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⑦① Applicant: **Sawyer, Philip Nicholas, 7600 Ridge Boulevard, Brooklyn New York (US)**

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⑦② Inventor: **Sawyer, Philip Nicholas, 7600 Ridge Boulevard, Brooklyn New York (US)**

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⑦④ Representative: **Vossius, Volker, Dr. et al, Vossius, Vossius, Hiltl, Tauchner, Heunemann Patentanwälte Siebertstrasse 4, D-8000 München 86 (DE)**

⑤④ **Cardiac and vascular prostheses and methods of making the same.**

⑤⑦ Cardiac and vascular prostheses and methods of making the same. The prostheses are suitable for implantation in a bloody environment. The prosthesis includes a surface material adapted for exhibiting a regular and periodic surface charge characteristic in the bloody environment (See Fig. 1A: scanning electron micrographs of an albumin Dacron hybrid graft). A prosthesis means supports the surface material and is adapted to perform a prosthetic function in the bloody environment. In cases where cross-linking is desired and the surface material cannot cross-link with the substrate material, an adaptive material is provided which cross-links both to the surface material and to the material of the prosthesis.



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PATENTANWÄLTE
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Philip Nicholas Sawyer
Brooklyn, New York, U.S.A.

"Cardiac and Vascular Prostheses and Methods of Making
the Same"

Priority: August 26, 1977, U.S.A., No. 827,952

Field of Invention

This invention relates to cardiac and vascular prostheses
and methods of making the same and materials therefor.

1 Background of the Invention

2 The major emphasis in early research on implants was per-
3 formed within the field of orthopedics. (Bechtol, C. D.,
4 Ferguson, Jr., A. B., and Laing, P. G. Metals and Engi-
5 neering in Bone and Joint Surgery. Williams and Wilkins
6 Co., Balt. Chapter 1, 1-18 (1959); Scales, J. T.
7 Arthroplasty of the Hip Using Foreign Materials: A
8 History, Paper 13, A Symposium, Institute of Mechanical
9 Engineers, Vol. 181 Part 3J. 63-84, (1967); Weisman. S.
10 Metals for Implantation in the Human Body. Ann. N.Y.
11 Acad. Sci. 146, 80-95, (1968)). The most significant
12 addition to implant surgery in the last twenty years has
13 been the development of prostheses for vascular and
14 cardiac reconstruction (Wesolowski, S.A., Martinez, A.,
15 and McMahon, J. D. The Use of Artificial Materials in
16 Surgery, "Current Problems in Surgery." Year Book Me-
17 dical Publishers, Inc., Chicago (1966). A major problem
18 involved in the use of prosthetic alloplastic materials
19 has been severe occlusive thrombogenicity (Hufnagel, C.
20 A. The Use of Rigid and Flexible Plastic Prostheses for
21 Arterial Replacement, Surgery, 37:165, (1955)). The
22 search for acceptable materials and designs for vascular
23 prostheses was of great importance because of a number of
24 problems which were encountered with the use of polymeric
25 and metal prostheses, autologous, homologous and hetero-
26 logous bypass grafts in man. (Johnson, W. D. Auer, J. E.
27 and Tector, A. T., Late Changes in Coronary Vein Grafts,
28 Am. Jour. of Cardiol. 26: 640, (1970) and Artegraft Con-
29 ference, Johnson and Johnson, Toronto, Ontario, Canada,
30 June 23, (1973)).

31 Following the development of open heart surgery, an
32 enormous effort was made in the field of prosthetic
33 heart valves. The first successful development in this
34 area was made by Goodman, Berg and Stuckey (Wesolowski,
35 S. A. et al. The Use of Artificial Materials in Surgery,

