Figure 8 for percent of luminal stenosis. In order to avoid confusion, the percent of luminal stenosis is represented, in Figure 8, as one-hundred minus the percent of luminal stenosis. The same trend of prosthesis performance observed for % TFS was observed for percent of luminal stenosis. The USCI® Sauvage™ noncrimped supported Dacron® and the 20% HEMA/2% MMA/20% methanol impregnated Dacron® grafts had the lowest percent of stenosis. Both 20% HEMA/2% EGDM impregnated Dacron® graft formulations

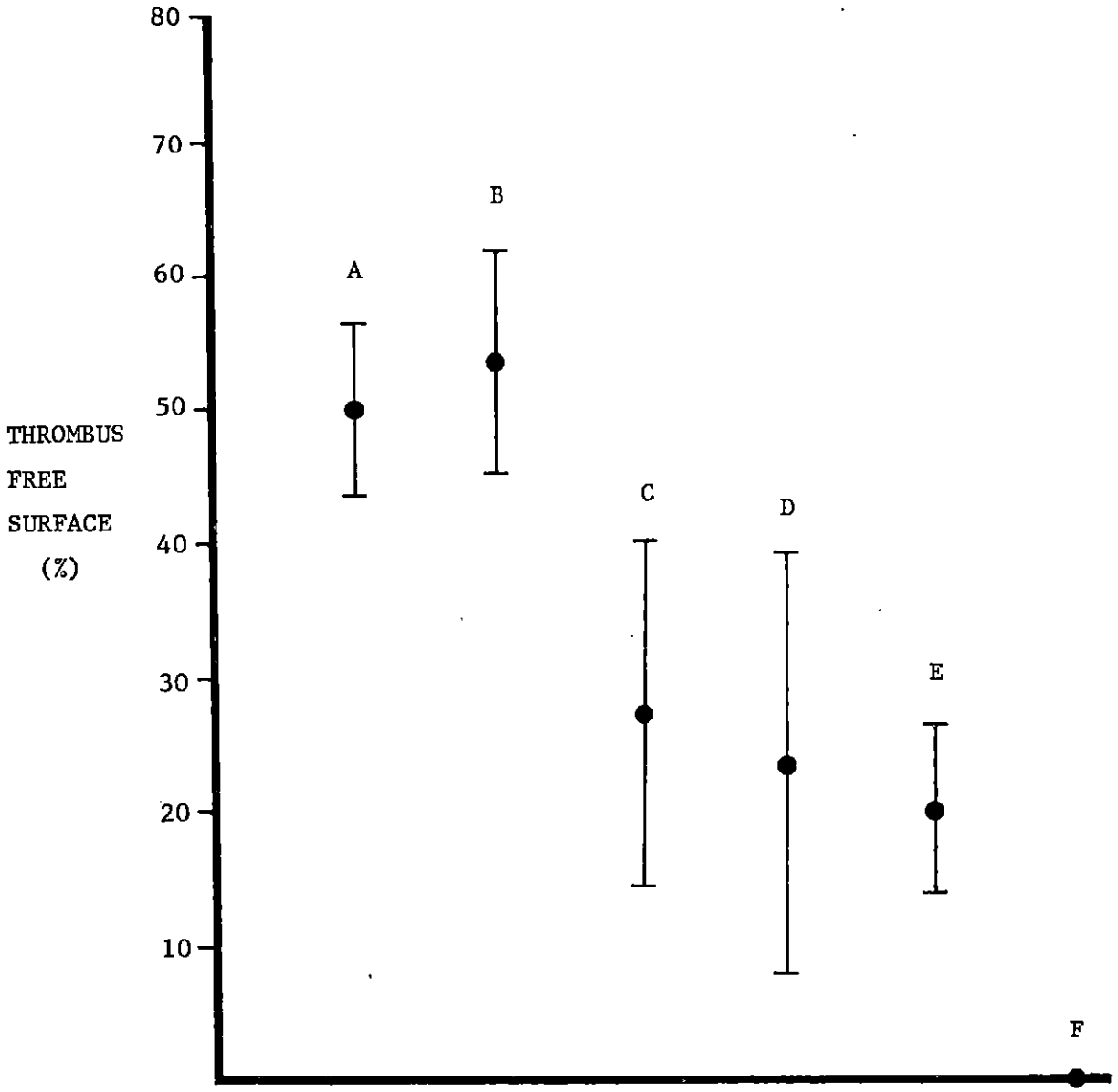

Figure 2. Relationship of the percent of thrombus free surface for each type of prosthesis. Data were taken from Table II. Sauvage noncrimped Dacron® is A, 20% HEMA/2% MMA/20% is B, 20% HEMA/2% MMA/15% is C, Gore-tex® is D, 20% HEMA/2% EGDM/39% is E and 20% HEMA/2% EGDM/19% is F. Range represents one standard deviation

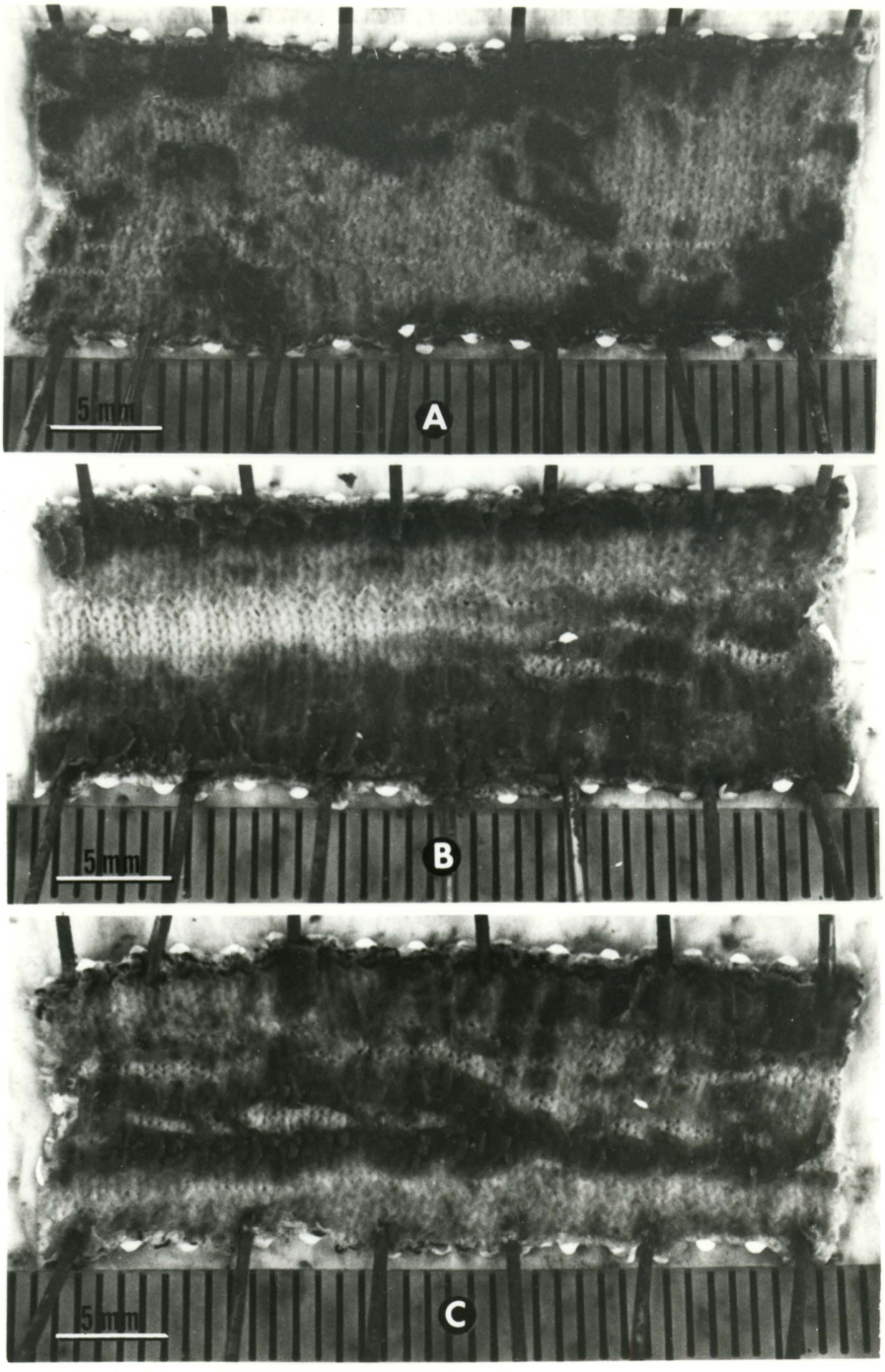

Figure 3. Luminal surface of USCI® Sauvage™ noncrimped supported Dacron® prostheses. Sample 3s is A, 4s is B and 5s is C. Scale bar = 5 mm

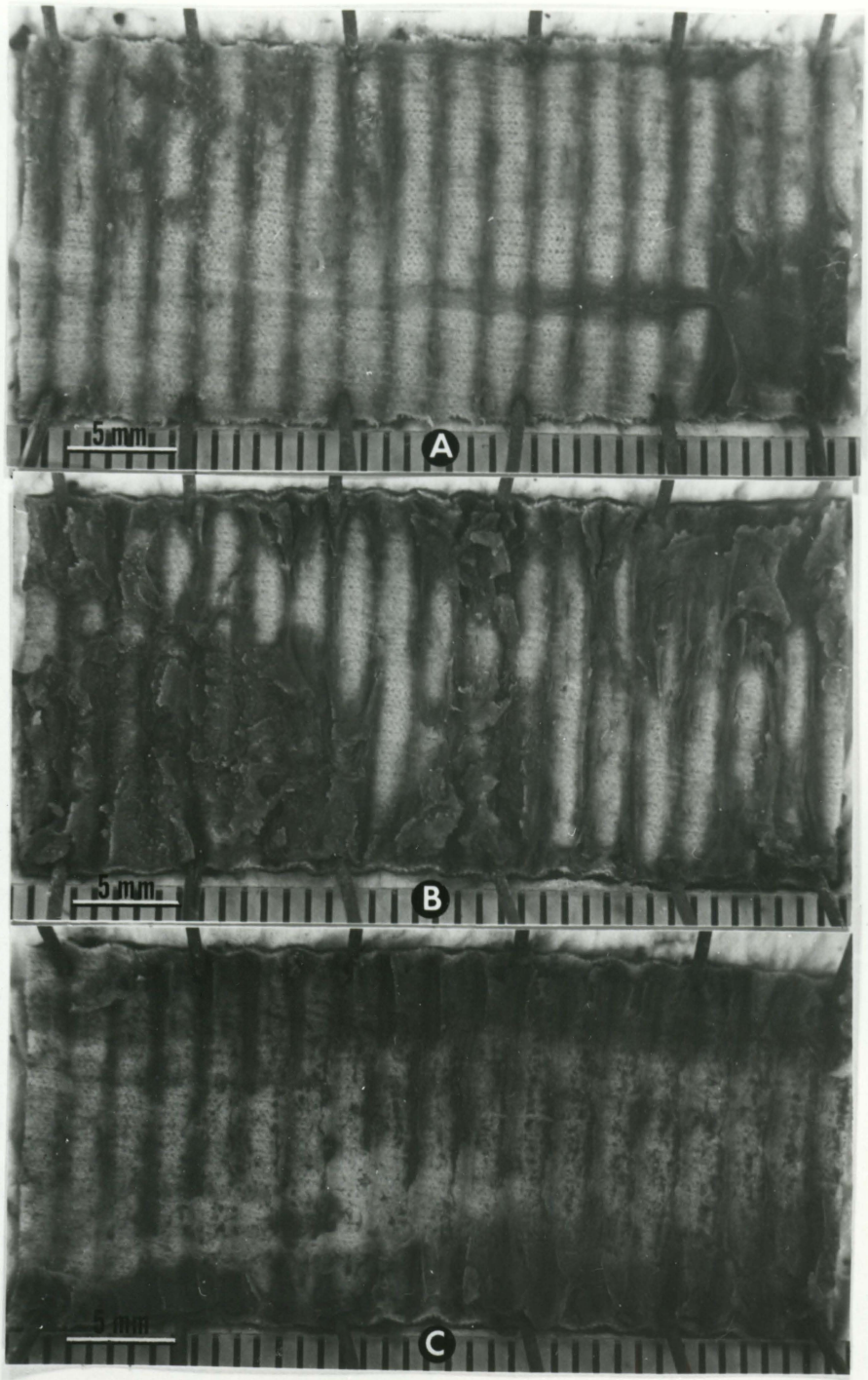

Figure 4. Luminal surface of 20% HEMA/2% MMA/20% methanol impregnated Dacron® prostheses. Sample 21 is A, 26 is B and 27 is C. Scale bar = 5 mm

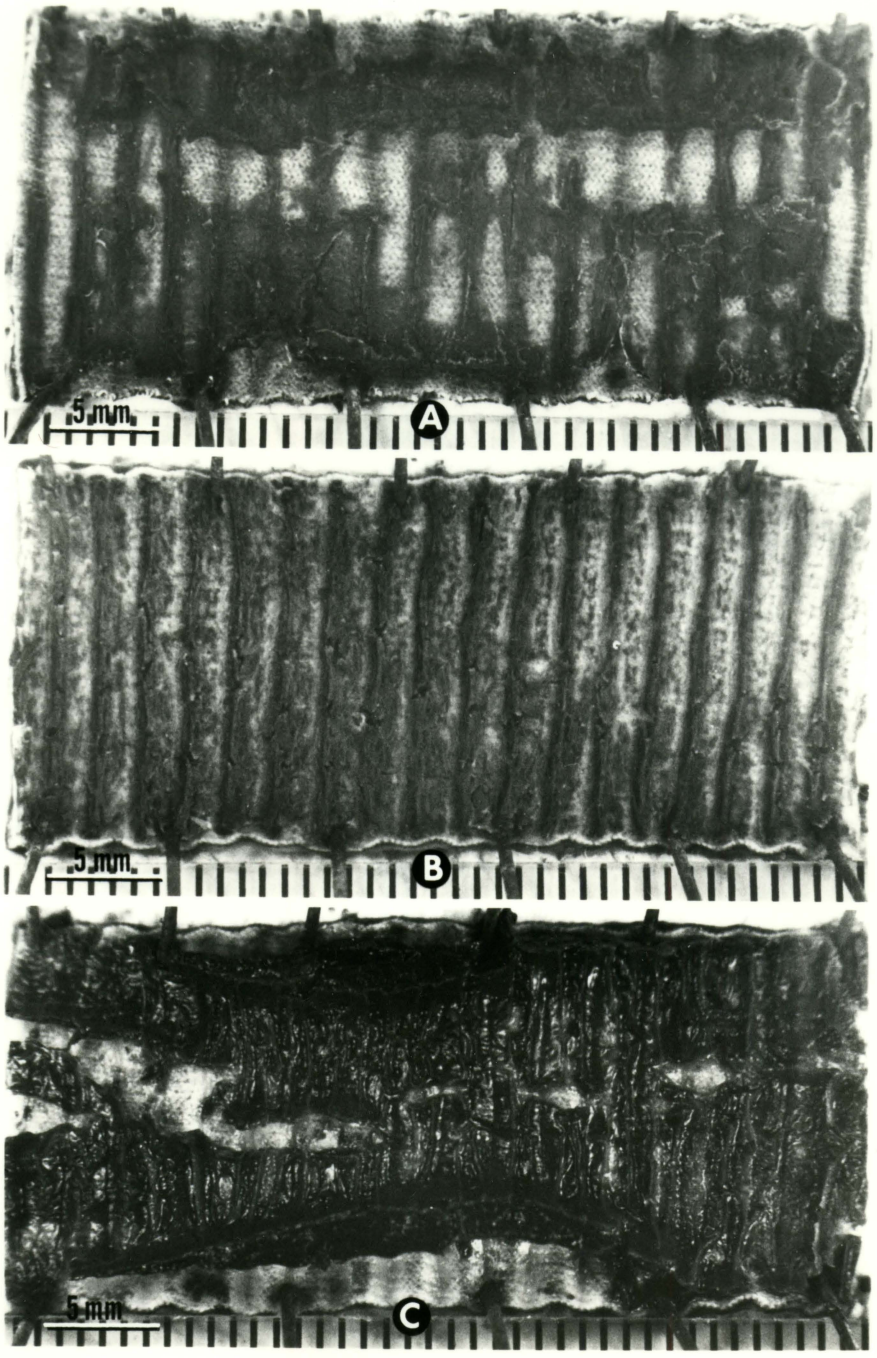

Figure 5. Luminal surface of 20% HEMA/2% MMA/15% methanol impregnated Dacron® prostheses. Sample 23 is A, 28 is B and 29 is C. Scale bar = 5 mm

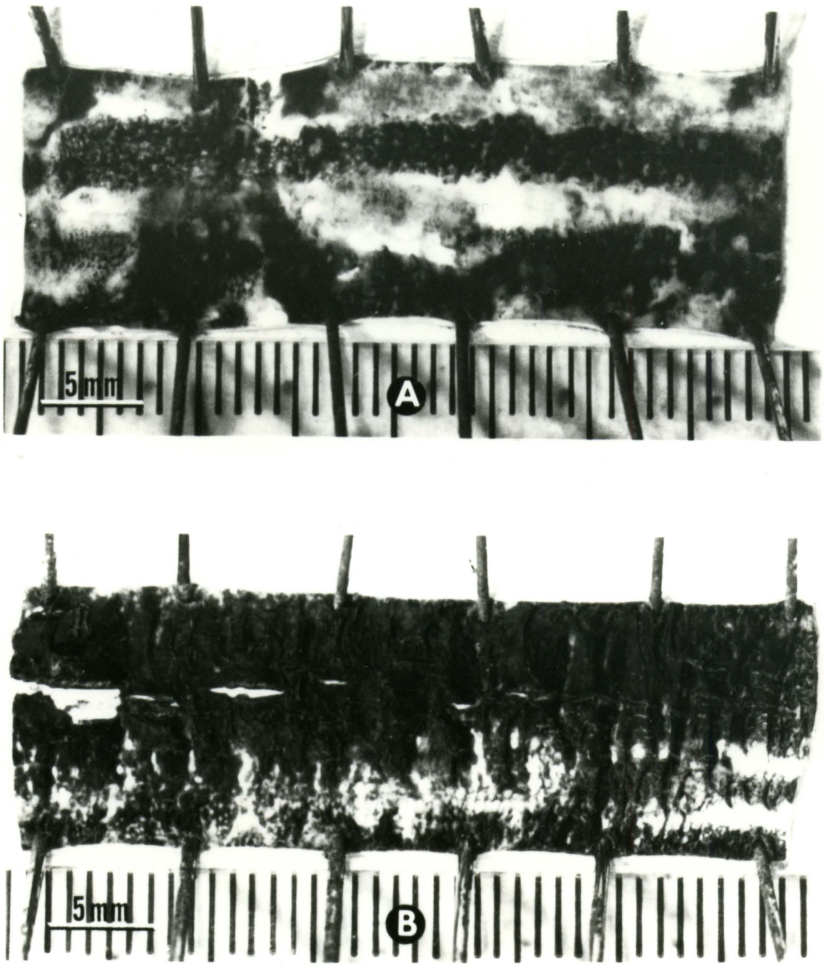

Figure 6. Luminal surface of Gore-tex® grafts. Sample 1g is A and 2g is B. Scale bar = 5 mm

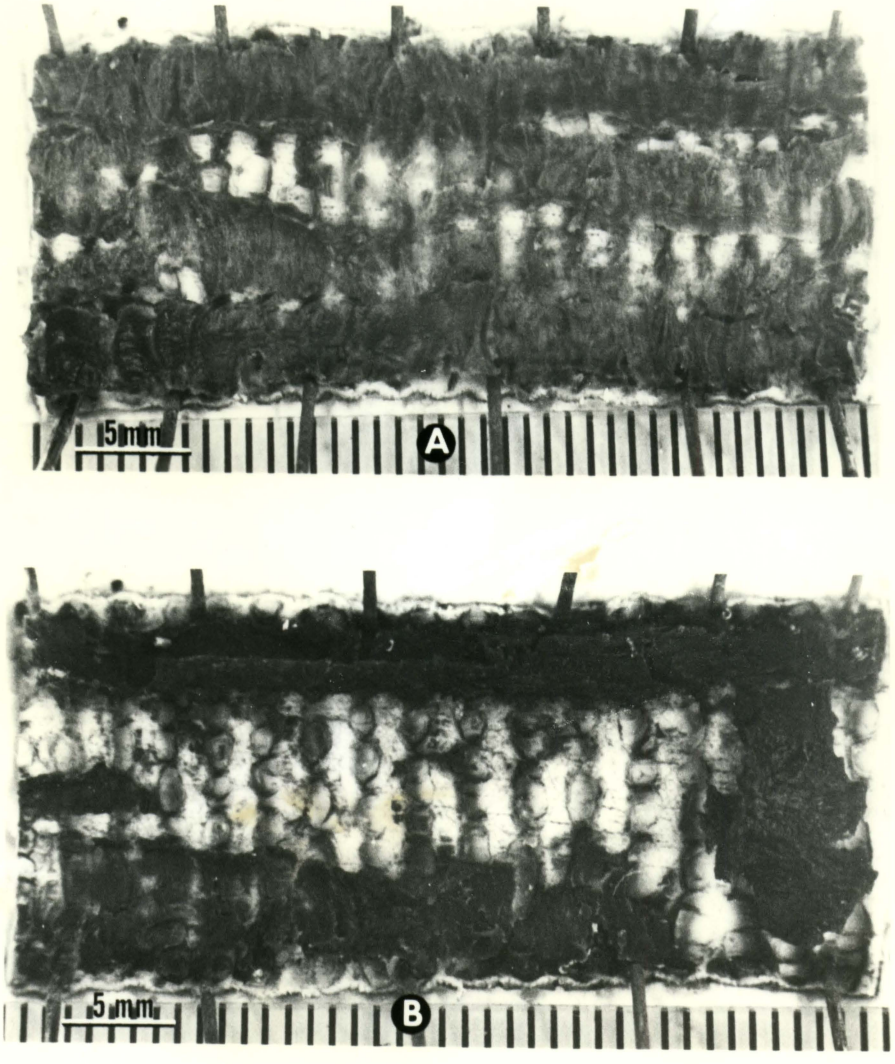

Figure 7. Luminal surface of 20% HEMA/2% EGDM/39% methanol impregnated Dacron® prostheses. Sample 4 is A and 7 is B. Scale bar = 5 mm