- 1 "Current Problems in Surgery" Year Book Medical Publishers, Inc., Chicago (1966). They produced the first completely thromboresistant prosthetic heart valve. This valve was later improved by Dr. Albert Starr and Edwards Laboratories (Edwards, W. S. and Smith, L. Aortial Valve Replacement with a Sub Coronary Ball Valve. Sur. Forum 9:309 (1958); Starr, A. Mitral Valve Replacement with a Ball Valve Prosthesis. British Heart Journal, 33 Supp. 47 (1971). A number of valves were further developed by several corporations. These included the Bjork-Shiley valve which is the most successful of the prosthetic heart valves available today. More recently, glutaraldehyde tanned porcine collagen prosthetic heart valves developed by the Hancock Laboratory (Sauvage, L. R. Wesolowski, A. S., Sawyer, P. N. et al. Prosthetic Valve Replacement Ann. of the N. Y. Academy of Sci. 146:289 (1968)) have proven useful. They are resistant to intravascular thrombosis and have maintained their tensile strength following several years of implantation in man. Thus, a host of glutaraldehyde tanned collagen valvular prostheses have proven useful in man with respect to long term function and resistance to thrombosis.
- 25 Early attempts to replace blood vessels in man involved the use of rigid tubes of gold, silver, aluminum, magnesium, as well as the later development of polyethylene and polymethyl acrylic tubes (Szilagyi, D. E. Long Term Evaluation of Plastic Arterial Substitutes: An Experimental Study, Surg. 55:165 (1964); Woodward, S. C. Biological End Points for Compatibility, Plastics in Surgical Implants ASTM-STP 386, A Symposium on Surg. Implants, Indianapolis, Indiana (1964); Sawyer, P. N., Wu, K. T., Wesolowski, A. S., Brattain, W. H. and Boddy, P. J. Long Term Patency of Solid Wall Vascular Prostheses, Arch. Surg. 91:735, (1965). In the majority of cases, these prostheses did not function satisfactorily. A major

1 breakthrough came in 1952 when Voorhees and Blakemore
described their experiments with a cloth prosthesis of
Vinyon. (Voorhees, A. B., Jr., Jaretski, 111, A. and
Blakemore, A. H. Use of tubes constructed from Vinyon-"N"
5 Cloth in bridging Arterial Defects. Ann. Surg. 135; 332
(1952)). This material was easy to handle, preclottable
and resistant to thrombosis following implantation,
although tensile strength, in situ, was lost with the
passage of time. Preclotting the graft with the patient's
10 blood produced a compound prosthesis, whereby, the lumen
was covered by a fibrous neo-intima and the adventitia
was enclosed by a fibrous capsule, which resulted in a
well tolerated graft. Presently, the Dacron graft is
considered to be the most successful cloth type graft.
15 (Sawyer, P. N. et al. Vascular Graft Symposium, Current
Status and Future Trends. NIH (1976)).

Early attempts at vessel substitution with materials of
biological origin were carried out using arteries from
20 cadavers (Wesolowski, S. A. and Sauvage, L. R. Hetero-
logus Aortic Hetergrafts with Special Reference to Re-
cipient Site, Ethylene Oxide Freeze Dry Preparation and
Specied of Origin, Ann. Surg. 145:187 (195). On the
whole, these homografts functioned well for a period of
25 time, but were soon replaced by fibrous tissue and calcium
salts. This resulted in an inelastic structure, susceptible to
thrombus, fracture, and aneurysm formation (Sauvage, L.
R. and Wesolowski, A. S. Healing and Fate of Arterial
Grafts, Surg. 38:1090 (1955)).

30 Following failure of the homografts, autografts from a
patient's veins (saphenous) were used. Currently, the
implantation statistics show that the saphenous vein is
superior to synthetic implants (Sawyer, P. N. et al,
35 Vascular Graft Symposium; Sauvage, L. R. and Wesolowski,
A. S. Healing and Fate of Arterial Grafts, supra, and
Vladamer, C. and Edwards, J. E. Pathological Changes
in Aortocoronary Arterial Saphenous Vein Grafts. Circula-

1 tion 44:719 (1971)). When the saphenous vein is not
available, i.e., the question arises as to which implant
should be used. Experience has shown that a vascular
graft must: (i) have an appropriate porosity (10,000-
5 20,000 ml of water/square cm/minute) Sawyer, P. N., Wu,
K. T., Wesolowski, S. A. Brattain, W. and Boddy, P. J.
An Aid in the Selection of Vascular Prosthesis Proc.
Natl. Acad. Sci., U.S.A., 53:1965), (ii) be blood comp-
atible, (iii) possess tensile strength and (iv) be easy
10 to handle with respect to sewing characteristics (Sawyer,
P. N. and Srinivasan, S. New Approaches in the Selections
of Materials Compatible with Blood. Artificial Heart
Prog.Conf.Proc.Chapter 2,1969).

15 In an effort to overcome the problems of porosity,
junctional thrombosis, and small diameter compliance
exhibited by most implants in the periphery, I have con-
ducted research on copolymeric collagen remnants from
bovine carotid and brachial arteries. The collagen re-
20 mains intact following enzymatic digestion (ficin) and
is then glutaraldehyde tanned and negatively charged.

When implanted, these grafts reveal a striking tendency
to remain thromboresistant and maintain their tensile
25 strength as opposed to other commercially available
collagen grafts that possess a limited degree of patency
with early loss of tensile strength. My copolymeric
grafts have remained patent in the femoral-popliteal, ca-
rotid and coronary positions (Vascular Graft Symposium,
30 supra).