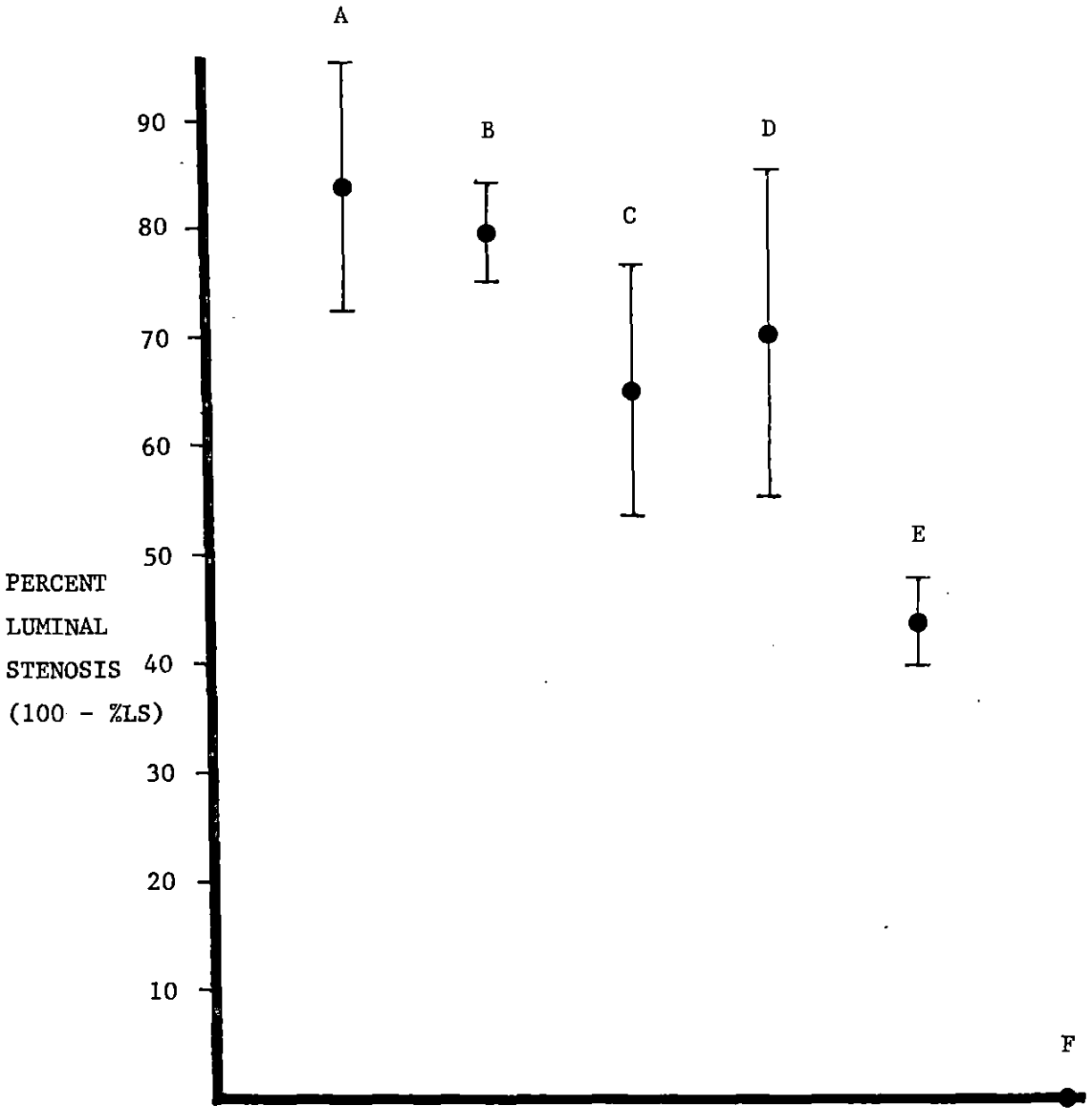

Figure 8. Relationship of the percent of luminal stenosis for each type of prosthesis. Data were taken from Table II. Sauvage non-crimped Dacron® is A, 20% HEMA/2% MMA/20% is B, 20% HEMA/2% MMA/15% is C, Gore-tex® is D, 20% HEMA/2% EGDM/39% is E and 20% HEMA/2% EGDM/19% is F. Range represents one standard deviation

developed large percents of stenoses. The lumens of all patent prostheses are shown in Figure 9.

Percent of total volume occlusion

The percent of total volume occlusion was calculated using the following equation:

$$\frac{\text{luminal stenosis (decimal)} \times \text{luminal area } (0.13\ \text{cm}^2) \times \text{length } (4\ \text{cm}) \times (1 - \text{TFS})}{\text{total luminal volume } (0.50\ \text{cm}^3)}$$

Table II contains the values for each type of prosthesis while Figure 10 provides a graphical comparison. Percent of total volume occlusion provides a better representation of the overall performance of a graft than does percent of thrombus free surface or percent of luminal stenosis.

Histology of interfacial thrombus

The histological analysis of the slides presented in Figures 11-14 is summarized in Table III. The hydrogel and expanded Teflon® surfaces seem to have a greater attraction for white blood cells (WBCs) than the preclotted fibrin surface. The Gore-tex® surface developed an extensive fibrin network that penetrated 40% of the wall thickness. A well-developed fibrin network was formed over the samples containing the largest voids (Gore-tex® and 20% HEMA/2% EGDM/19% methanol). The preclotted fibrin and 20% HEMA/2% MMA/20% methanol polymer surfaces seem to be the least reactive to blood components.

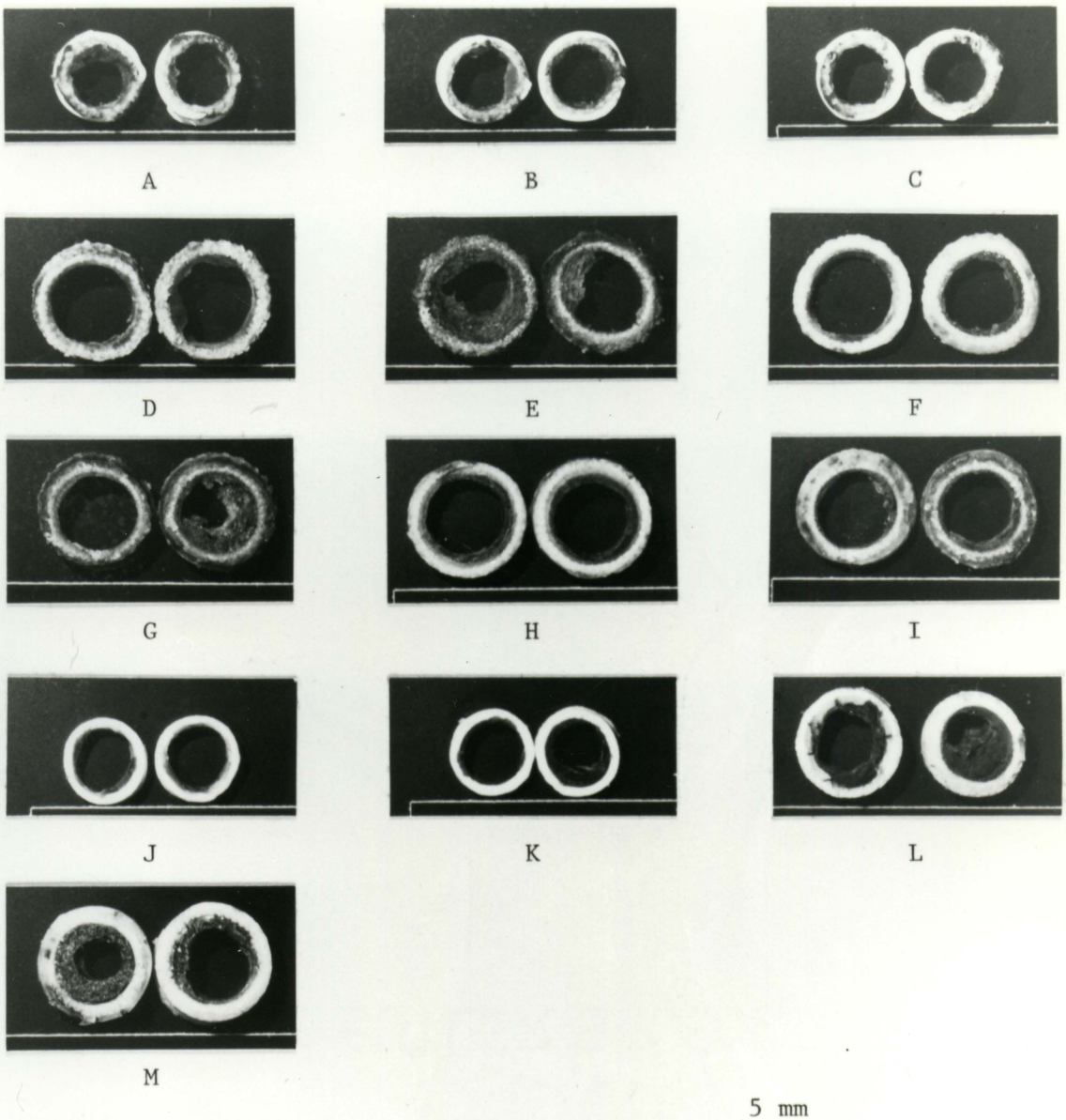

Figure 9. Cross sections of patent prostheses one centimeter from each anastomosis. Sample 3s is A, 4s is B, 5s is C, 21 is D, 26 is E, 27 is F, 23 is G, 28 is H, 29 is I, 1g is J, 2g is K, 4 is L and 7 is M

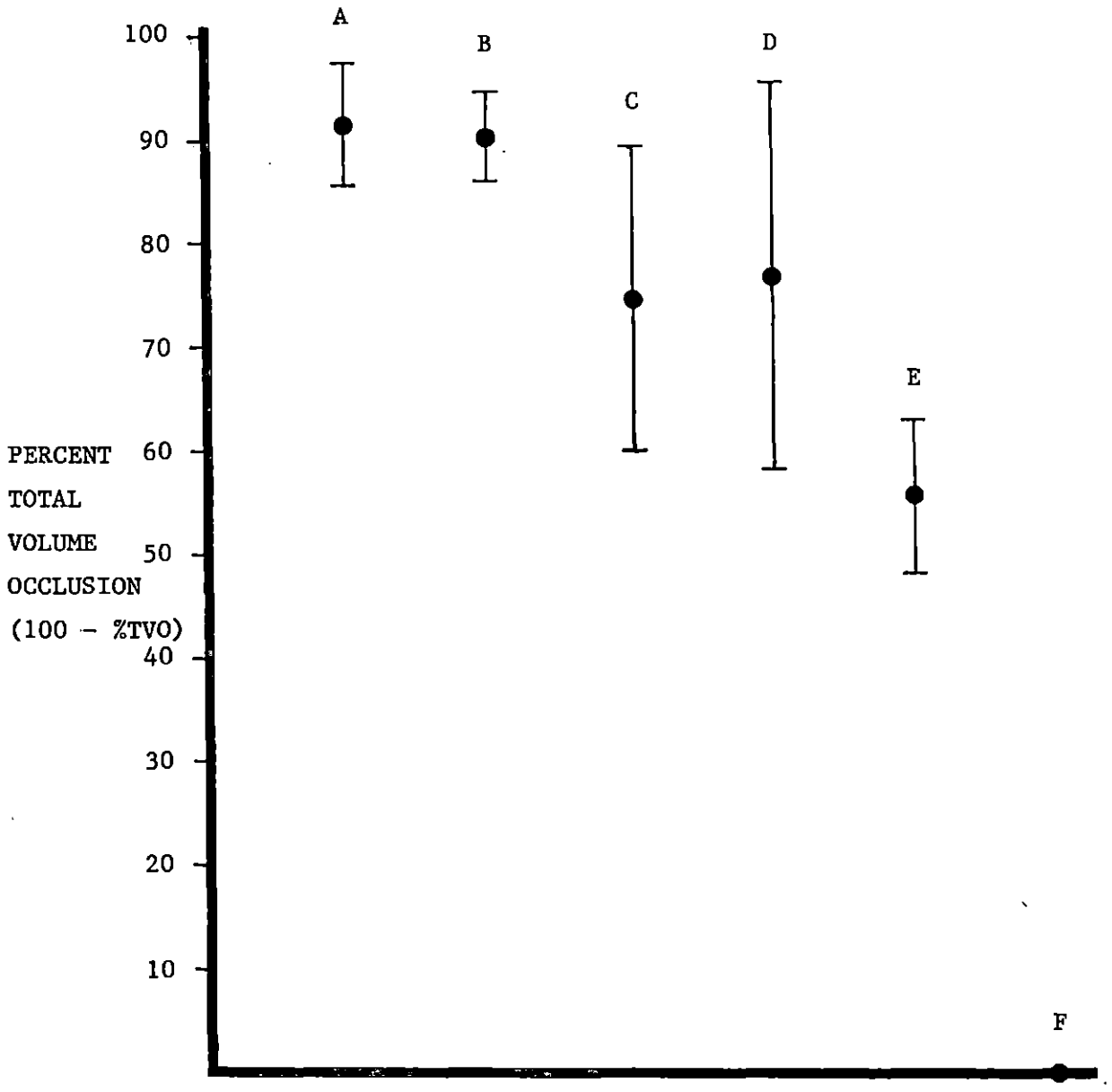

Figure 10. Relationship of the percent of total volume occlusion for each type of prosthesis. Data were taken from Table II. Sauvage noncrimped Dacron® is A, 20% HEMA/2% MMA/20% is B, 20% HEMA/2% MMA/15% is C, Gore-tex® is D, 20% HEMA/2% EGDM/39% is E, and 20% HEMA/2% EGDM/19% is F. Range represents one standard deviation

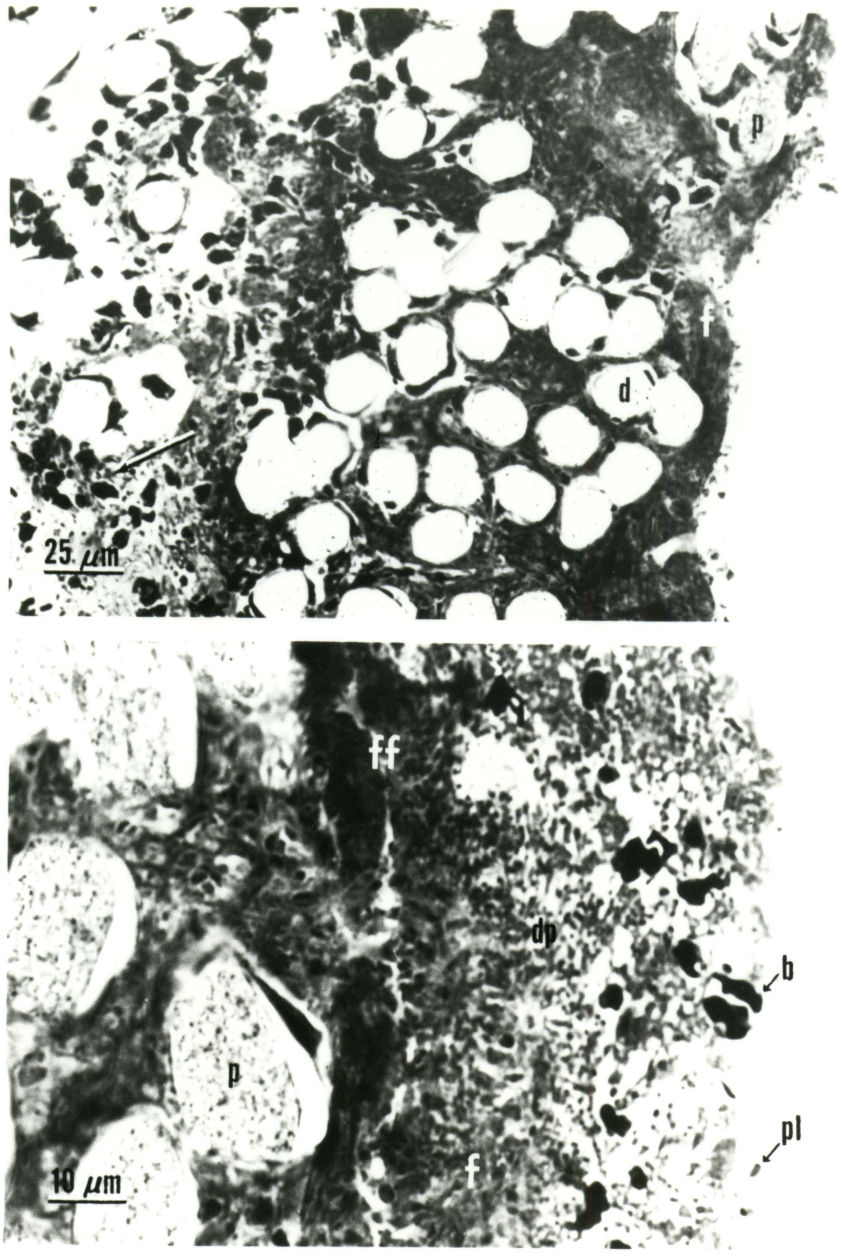

Figure 11. Light micrographs of USCI® Sauvage™ noncrimped supported Dacron® sections stained with toluidine blue. A Dacron®-fibrin flow surface is at ff, degranulated platelets at dp, fibrin at f, RBC at b and platelet at pl. Notice the blood penetration into the graft (arrow). Dacron® fiber (d) and polypropylene fiber (p)

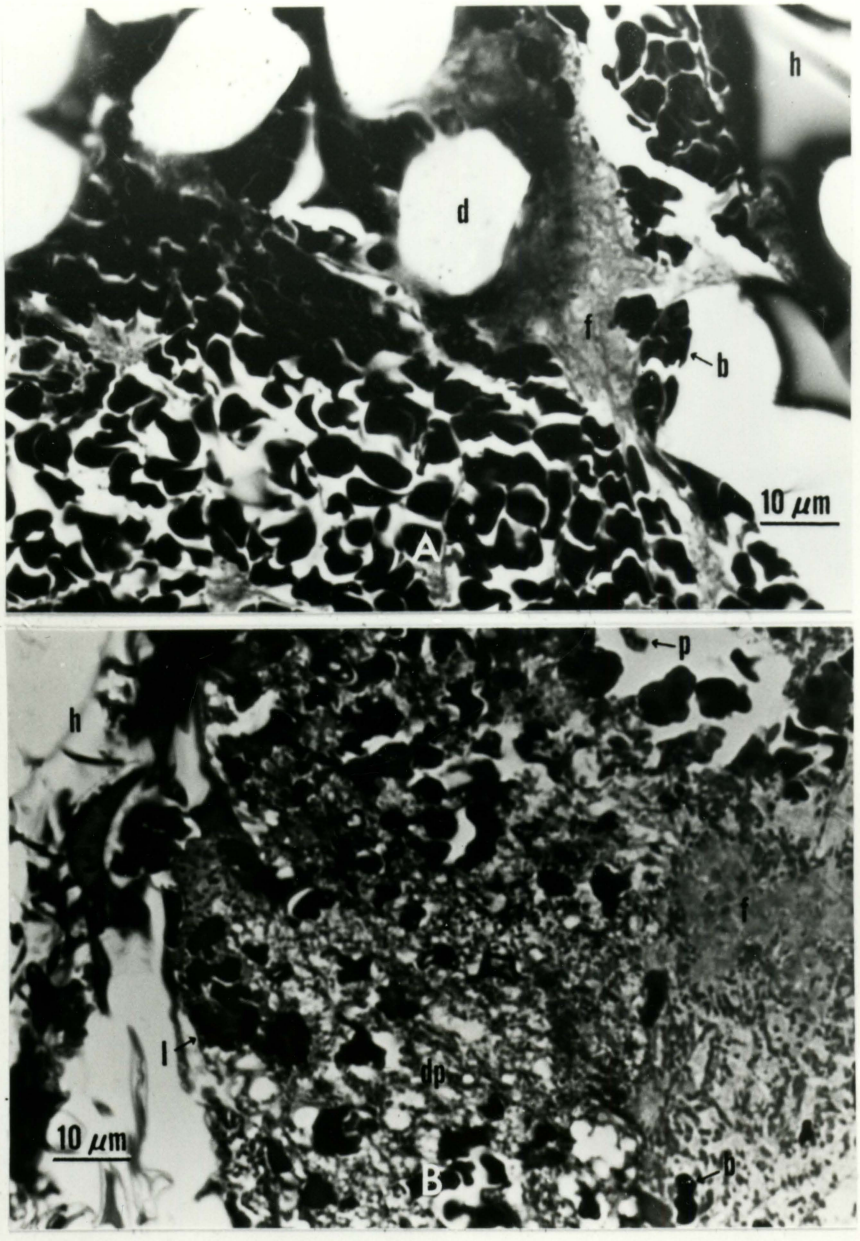

Figure 12. Light micrographs of 20% HEMA/2% MMA/20% methanol (A) and 20% HEMA/2% MMA/15% methanol (B) impregnated Dacron® sections stained with toluidine blue. A Dacron® fiber is at d, hydrogel polymer at h, fibrin at f, RBC at b, degranulated platelets at dp, platelet at p and leukocyte at l

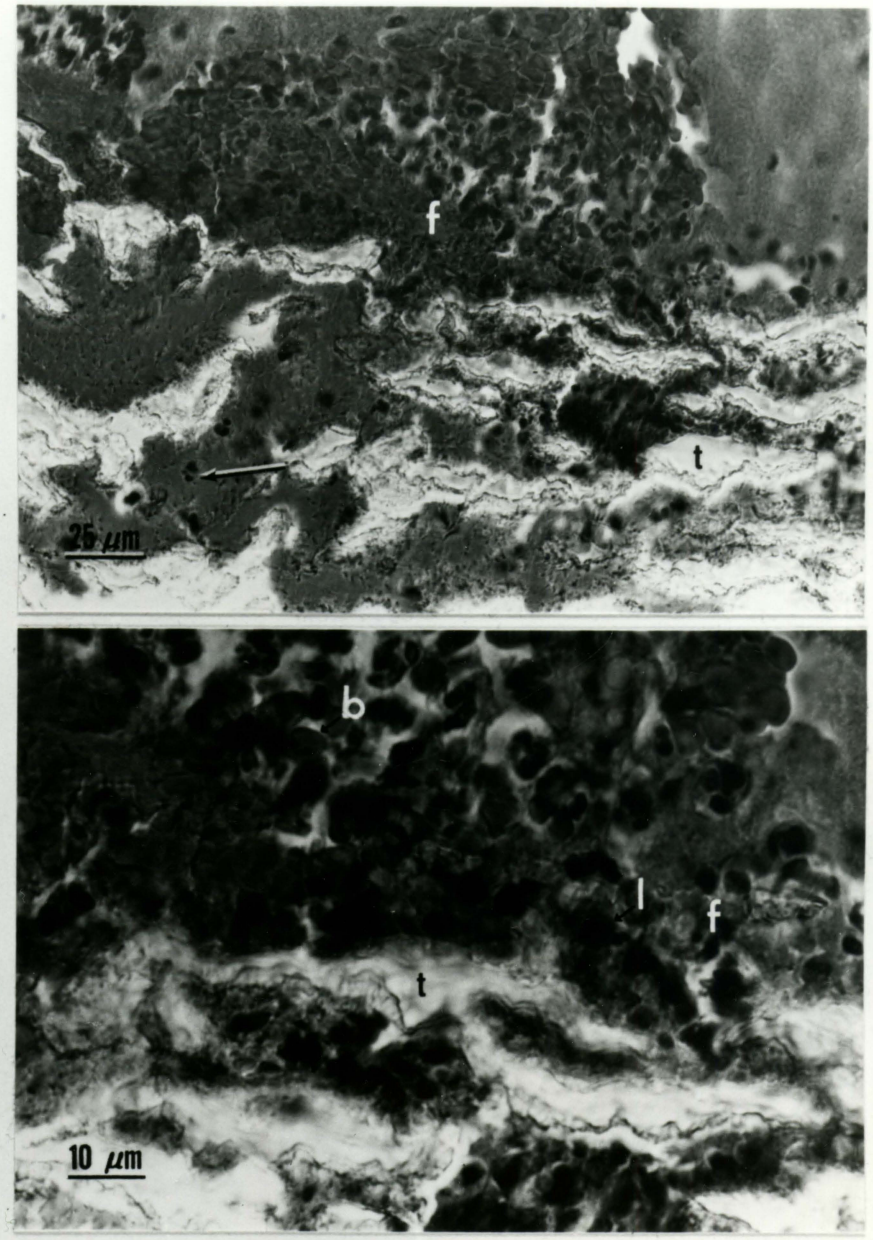

Figure 13. Light micrographs of sectioned Gore-tex® stained with hematoxylin and eosin. Teflon® is at t, fibrin at f, RBC at b and leukocyte at 1. Notice the blood cells and fibrin penetration into the graft (arrow)