As to why these types of grafts perform so well, conclu-
sive answers are not yet available. Scanning electron
microscopic studies indicate that when the grafts fail,
35 they become occluded by an atypical thrombus. It is there-
fore obvious that the success of this type of graft
depends upon (i) the surface modification characteristics
of the collagen (ii) blood interfacial reactions'

1 occurring, (iii) structural aspects of the collagen surface.

5

SUMMARY OF THE INVENTION

I have heretofore developed a negatively charged, glutar-
aldehyde tanned collagen copolymeric vascular bypass graft
This new graft appears to be superior to other commercially
10 available bypass heterografts based upon life analysis
tables. The processes used to chemically modify the
collagen are uniquely versatile.

I am now working with second and third generation grafts
15 and have produced Dacron* hybrid grafts of several types.
The application of this graft is aimed at specific clinical
problems where the saphenous vein or conventional
heterografts would be considered unsatisfactory. The
hybrid graft can also be applied to the total artificial
20 heart and bypass pump chambers and Dacron skirt of heart
valves providing a more satisfactory blood compatible
surface.

Dacron vascular grafts have been altered, in accordance
25 with the invention, by coating them and filling their
interstitial spaces with a variety of agents, in a variety
of ways, to produce grafts with superior anti-
thrombogenic characteristics.

30 The techniques utilized include: (1) copolymerization of
proteins to the Dacron with glutaraldehyde and subsequent
negative charging, (2) aluminization of the grafts,

35 *Registered Trademark for a Polyester of terephthalic acid
and ethylene glycol manufactured by DuPont de Nemours
& Co., Inc., Wilmington, Delaware, U.S.A.

1 (3) the deposit of substances which may accept from a
bloody environment materials which are compatible with
the environment.

5 It is an object of the invention to provide an improved
prosthesis material for making an improved prosthesis
and method for making the same.

It is another object of the invention to provide for
10 improved prosthesis characteristics in a blood environ-
ment.

To achieve the above and other objects of the invention
there is provided a prosthesis suitable for implantation
15 in a bloody environment, said prosthesis comprising a
surface material adapted for exhibiting a regular and
periodic surface charge characteristic in said bloody
environment, and prostheses means supporting said surface
material and adapted to perform a prosthetic function in
20 this environment.

In accordance with one embodiment of the invention, the
surface material may be aluminum. In accordance with
another embodiment, the surface material may be albumin.
25 In accordance with still other embodiments, the surface
material may be agar or acrylamide.

In accordance with one aspect of the invention, the
prosthetic means is of a material normally incapable of
30 crosslinking to said surface material. In this case,
there is provided an adaptive material to cross-link to
the material of said prosthetic means and to said surface
material to provide architectural continuity therebetween
while preserving said surface charge characteristics.
35

In accordance with the invention and the preferred

- 1 embodiment thereof, the surface charge is preferably equivalent to approximately one electron per thousand square Angstroms in a regular three-dimensional pattern.
- 5 According to the invention, there is provided a method for preparing a prosthesis for functioning in a bloody environment with minimal thrombogenicity. This method comprises depositing on the surface of the prostheses a material adapted for exhibiting a regular and periodic
- 10 thrombosis resistant surface charge in the bloody environment. Thus, for example, a synthetic material may be adapted for use as a prosthetic material which is adapted to function in a bloody environment. This may be achieved, for example, by metallizing the material
- 15 at the surface thereof.

According to one embodiment of the invention, the metallizing may take place by the vapor depositing of aluminum on the surface.

20

According to another embodiment, the aluminum may be sprayed in an aerosol on the surface.

- The prosthesis may be, for example, of woven Dacron.
- 25 Thereupon may be deposited a surface material in the form of albumin, combined with gelatin. The thusly coated prosthesis may, for example, thereafter be treated with glutaraldehyde. The material deposited on the prosthesis may be , for example, of the general category
- 30 of proteins and may be applied preferably in, for example, about a 3 % solution.

- According to the further feature of the invention, a thusly coated prosthesis may be further treated with a
- 35 succinic acid compound.

1 According to still further aspects of the invention, the material may be applied by immersing the prosthesis in a slurry of agar. As an alternative, the prosthesis may be immersed in a solution of acrylamide.

5

The above and further objects and features of the invention will be found in the detailed description thereof as follows hereinafter.