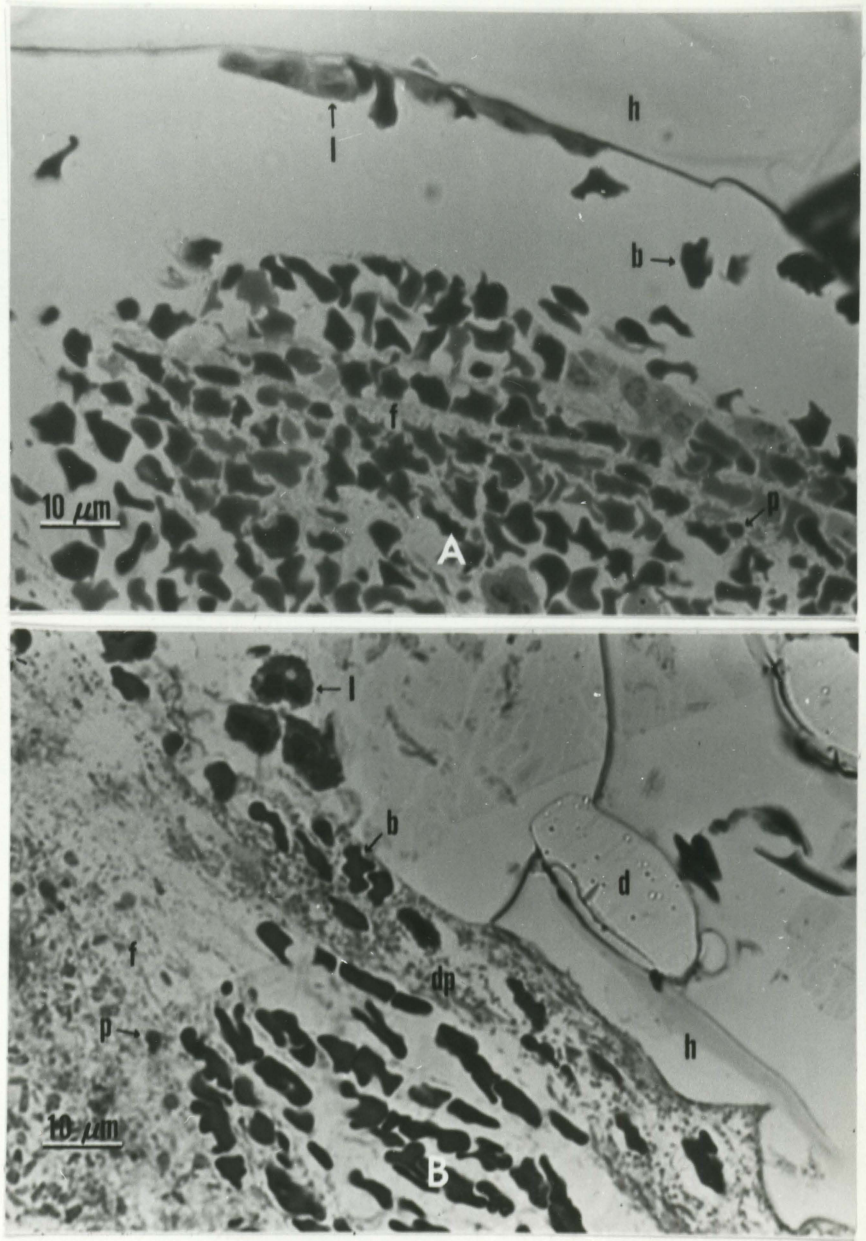

Figure 14. Light micrographs of sectioned 20% HEMA/2% EGDM/39% methanol (A) and 20% HEMA/2% EGDM/19% methanol (B) impregnated Dacron® prostheses stained with toluidine blue. A Dacron® fiber is at d, hydrogel polymer at h, fibrin at f, RBC at b, degranulated platelets at dp, platelet at p and leukocyte at l

Surface morphology

The surface texture data (micro and macro voids) found in Table III were determined from the scanning electron micrographs (SEM) presented in Figures 15-17. The copolymer surfaces of polyhydroxyethyl methacrylate demonstrate greater thrombogenicity with increasing pore size. The least thrombogenic formulation of 20% HEMA, 2% MMA and 20% methanol possesses a smooth nonporous surface at 5,000X magnification while the most thrombogenic formulation of 20% HEMA, 2% EGDM and 19% methanol has void diameters between 1 - 4 μm. Gore-tex® was an exception showing compatibility similar to the formulation of 20% HEMA, 2% MMA and 15% methanol that possessed a flakey, nonporous surface at 5,000X magnification. Since Gore-tex® had voids between 9 - 14 μm in diameter and fiber lengths < 30 μm, the shape of the voids and the hydrophobic nature of Teflon® must influence overall compatibility.

Water imbibement

Water imbibement data are recorded in Table III. The polymer formulations of 20% HEMA/2% MMA with the largest water contents demonstrate the best hemocompatibility, while the most thrombogenic polymer formulations of 20% HEMA/2% EGDM had the lowest water content. Gore-tex® showed moderate thromboresistance and a zero water content.

Solid-liquid contact angles

An example of the sessile drop contact angle (θ) measurement used in this study is represented in Figure 18. The wettability of water on the hydrogel copolymers, expanded Teflon® (Gore-tex®) and silicone rubber (Silastic®) was observed with the use of solid-liquid contact

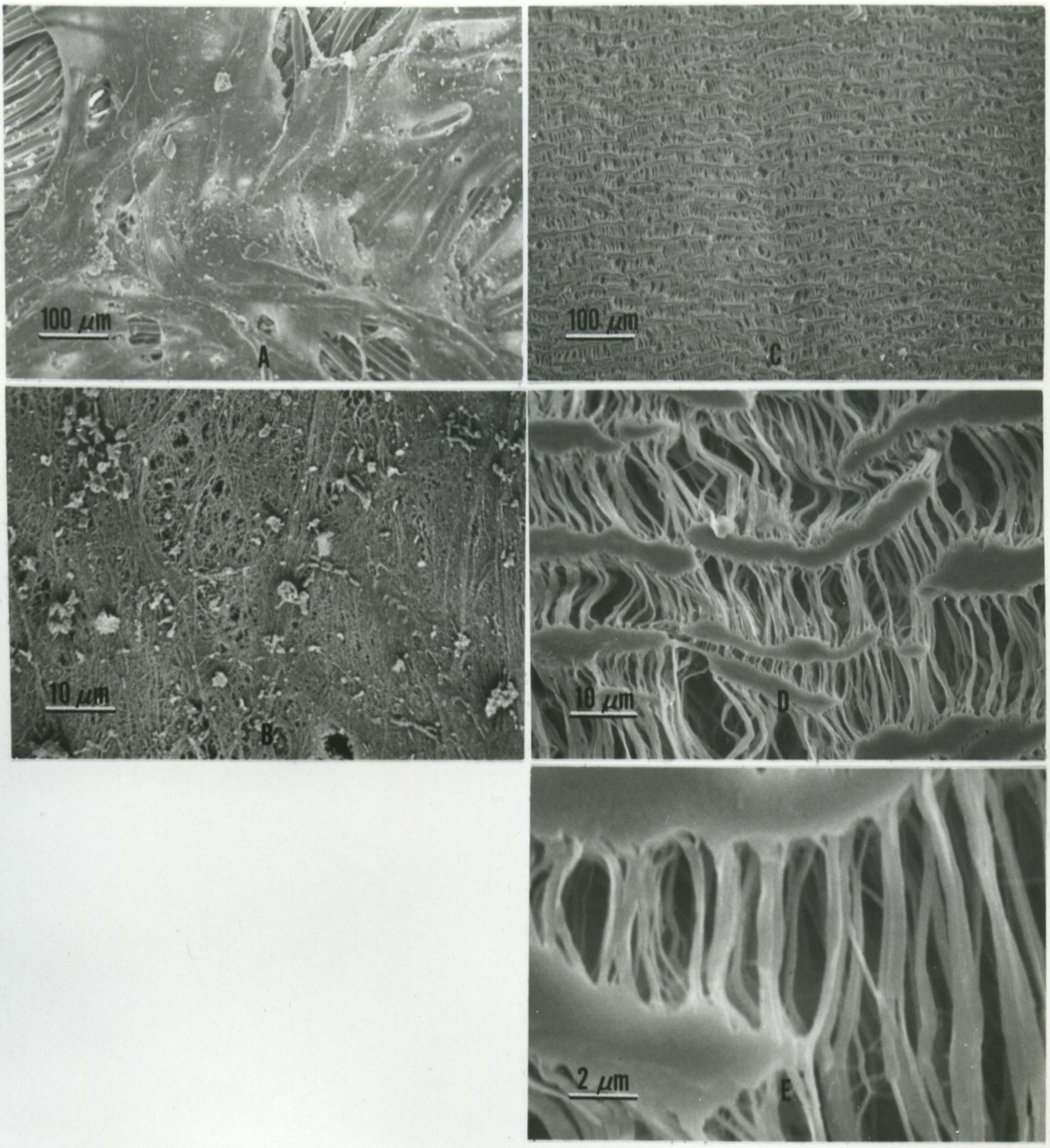

Figure 15. Scanning electron micrographs of a preclotted fibrin surface (A and B) and a Gore-tex® surface (C, D and E). Fibrin forms a thin sheet over the Dacron®. Gore-tex® contains voids between 9-14 µm with fiber lengths of 30 µm. 15 KeV (A), 5 KeV (B) and 10 KeV (C, D and E)

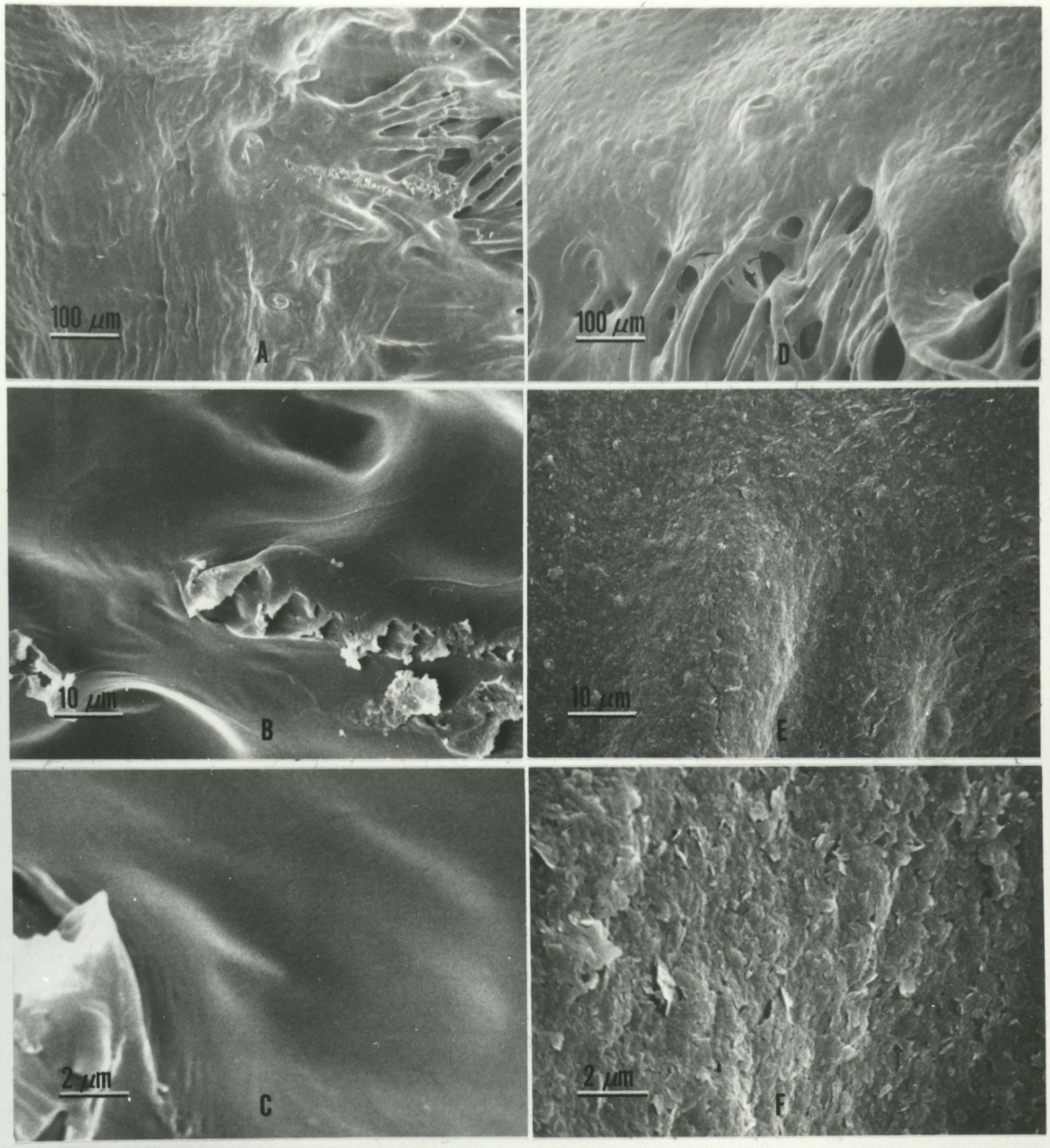

Figure 16. Scanning electron micrographs of 20% HEMA/2% MMA/20% methanol (A, B and C) and 20% HEMA/2% MMA/15% methanol (D, E and F) impregnated Dacron® grafts. 10KeV

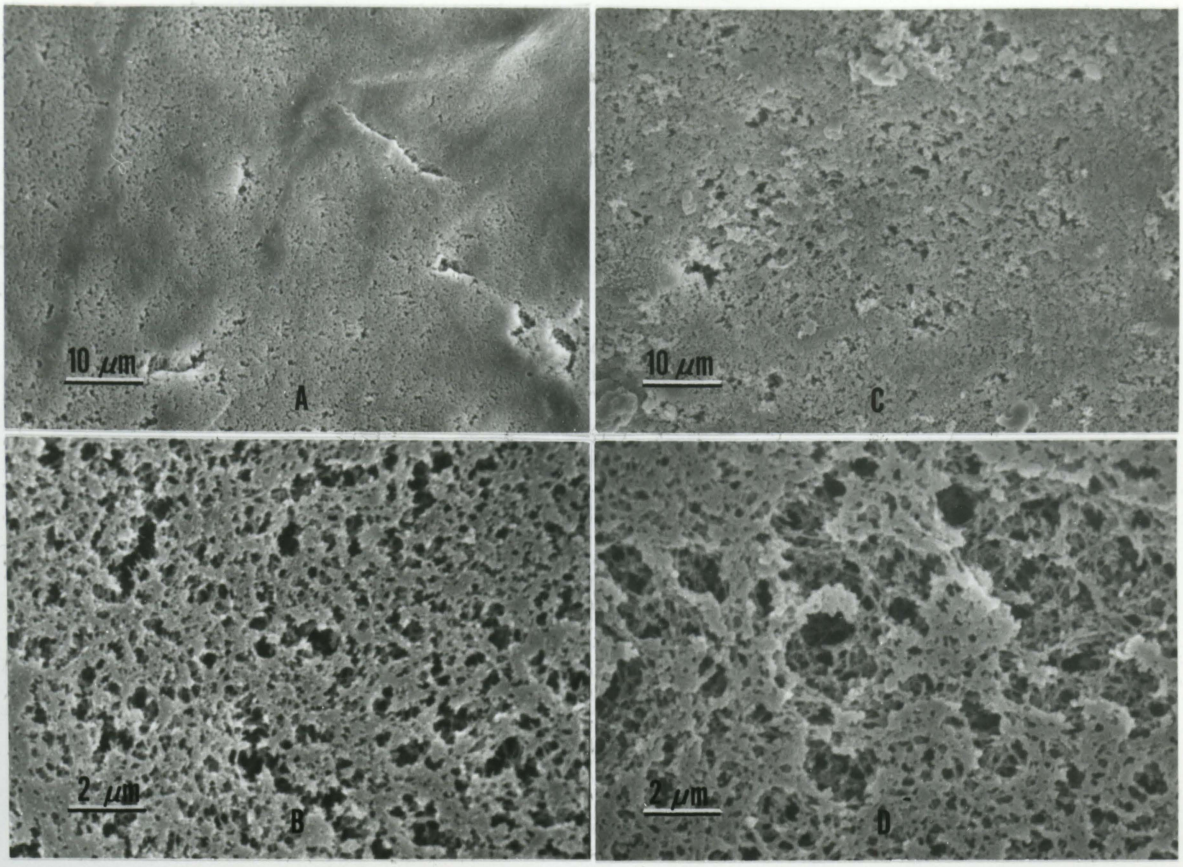

Figure 17. Scanning electron micrographs of 20% HEMA/2% EGDM/39% methanol (A and B) and 20% HEMA/2% EGDM/19% methanol (C and D) impregnated Dacron® grafts. Grafts of HEMA/EGDM/39% contain voids between 0.2-1 μm. Grafts of HEMA/EGDM/19% contain voids between 1-4 μm. 10 KeV

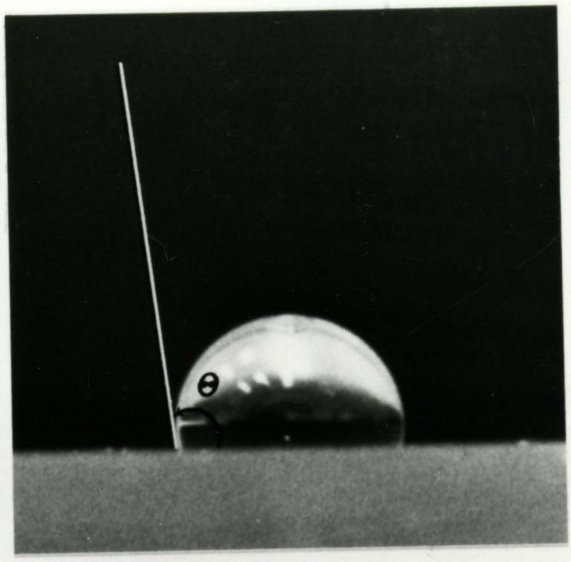

Figure 18. Sessile drop method for contact angle (θ) measurements

angles (θ) and recorded in Table IV. Figure 19 compares the polymer-water contact angles (θ) of the materials listed in Table IV.

The most wettable (lowest θ) materials were the copolymers of HEMA/EGDM and the least wettable (highest θ) was expanded Teflon® (Gore-tex®). The water contact angle of 101° for silicone rubber (Silastic®) was the same as the contact angle reported by Owens and Wendt (1969). Literature values for the hydrogel formulations and expanded Teflon® (Gore-tex®) are not available for comparison.

Critical surface tension

Contact angle information from Table IV was utilized in Figure 20 to obtain a critical surface tension (γ_c) for each polymeric material. Silicone rubber (Silastic®), Gore-tex® and the polymer formulation of 20% HEMA/2% MMA/20% methanol had critical surface tensions in the 20-31 dynes/cm range. The highest critical surface tensions were observed for 20% HEMA/2% EGDM copolymer formulations. The critical surface tension (γ_c) of Gore-tex® (28 dynes/cm) cannot be compared with Teflon® (18.5 dynes/cm) due to structural differences. The silicone rubber value of γ_c = 26 dynes/cm showed good agreement with the reported value of γ_c = 24 dynes/cm (Owens and Wendt, 1969).

General observations

Table I shows the relationship between artery diameter and percent of stenosis. Every sample, except #23, that used the smallest flow meter (2.5 mm, indicating an arterial diameter of ~ 3.2 mm) had the largest percent of luminal stenosis within each graft type.

Differences in activated clotting times, platelet counts and

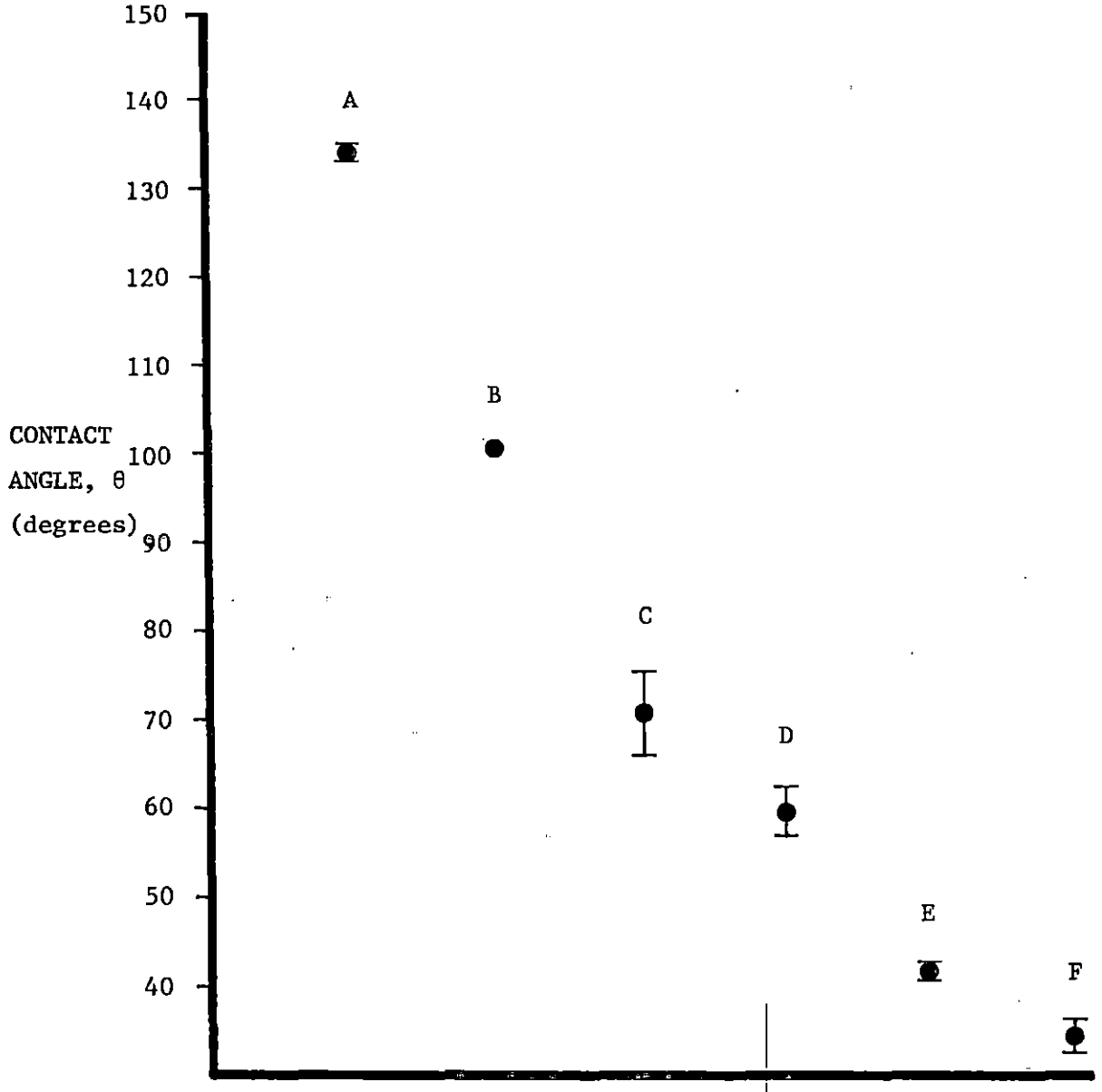

Figure 19. Water wettability for each polymer formulation using contact angle comparisons. Gore-tex® is A, silicone rubber is B, 20% HEMA/2% MMA/15% methanol is C, 20% HEMA/2% MMA/20% methanol is D, 20% HEMA/2% EGDM/39% methanol is E and 20% HEMA/2% EGDM/19% methanol is F. Silicone rubber was used as a standard