10

BRIEF DESCRIPTION OF DRAWING

IN THE DRAWING:

15 Figure 1(A) is a scanning electron microphotograph group of a polarized protein;

Figure 1(B) is a collection of scanning electron micrographs of a protein-gelatin Dacron hybrid graft;

Figure 2(A) shows scanning electron micrographs of a gelatin-Dacron hybrid graft;

20 Figure 2(B) is a group of scanning electron micrographs of a gelatin-Dacron hybrid graft;

Figure 3(A) shows the structure of an agar coated graft;

Figure 3(B) shows the implantation results of a protein-gelatin Dacron hybrid graft;

25 Figure 4 is a group of scanning electron micrographs of an aluminum coated Dacron hybrid graft;

Figure 5 shows scanning electron micrographs of an agar coated Dacron hybrid graft;

30 Figure 6(A) shows an aluminum coated Dacron prosthesis exposed to blood for one second; and

Figure 6(B) shows further magnification of the prosthesis illustrated in Fig. 6(A).

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DETAILED DESCRIPTION

As has been stated hereinabove, the invention relates to
5 the provision of a prosthesis having the capability of
functioning in a bloody environment. The prosthesis may be
of varying types but it can be for example, a vascular
prosthesis consisting of a tube adapted to function as a
connection for bypass or a valve functioning to operate
10 in the cardiac environment.

The invention is based in general on having the prosthesis
exhibit a regular and periodic negative surface charge
characteristic in the preferred case approximating one
electron per thousand square Angstroms of surface in a
15 regular threedimensional pattern. Under certain circum-
stances, the regular and periodic surface charge may be
positive.

A surface material will generally be employed to exhibit
the above-noted characteristic. For this reason, the
20 present invention is concerned with hybrid prostheses
which are prostheses consisting of a basic material
forming a prosthesis so that it is capable of performing
the desired function and a surface material which exhibits
the aforementioned charge.

25 In case the basic material is incapable of crosslinking
with the surface material, an adaptive material may be
provided to offer architectural continuity and cross-link
the surface material and the supporting material by being
adapted to cross-link itself respectively to each of these
30 materials.

The invention offers, for example, copolymerization for a
form of protein deposition, metallizing, agar application
in the form of a uniform coating and the application of,
for example, acrylamide. The invention offers further se-
35 lective processing of certain of the above materials by
treatment with succinic acid or the like or, for example,
by treatment with glutaraldehyde.

1 The following are details of materials and methods which
may be employed in accordance with the above:

Copolymerization (a method of protein deposition) Frac-
tion V Albumin (purified bovine or other mammalia albumin)
5 purchased from Sigma Corporation, St. Louis, Mo., USA was
dissolved (1, 3, 5 and 10 grams in 100 ml. of water) to
produce several solutions of 1 % - 10 % with distilled
and deionized water Gelatin (cutaneous), purchased from
Baker Corporation*, was dissolved at room temperature in
10 water, producing solutions ranging in concentration
(1, 3, 5 and 10 grams in 100 ml of water) from 1 % - 10%.

A Microknit[®] knitted or woven vascular prosthesis (Go-
laski Labs. Inc. of Philadelphia, Penna. USA) possessing
15 18 ridge to the inch (50 interstices per inch) was cut
into 10 cm. segmen (collapsed length) and cleaned in
distilled and deionized water. It is then immersed in
10-20 % (preferably 20%) glutaraldehyde (purchased from
Electron Microscope Science Corporation of Fort Washington
20 Penna., USA) with distilled and deionized water (40 ml of
50 % glutaraldehyde in 60 ml of water) and was allowed to
stand for three hours.

The graft materials were then immersed in the appropriate
protein (albumin, gelatin, collagen or the like) solution
25 until thin (1 to 2,000 microns) coats of polymerized
protein surrounded the Dacron tube. They were then
suspended in air at room temperature and rinsed with
water at room temperature for a minimum of 2 minutes. Care
was taken to rinse the lumen of the grafts with water.
30 The graft-protein "hybrid" prostheses were then reintro-
duced into fresh (20%) glutaraldehyde (40 ml of 50 %
glutaraldehyde in 60 ml of water) for 5-15 minutes. The
process was repeated several times (three to ten times)
until thin (1 to 2000 microns), uniformly coated grafts
35 with their interstitial spaces filled were produced.

* J. T. Baker Chemical Co., Phillipsburgh, New Jersey, USA.

1 Dacron Backbone Structure

Several types of grafts and varying concentrations of proteins were utilized to determine optimal reaction condition. They included 3 and 6 mm. I.D. (2-20 mm is the range), 18 and 32 ridges/inch (10-32 ridges would be the range), and 1, 3, 5 and 10 % protein solutions. It was found that the grafts possessing 18 ridges/inch (half crimp) were the optimal type of graft to modify, as they possessed the best surface configuration to provide for an even deposition of protein. The grafts having 32 ridges/inch (full crimp) had to be stretched on a glass rod of the same I.D. as the grafts in order to coat them properly. This prevented intra lumen coating and produced cracking when the rod was removed and the grafts regained their original shape. It was further found that a 3 % solution of protein provided the most effective coating both in optimizing time to coat and in thickness. One percent took a much longer time period to coat (greater than 1 hour). A 10 % coating was achieved within 30 seconds but was very thick and did not permit obtaining the amount of flexibility required. In the foregoing, the range of depth of the crimps is about 1 to 2 mm and the wall thickness of the grafts is about 1/2 to 1 mm.

Using the techniques described above (i.e., 3 % protein solutions and half crimped grafts) it was possible to coat both 3 mm and 6 mm I.D. grafts without difficulty.

Chemical Modification

The protein copolymerized hybrid grafts were further modified to enhance their net negative surface charge.

30 The end terminal amino acid residues were covalently coupled to succinic acid under acetylation reaction conditions (see Pat. No. 3,927,422). The grafts were rinsed thoroughly with distilled deionized water, and placed into a 10 % (8-10 % range) sodium bicarbonate buffer at a pH 8.5 (8.2-8.7 range) (8-10 grains in 100 ml of water).

35 The grafts were allowed to equilibrate with the buffer

- 1 (5-30 minutes at 17-27°C) then ground solid succinic an-
hydride was added in 5 mg portions (range 2-5 grams) to
the buffer such that the final succinate solution con-
centration would be 1 M, (range: 1 to 5 molar) (2 to 8
5 micromoles of succinate per gram of protein).
The reaction was sufficiently vigorous to perfuse the
lumen of the grafts. Several aliquots (range: 3 to 5) of
the solid anhydride were added directly into the lumens
of the grafts which were then placed into the buffer.
10 At the conclusion of the reaction (i.e., cessation of
effervescence), the grafts were retested on a Zarb
hydrostatic testing apparatus to ascertain structural
integrity of copolymerization. The grafts were recoated
if necessary and then stored and sterilized in 10 %
15 (range 3 - 10 %) glutaraldehyde.

Acrylamide Hybridization

- Acrylamide was prepared in accordance with the method
of Davis et al. A solution which would yield 7 % (range:
5 to 8 %) gel was utilized. The graft was immersed into
20 solution and was allowed to polymerize. The graft was re-
moved, washed with distilled deionized water and stored
in 40 % ETOH and water.

- More particularly, a 20 ml solution of 28.0 g of acryl-
amide (Bio-Rad Labs of Richmond, Ca.) and .735 g of bis-
25 acrylamide (Bio-Rad Labs of Richmond, Ca.) were added
to a 20 ml solution containing 1 N. HCl, 48.0ml/100 ml.,
36.3 g/100 ml of TRIS (Bio-Rad Inc. of) and
0.723 ml of TEMED/100 ml.

- A segment of graft material was added to the acrylamide
30 solution and allowed to equilibrate for one hour (1/2-1
hours at room temperature). At that time, a 40 ml solu-
tion of ammonium persulfate, 1 mg/ml, (Bio-Rad Labs of
Richmond, Ca.) was added to the acrylamide-bis-acrylamide
solution in order to polymerize the solution. The solu-
35 tion was allowed to gel at room temperature for 1/2 hour
(range: 10-40 minutes). During this time interval, the

1 graft was removed and reintroduced into the gelling
medium 10 (range: 10 to 20) times. The graft was removed
after the solution began to gel but before a solidified
gel could form. The graft was then placed into a new test
5 tube, and the acrylamide which had coated the Dacron
fibers was allowed to gel. After the acrylamide polymer-
ized, the graft was perfused with water at room tempera-
ture for 1 hour (range: 1/2 to 1 hour).

Agar Hybridization

10 A 3 % agar-water mixture (Difco Labs, Detroit, Mich. USA) was
made and heated to 100°C until a uniform (aggregate free)
slurry was evident. The graft material, washed three to
ten times in distilled and deionized water, was immersed
in the agar slurry until it was uniformly coated (100-
15 2,000 microns). It was then kept at 4°C (range: 4-10°C)
until the agar congealed. The graft was removed, washed
and stored in 40 % ethanol-H₂O until used.

Method of In Vitro Testing

All hybrid grafts underwent hydrostatic testing using
20 a Zarb hydraulic pressure tester. The criterion used was
the ability to withstand 30 mm (range 20-50) of Hg
pressure without showing evidence of air leaks through
the graft pores.

If a graft failed because of leaks during the test pro-
cedure, it was recoated by the process described above
25 until there was no evidence of leakage. The hybrid grafts
were stored in 10 % glutaraldehyde until used.