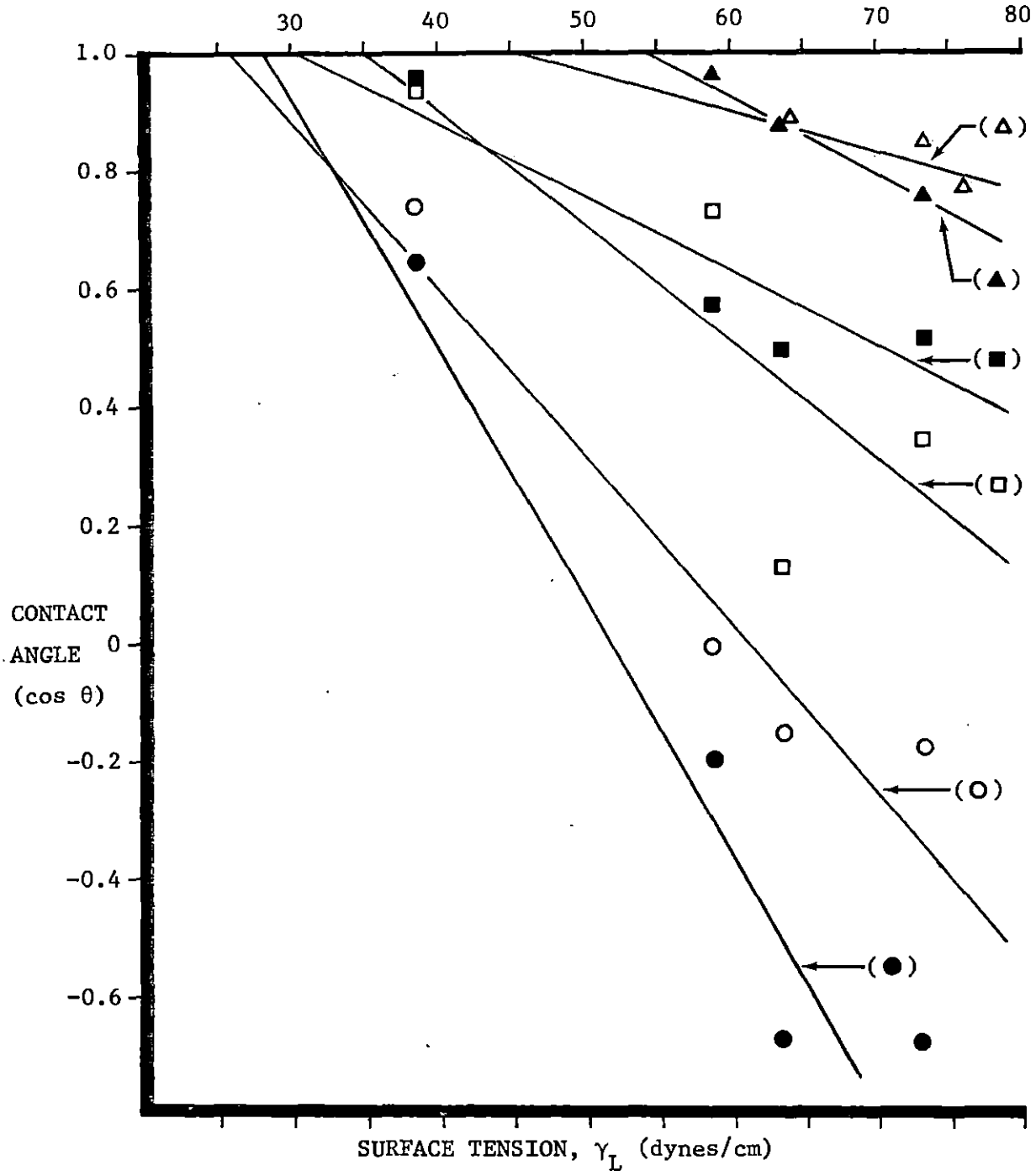

Figure 20. Zisman plot for determining the critical surface tension of each polymer formulation. (○) is silicone rubber, (●) is Gore-tex®, (■) is HEMA/MMA/20%, (□) is HEMA/MMA/15%, (▲) is HEMA/EGDM/39% and (△) is HEMA/EGDM/19%. Linear regression was utilized for line placement.

systolic/diastolic flow rates did not seem to cause any significant changes with respect to graft performance.

Statistical analysis of data

Table V shows a comparison of the statistical interdependence of each type of graft with respect to percent of thrombus free surface (TFS), percent of luminal stenosis (LS) and percent of total volume occlusion (TVO). A t-test for determining the significance between two sample means was used to determine sample independence. A t-score with a probability of 0.05 or greater would indicate an insignificant difference between the two means measured (dependent). If a t-score has a probability of 0.01 or less then the difference between the two means is highly significant (independent). A t-score with a probability between 0.05 and 0.01 would possibly indicate a significant difference, but would be inconclusive.

The difference between the USCI® Sauvage™ noncrimped supported Dacron® and 20% HEMA/2% MMA/20% methanol impregnated Dacron® grafts was not significant (dependent) with respect to percent of thrombus free surface, percent of luminal stenosis and percent of total volume occlusion. The differences between the performances of the above grafts and those of Gore-tex® and 20% HEMA/2% MMA/15% methanol impregnated Dacron® grafts were questionably significant. The 20% HEMA/2% EGDM/39% methanol impregnated Dacron® graft showed a highly significant difference (independence) with respect to USCI® Sauvage™ noncrimped supported Dacron® and 20% HEMA/2% MMA/20% methanol impregnated Dacron® grafts. The difference between Gore-tex® and 20% HEMA/2% MMA/15% methanol impreg-

Table V. t-test comparison of grafts with respect to percent of thrombus free surface (TFS), percent of luminal stenosis (LS) and percent of total volume occlusion (TVO)

		USCI® Sauvage™ supported noncrimped Dacron®		20% HEMA 2% MMA 20% methanol		20% HEMA 2% MMA 15% methanol		Gore-tex®, expanded PTFE	
		t_p[a]	ν[b]	t_p	ν	t_p	ν	t_p	ν
20% HEMA	TFS[c]	0.7	4	–	–	–	–	–	–
2% MMA	LS[d]	0.6	4	–	–	–	–	–	–
20% methanol	TVO[e]	0.7	4	–	–	–	–	–	–
20% HEMA	TFS	0.05	4	0.05	4	–	–	–	–
2% MMA	LS	0.1	4	0.1	4	–	–	–	–
15% methanol	TVO	0.02	4	0.02	4	–	–	–	–
Gore-tex®,	TFS	0.1	3	0.1	3	0.8	3	–	–
expanded	LS	0.4	3	0.4	3	0.7	3	–	–
PTFE	TVO	0.05	3	0.05	3	0.8	3	–	–
20% HEMA	TFS	0.01	3	0.02	3	0.5	3	0.8	2
2% EGDM	LS	0.01	3	< 0.01	3	0.05	3	0.2	2
39% methanol	TVO	< 0.01	3	< 0.01	3	0.02	3	0.05	2

[a]Probability of t-score.

[b]Degrees of freedom (N - 2).

[c]Percent of thrombus free surface.

[d]Percent of luminal stenosis.

[e]Percent of total volume occlusion.

nated Dacron® grafts was not significant with respect to percent of thrombus free surface, percent of luminal stenosis and percent of occluded volume.

Discussion

To predict a graft's patency several surface parameters such as texture (micro and macro), degree of hydrophilicity and blood-solid interfacial energy must be considered. Other long-term factors to be considered are tissue biocompatibility (immune response), structural strength, circumferential flexibility, chemical stability and neointimal healing (endothelial development). Long-term considerations were not included in this study.

The criteria for evaluating graft performance included percent of thrombus free surface, percent of luminal stenosis and percent of total volume occlusion.

Surface texture

Variations in surface texture produced significant deviations in thromboresistance. The smoothest and least porous hydrogel surface (20% HEMA, 2% MMA, 20% methanol, and 58% water) demonstrated superior blood compatibility. The roughest and most porous hydrogel (20% HEMA, 2% EGDM, 19% methanol and 59% water) was the most thrombogenic. Cumming (1980) attributed cellular adhesion and aggregation, with respect to surface texture, to flow effects.

Large surface imperfections caused by suture lines, artery-graft mismatch and crimps of hydrogel impregnated grafts may initiate thrombus

formation. This is in agreement with the literature cited (Herzlinger and Cumming, 1980).

Gore-tex® contained the largest voids but was omitted from the texture discussion because of the fiber connected elongated pores and the hydrophobic character of Teflon®.

Hydrophilicity

The degree of surface hydrophilicity, with respect to blood coagulation phenomena, is influenced by the following: surface energy, water content and hydrophilic-hydrophobic microphase separation.

The surface energy of materials can be compared using contact angle information. The 20% HEMA/2% EGDM formulations are the most hydrophilic (smallest θ) while Gore-tex® is the most hydrophobic (largest θ). A decrease in thromboresistance with increasing hydrophilicity was observed by Baier and Dutton (1969). The increase in thrombogenicity with increasing hydrophilicity may influence plasma proteins, as previously suggested. This seems to disagree with the increasing protein denaturation with decreasing hydrophilicity observed by Hoffman (1974).

Water content and structuring seem to affect hemocompatibility. Hemocompatibility increases with increasing water content, see Table III. Water content may directly influence the extent of water structuring. The importance of water within a material is presently unknown.

There seems to be good blood compatibility with materials containing hydrophilic-hydrophobic microphase separations. This study was in agreement with the observations of Nakashima, et al. (1977) of improved blood compatibility with hydrophilic-hydrophobic microphase

separated copolymers. It is unknown whether water formations or protein phenomena are responsible for improved blood compatibility.

Blood-solid interfacial energy

Interfacial energy, represented by critical surface tension (γ_c), provides significant information with respect to thrombogenic character. The least thrombogenic surface had a γ_c = 31 dynes/cm which approached the desired range of 20-30 dynes/cm suggested by Andrade. The most thrombogenic materials had critical surface tensions (γ_c) above 35 dynes/cm. A material with a critical surface tension (γ_c) between 20-35 dynes/cm seems to be desirable for hemocompatibility.

CONCLUSION

The patency of the small caliber prostheses tested in this study could be predicted by observed hemocompatibility. The USCI® Sauvage™ noncrimped supported Dacron® and 20% HEMA/2% MMA/20% methanol impregnated Dacron® grafts would be predicted to demonstrate superior patency in small caliber, low flow arterial applications. Gore-tex® and 20% HEMA/2% MMA/15% methanol impregnated Dacron® grafts would be predicted to show moderate patency. The 20% HEMA/2% EGDM impregnated Dacron® formulations would show the least patency. This type of graded evaluation of graft performance could be used as a valuable tool for surgeons, as well as investigators working on a correlation between surface parameters and blood compatibility.

Several surface parameters such as texture, degree of hydrophilicity (with respect to surface energy, water content and microphase separation of hydrophilic-hydrophobic sites) and blood-solid interfacial energy (critical surface tension, γ_c) were found to be significant with respect to blood compatibility. The evaluation of hemocompatibility was measured by means of percent thrombus free surface, percent of luminal stenosis and percent of total luminal occlusion. The determination of which surface parameter (texture, hydrophilicity or interfacial energy) had the most influence on the hemocompatibility performance (percent thrombus free surface, percent luminal stenosis and percent of total volume occlusion) of each graft was not obtainable. A study of each independent parameter (texture,

hydrophilicity and interfacial energy) should be performed before assigning a level of significance to each parameter with respect to hemocompatibility.

RECOMMENDATIONS FOR FUTURE RESEARCH

Three- and six-week implantation studies of the grafts presented in this investigation would further indicate the significance of the percent of thrombus free surface, percent of luminal stenosis and percent of total volume occlusion with respect to patency prediction.

The significance of artery-prosthesis mismatch could be determined by implanting dual coated hydrogel impregnated grafts, with 0.5 mm incremented diameters, in the canine carotid artery. This would allow varying degrees of mismatch. The percent of luminal stenosis and percent of total volume occlusion could be utilized as modes of evaluation.

Blood compatibility studies of several polymers, using the evaluation methods of this investigation, would be desirable. The best polymer formulation could then be maximized with respect to surface parameters. Of interest would be copolymers with microphase separated hydrophilic-hydrophobic domains, such as HEMA/MMA and HEMA/EMA copolymers.

The following surface parameters deserve further study: texture or microstructure, hydrophilicity and critical surface tension.

Microstructure can be controlled by solvent ratios, as reported by Knoll (1980) and observed for several polymer systems. A correlation between microstructure and hemocompatibility for each polymer system would provide significant information for choosing microstructure.

Hemocompatibility in relation to hydrophilicity, as measured by water content and wettability, warrants further investigation.

It is possible that porous polymer networks, such as Gore-tex® or 20% HEMA/2% EGDM/19% methanol, may demonstrate superior compatibility if preclotted prior to utilization. A thin fibrin surface over the compatible hydrogel would make exposure of the thrombogenic Dacron® fibers less likely. The preclotted Gore-tex® may reduce incidence of wound edema.

LITERATURE CITED

Andrade, J. D., H. B. Lee, M. S. Jhon, S. W. Kim, and J. B. Hibbs. 1973. Water as a biomaterial. Trans. Amer. Soc. Artif. Int. Org. 19: 1-7.

Andrew, W. D., and M. R. Lewis. 1976. Further experiences with bovine arterial grafts. Surgery 80: 711-721.

Baier, R. E., and R. C. Dutton. 1969. Initial events in interactions of blood with a foreign surface. J. Biomed. Mater. Res. 3: 191-206.

Boffa, G. A., N. Lucien, A. Faure, M. C. Boffa, J. Jozefonvicz, A. Szubarga, P. Mandon, and M. J. Larrieu. 1977. Polytetrafluoroethylene-N-vinylpyrrolidone graft copolymers: Affinity with plasma proteins. J. Biomed. Mater. Res. 11: 317-337.

Bruck, S. D. 1977. Interactions of synthetic and natural surfaces with blood in the physiological environment. J. Biomed. Mater. Res. Symp. 11: 1-22.

Campbell, C. D., D. H. Brooks, M. W. Webster, D. L. Diamond, R. L. Peel, and H. T. Bahnson. 1979. Expanded microporous polytetrafluoroethylene as a vascular substitute: A two year follow-up. Surgery 85: 177-183.

Cumming, R. D. 1980. Important factors affecting initial blood-material interactions. Trans. Amer. Soc. Artif. Int. Org. 26: 304-308.

Dale, W. A., and M. R. Lewis. 1976. Further experiences with bovine arterial grafts. Surgery 80: 711-721.

Davidsohn, I., and J. B. Henry. 1969. Todd-Sanford Clinical Diagnosis by Laboratory Methods. 14th Edition. Saunders Company, Philadelphia, Pa.

DeBakey, M. E. 1979. The development of vascular surgery. Am. J. Surg. 137: 697-738.

Edwards, W. S. 1978. Arterial grafts — Past, present and future. Arch. Surg. 113: 1225-1233.

Eskin, S. G., L. Trevino, and J. E. Chinoskey. 1978. Endothelial cell culture on Dacron fabrics of different configurations. J. Biomed. Mater. Res. 12: 517-524.

Garcia, C., J. M. Anderson, and S. A. Barenberg. 1980. Hemocompatibility: Effect of structured water. Trans. Amer. Soc. Artif. Int. Org. 26: 294-298.

Guidoin, R. G., C. Gosselin, D. Domurado, M. Marois, P. A. Levaillant, J. Awad, C. Rouleau, and L. Levasseur. 1977. Dacron as arterial prosthetic material: Nature, properties, brands, fate and perspectives. Biomat. Med. Dev. Art. Org. 5: 177-203.

Guyton, A. C. 1976. Textbook of medical physiology. Fifth Edition. W. B. Saunders Company, Philadelphia, Pa.

Hersh, L. S., V. L. Gott, and F. Najjar. 1972. Thermal and ionic methods of heparinizing small-diameter Dacron grafts. J. Biomed. Mater. Res. Symp. 3: 85-96.

Herzlinger, G. A., and R. D. Cumming. 1980. Role of complement activation in cell adhesion to polymer blood contact surfaces. Trans. Amer. Soc. Artif. Int. Org. 26: 165-170.

Hiratzka, L. F., and C. B. Wright. 1978. Experimental and clinical results of grafts in the venous system: A current review. J. Surg. Res. 25: 542-561.

Hiratzka, L. F., J. A. Goeken, R. A. White, and C. B. Wright. 1979. In vivo comparison of replimineform Silastic and bioelectric polyurethane arterial grafts. Arch. Surg. 114: 698-702.

Hoffman, A. S. 1974. Principles governing biomolecular interactions at foreign interfaces. J. Biomed. Mater. Res. Symp. 8: 77-83.

Hoffman, A. S. 1975. Hydrogels - A broad class of biomaterials. Pages 33-44 in R. L. Kronenthal, et al., Eds. Polymers in medicine and surgery. Plenum Press, New York, N.Y.

Hollier, L. H., R. C. Batson, F. M. Gonzalez, W. A. Rock, Jr., and J. E. Pearson. 1980. Causes of thrombosis in angioaccess models in the goat. Trans. Amer. Soc. Artif. Int. Org. 26: 82-86.

Holly, F. J., and M. F. Refojo. 1976. Water wettability of hydrogels. Pages 252-266 in J. D. Andrade, ed. Hydrogels for medical and related applications. American Chemical Society, Washington, D.C.

Jhon, M. S., and J. D. Andrade. 1973. Water and hydrogels. J. Biomed. Mater. Res. 7: 509-522.

Kaetsu, I., M. Kumakura, M. Asano, A. Yamada, and Y. Sakurai. 1980. Immobilization of enzymes for medical uses on plastic surfaces by radiation-induced polymerization at low temperatures. J. Biomed. Mater. Res. 14: 199-210.

Kenny, D. A., K. Berger, M. W. Walker, S. B. Robel, L. Boguslavsky, L. I. Ray, M. M. Lischko, and L. R. Sauvage. 1980. Experimental comparison of the thrombogenicity of fibrin and PTFE flow surfaces. Ann. Surg. 191: 355-361.

Kim, S. W., R. G. Lee, H. Oster, D. Coleman, J. D. Andrade, D. J. Lentz, and D. Olsen. 1974. Platelet adhesion to polymer surfaces. Trans. Amer. Soc. Artif. Int. Org. 20: 449-455.

Knoll, R. L. 1980. Analysis of polyhydroxyethyl methacrylate coatings on polyethylene terephthalate fabric substrates for cardiovascular prosthetic applications. Ph.D. dissertation. Iowa State University, Ames, Iowa. 230 pp.

Lindsay, R. M., R. G. Mason, S. W. Kim, J. D. Andrade, and R. M. Hakim. 1980. Blood surface interactions. Trans. Amer. Soc. Artif. Int. Org. 26: 603-610.

Mansfield, P. B., A. R. Wichnezak, and L. R. Sauvage. 1975. Preventing thrombus on artificial vascular surfaces: True endothelial cells. Trans. Amer. Soc. Artif. Int. Organs 21: 264-272.

Mindich, B., M. Silverman, A. Elguezabel, L. Flores, R. P. Sheka, and B. S. Levowitz. 1977. Human umbilical cord vein for vascular replacement: Preliminary report and observations. Surgery 81: 152-160.

Nakashima, T., K. Takakura, and Y. Komoto. 1977. Thromboresistance of graft-type copolymers with hydrophilic-hydrophobic microphase-separated structure. J. Biomed. Mater. Res. 11: 787-798.

Nyilas, E., W. A. Morton, R. D. Cumming, D. M. Lederman, and T. H. Chiu. 1977. Effects of polymer surface molecular structure and force-field characteristics on blood interfacial phenomena. J. Biomed. Mater. Res. Symp. 11: 51-68.

O'Brien, B. 1977. Microvascular reconstructive surgery. Churchill Livingston, London. 359 pp.

Owens, D. K., and R. C. Wendt. 1969. Estimation of the surface free energy of polymers. J. Appl. Polym. Sci. 13: 1741-1747.

Ratner, B. D. 1980. Characterization of graft polymers for biomedical applications. J. Biomed. Mater. Res. 14: 665-687.

Ratner, B. D., and A. S. Hoffman. 1975. Radiation grafted hydrogels on silicone rubber as new biomaterials. Pages 159-171 in H. P. Gregor, ed. Biomedical applications of polymers. Plenum Publication Corp., New York.

Ratner, B. D., A. S. Hoffman, and J. D. Whiffen. 1978. The thrombogenicity of radiation grafted polymers as measured by the vena cava ring test. J. Bioeng. 2: 313-323.

Salzman, E. W. 1972. Surface effects in hemostasis and thrombosis. Pages 489-522 in M. L. Hair, ed. The chemistry of biosurfaces, Vol. II. Marcel Dekker, Inc., New York.

Sauvage, L. R., K. E. Berger, P. B. Mansfield, S. J. Wood, J. C. Smith, and J. B. Overton. 1974. Future directions in the development of arterial prostheses for small and medium caliber arteries. Surg. Clin. North America 54: 213-228.

Sauvage, L. R., K. E. Berger, S. J. Wood, L. G. Fernandez, P. B. Mansfield, J. C. Smith, C. C. Davis, D. G. Hall, and E. A. Rittenhouse. 1976. Healing of arterial prostheses: Goal of design and clinical use. Providence Medical Center, Seattle, Washington. 23 pp.

Sauvage, L. R., M. W. Walker, K. Berger, S. B. Robel, M. Lischko, S. G. Yates, and G. A. Logan. 1979. Current arterial prostheses. Arch. Surg. 114: 687-691.

Sawyer, P. N., B. Stanczewski, G. P. Hoskin, Z. Sophie, R. M. Stillman, R. J. Turner, and H. L. Hoffman. 1979. In vitro and in vivo evaluations of Dacron velour and knit prostheses. J. Biomed. Mater. Res. 13: 937-956.

Schoen, F. J., S. J. Normann, R. A. Brunswick, and G. R. Diacoff. 1979. Can a small blood vessel prosthesis be derived from heterologous foreign body reactive tissue? J. Biomed. Mater. Res. 13: 149-154.

Schultz, J. S., S. M. Lindenauer, J. A. Penner, and S. Barenberg. 1980. Determinants of thrombus formation on surfaces. Trans. Amer. Soc. Artif. Int. Org. 26: 279-283.

Selman, S. H., R. S. Rhodes, J. M. Anderson, R. G. DePalma, and A. W. Clowes. 1980. Atheromatous changes in expanded polytetrafluoroethylene grafts. Surgery 87: 630-637.

Veith, F. J., S. Gupta, and V. Daly. 1980. Management of early and late thrombosis of expanded polytetrafluoroethylene (PTFE) femoropopliteal bypass grafts: Favorable prognosis with appropriate reoperation. Surgery 87: 581-587.

Voorhees, A. B., A. Jarentski, and A. H. Blakemore. 1952. The use of tubes constructed from Vinyon "N" cloth in bridging arterial defects. Ann. Surg. 135: 332-336.

Weast, R. C., ed. 1976. CRC handbook of chemistry and physics. 48th edition. Chemical Rubber Co., Cleveland, Ohio.

Weathersby, P. K., T. A. Horbett, and A. S. Hoffman. 1977. Surface analysis of methacrylate graft copolymers varying in hydrophilicity. J. Bioeng. 1: 381-394.

Yates, S. G., A. B. Barros, K. Berger, L. G. Fernandez, S. J. Wood, E. A. Rittenhouse, C. C. Davis, P. B. Mansfield, and L. R. Sauvage. 1978. The preclotting of porous arterial prostheses. Ann. Surg. 188: 611-622.

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APPENDIX: INTRINSIC AND EXTRINSIC PATHWAYS

This diagram is from Salzman (1972) and provides an overview of important factors involved in the clotting cascade with foreign materials.