Aluminization

Two techniques of coating a Dacron prosthesis with alumi-
30 num were utilized. They were: (1) vapor deposition and
(2) hot plasma spray technique.

Vapor Deposition

Vapor deposition was accomplished in vacuum at 10⁻⁵ Torr.
The aluminium vapor was forced through a small opening
35 (.25 to 20 mm) in a heat shield and deposited on a
Dacron graft in a thin, uniform coat of 1/2 to 10 microns.

1 This technique proved to be the best technique for coating Dacron tubular material with aluminum as it provides a vapor which coats the graft completely with localized over concentration.

5 Hot Plasma Spray

Aluminium powder is heated to its melting points and is then sprayed by a pressurized aerosol-like container. This is repeated until a thin coating (1/2 to 10 microns) of aluminum is observed on the Dacron.

10 Implantation Studies

Implantation studies were divided into chronic and short term evaluation of hybrid graft materials.

Three and four mm hybrid grafts were sutured (implanted) into the carotid and femoral arteries of dogs for periods ranging from 1 second to 2 months. (1 second, 2 hours, 1 week, 1 month, 2 months).

Six mm hybrid grafts were implanted into the abdominal aorta of dogs for time periods ranging from 1 month to 1 year (1 month, 6 months, 9 months, 1 year).

20 The hybrid grafts were removed from their sterile solutions and placed into a sterilized 500 ml beaker and sequential washed with 250 ml of sterile saline 20 x.

The grafts were then placed into a sterile stainless steel bin containing 1.5 liters of sterile saline, until placed in a sterile field and implanted.

Prior to implantation, the end pieces were cut to provide an even surface for anastomosis and samples for controls, culturing and SEM studies to determine efficiency of coating and function.

30 End-to-end anastomosis was carried out using a running Carrell suture with 4-0 cardiovascular Dacron or other material. Precise anastomosis was carried out in all cases.

35 Percent patency has been determined using a semiquantative scale. This has been determined by taking the cross sectional view of a graft and dividing it into equal

1 concentric circles of 1/4 D from the wall of the vessel.
Thus, it is now possible to quantify the amount of
thrombus formed by measuring the depth of the thrombus
then relating it to the total cross sectional area. The
5 numbers generated by this technique are expressed in
terms of percentage of the entire vessel.

Post Implantation Studies

The graft-hybrid materials were analyzed using (1)
scanning electron microscopy (SEM), (2) light micro-
10 scopy and (3) histological staining. Patency was deter-
mined using the aforementioned semi-quantitative
technique.

Scanning Electron Microscopy (SEM)

SEM's were performed on the grafts before implantation
15 and after removal using a Phillip's scanning electron
microscope at 200x, 10Kx and 20Kx magnifications. With
these magnifications, it is possible to resolve and
differentiate platelets, erythrocytes, and leukocytes,
and white blood cells and to visualize protein (inclu-
20 ding fibrin) deposition.

The SEM's were used to detect (1) efficiency of hybridi-
zation (to determine structural flaws in the coatings)
and (2) to determine visible evidence of the type of
blood prostheses interfacial reactions.

25 Histological and Light Microscopic Evaluations

Graft sections were evaluated by the use of several
standard histological stains including Hemotoxylan and
Eosin (H&E) Van Geisen (Elastic VG stain), PTAH and Tri-
chrome. Each stain specifically detects the type of ma-
30 terial adhering to the graft or removal from the animal
by the colors which are produced when the staining solu-
tion is in contact with the biologic material. H&E is
used to visualize cellular entities and to demonstrate
if an inflammatory response has occurred and if poly-
35 nucleated cells or lymphocytes are present. EVG stain
is used to illustrate fibroblastic invasion and to dye
the collagen present. For instance, Trichrome stains

1 collagen blue. PTAH stain is used to demonstrate the presence of fibrin.

Results

5 The following table illustrates the patency levels of the various hybrid grafts.

% Patency of Hybrid Grafts

	Type	1 Sec	2 Hr	1 Month	9 Months	Dacron Support
10	Agar	90	70	-	-	Microknit®
	Acrylamide	95	80	-	-	"
	A Unmod	99	99	95	-	"
	(-) 99	99	99	99	X	"
	G Unmod	99	80	80	-	"
15	(-) 99	99	99	99	X	"
	A&G Unmod	95	90	90	-	"
	(-) 99	99	99	99	X	"
	AL	99	99	99	X	WVE Old
20	AL	99	99	99	X	Wesolowski Weave Knit

A - Albumin

G - Gelatin

25 Table 1: Relates the type of hybridization, time of implantation and percent patency. Column 1 is the type of "coating" and subsequent negative charging. The next 4 columns are the time parameters at which they were analyzed. The numbers represent the % of patency. A check indicates that the graft is still functioning in-vivo. A - indicates that that parameter was not measured. The last column is the type of Dacron support used with that particular modification.

35 The following figures are SEM studies of the following hybrid grafts before and after implantation, negative charged and unmodified, of (i) Albumin (A) coated grafts,

- 1 (ii) Gelatin (G) coated grafts, (iii) a 50/50 mixture of
albumin and gelatin a concentration of 3 % each (AG),
(iv) acrylamide coated graft (ACD), (v) agar-agar coated
grafts (AGR), and CVD aluminum coated grafts (Al).
- 5 Fig. 1(a) shows scanning electron micrographs of a poly-
merized protein, namely, of an albumin Dacron hybrid
graft. This series of photographs demonstrates the homo-
genicity of a protein on Dacron surface.
Fig. 1(B) shows scanning electron micrographs of a pro-
tein-gelatin Dacron hybrid graft. The photographs show
10 a homogeneous surface prior to and after exposure of the
surface to blood for 1 second.
Fig. 2(A) shows scanning electron micrographs of a gela-
tin-Dacron hybrid graft. These photographs reveal minor
15 cracking of the polymerized surface at high (10Kx)
magnification.
Fig. 2(B) shows scanning electron micrographs of a gela-
tin-Dacron hybrid graft. Following polymerization of the
hybrid, the surface was chemically treated (succinylating)
20 to produce a net negative surface charge. These micrographs indicate
that the negative surface at 1 second, 2 hours and
1 month is far less reactive than the unmodified hybrid
surface. The unmodified surface has a dense amount of
cellular protein deposition thereon, while the negatively
25 charged surface attracts fewer cellular aggregates. The
patency rate for the negatively charged grafts was
approximately 90 % as opposed to 60 % for the unmodified
surface at 2 hours.
Fig. 3(A) shows the smooth structure of an agar coated
30 Microknit[®] Dacron graft and Fig. 3(B) shows implantation
results of a protein-gelatin Dacron hybrid graft. The
SEM photographs reveal that the protein-gelatin nega-
tively charged hybrid is less reactive at 1 second,
2 hours and 1 month than the unmodified graft. Many
35 erythrocytes entrapped in a protein matrix can be seen
in the unmodified surface while the negatively charged
surface possesses few red cells and no visible platelets.
Patency rates for the negatively charged grafts was

- 1 approximately 90 % as opposed to 70 % for the unmodified
grafts at 2 hours.
- Fig. 4 shows scanning electron micrographs of an aluminum
coated Dacron hybrid graft. Following implantation, the
5 graft was examined. Little protein deposition could be
seen. A layer of cells which appear to be erythrocytes
and monocytes bound to fibrinogen seem to be sparsely
coating the surface. The metallic coating may be toxic to
cellular elements, as the monocytes are atypical. The
10 patency rate in all cases was 100 %.
- Fig. 5 shows scanning electron micrographs of an agar
coated-Dacron hybrid. Implantation results indicate a
reactive surface with cellular and protein deposition.
Patency rates were 80 % at 1 second and 40 % at 2 hours.
- 15 Fig. 6(A) shows an aluminum coated Dacron prosthesis
exposed to blood for 1 second. The fibrils are virtually
free of cellular elements with the exception of a very
few erythrocytes and leukocytes with a few visible
platelets attached as well. At 10K X magnification, the
20 single large cell appears to be a toxic neutrophile. The
remainder of the photograph displays proteinated aluminum
substrate. At 2 hours, the cells are smaller and appear
to be crenating.
- Fig. 6(B) shows further magnifications at 1 K X and
25 these reveal a few crenating cells with a very thin layer
of protein attached to the aluminized Dacron fabric. These
cells are completely gone by 1 month, the fabric being
joined by what appears to be a thin amorphous layer of
protein. This may be proteinated aluminum.
- 30 Histological evaluation was carried out in parallel to
the SEM studies. In all cases, there was no evidence of
(1) polynucleated cells or lymphocytes or, (ii) inflama-
tory response. All grafts had fibroblastic invasion.
Type A presents the most explicit example. In all cases,
35 protein coating was firmly attached to the Dacron. Coating
of each strand of Dacron was also established.

1 The different histological responses to the various types of hybrid grafts are described in Table II.

5 TABLE II

A SUMMARY OF HISTOLOGICAL STUDY OF HYBRID GRAFTS

	GRAFT TYPE	PATENCY	DESCRIPTION *
10	A1	99 %	Revealed no evidence of cells or fibrin.
15	A	99 % (1 month)	Coating uniform with no evidence of separation. Unmodified: had few cellular elements which were loosely attached to proteineous material which is not fibrin. This may be a coincidental attachment of cells. Modified: revealed no evidence of cells. The globular objects which appear on the SEM are not cells. There is no trace of fibrin.
20			
25	G	80 % (unmodified) 2 Hr. & 1 Mon. 99 % (neg. charge) [1 sec.] 1 mon.	There is fibrin which is attached to the wall at a depth of .5 to 1 mm. Has few cellular elements which are widely dispersed and well passivated in a thin protein layer which is not fibrin.
30			
35	A & G	90 %	Unmodified: These are cellular elements which is followed by a fibrin layer. In 2 hours, there is evidence of a building new cellular and fibrin layer.

	GRAFT TYPE	PATENCY	DESCRIPTION
1		99 % (1 month)	Modified: There is evidence of a few cellular elements which are passivated by a thin non-fibrin protein layer.
5	Agar	75-80 %	In 1 sec., there is evidence of an instantaneous thrombus which is primarily a chain of fibrin. There are a few cells present at this time. At 2 hours - there is a large deposit of cellular elements plus fibrin formation on the graft.
10			
15	Acrylamide	75 %	In 1 sec. and 2 hours this graft produced a peculiar thrombus. The thrombus had many more cellular elements and markedly less fibrin than did any of the other thrombi producing surfaces.
20			

There will now be obvious to those skilled in the art many modifications and variations of the methods and materials set forth hereinabove. These modifications and variations will not depart from the scope of the invention if defined by the following claims.

30

35

1 What is claimed is:

1. A prosthesis suitable for implantation in a bloody environment, particularly for cardiac and vascular implants,
5 said prosthesis comprising a surface material adapted for exhibiting a regular and periodic surface charge characteristic in said environment, and prosthesis means supporting said surface material and adapted to perform a prosthetic function in said environment.

10

2. A prosthesis as claimed in claim 1, wherein said prosthesis means is of a material normally incapable of cross-linking to said surface material, comprising an adaptive material to cross-link to the material of said prosthesis
15 means and to said surface material to provide architectural continuity therebetween while preserving said surface charge characteristic.

20

3. A prosthesis as claimed in claim 2, wherein said adaptive material is glutaraldehyde or a succinic acid compound.

25

4. A prosthesis as claimed in claim 2, wherein said surface charge is equivalent to approximately one electron per thousand square Angstroms in a regular three-dimensional pattern.

30

5. A prosthesis as claimed in any of claims 1 to 4, wherein said surface material is aluminum, a protein, agar or acrylamide.

35

6. A prosthesis as claimed in claim 5, wherein said protein is albumin, gelatin or collagen.

7. A prosthesis as claimed in claim 5, wherein said surface material is albumin combined with gelatin.

- 1 8. A prosthesis as claimed in any of claims 1 to 7,
wherein said prosthesis means is of Dacron.
- 5 9. A prosthesis as claimed in claim 8, wherein said
Dacron is of a woven structure.
- 10 10. A method of preparing a prosthesis for functioning in
a bloody environment with minimal thrombogenicity said
method comprising depositing on the surface of the pros-
thesis means a material adapted for exhibiting a regular
and periodic surface charge in said environment.
- 15 11. A method as claimed in claim 10, wherein said sur-
face material is normally incapable of cross-linking to
said prosthesis means comprising cross-linking the said
material to said prosthesis means with an adaptive
material.
- 20 12. A method as claimed in claim 11, wherein said
adaptive material is glutaraldehyde or a succinic acid
compound.
- 25 13. A method as claimed in any of claims 10, 11 or 12,
wherein said surface material is aluminum, a protein,
agar or acrylamide.
- 30 14. A method as claimed in claim 13, wherein said pro-
tein is albumin, gelatin or collagen.
15. A method as claimed in claim 13, wherein the said
surface material is albumin combined with gelatin.
- 35 16. A method as claimed in claim 13, wherein said sur-
face material is a protein and is applied in about 3 %
solution.

1 17. A method of adapting a synthetic material for use
as a prosthetic material adapted to function in a
bloody environment, said method comprising metallizing
the material at the surface thereof.

5

18. A method as claimed in claims 13 or 17, comprising
vapor depositing aluminum on said prosthesis means.

19. A method as claimed in claims 13 or 17, comprising
10 spraying aluminum in an aerosol on said prosthesis means.

20. A method as claimed in any of claims 10 to 19, where-
in the prosthesis means is of woven Dacron.

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FIG. 1A

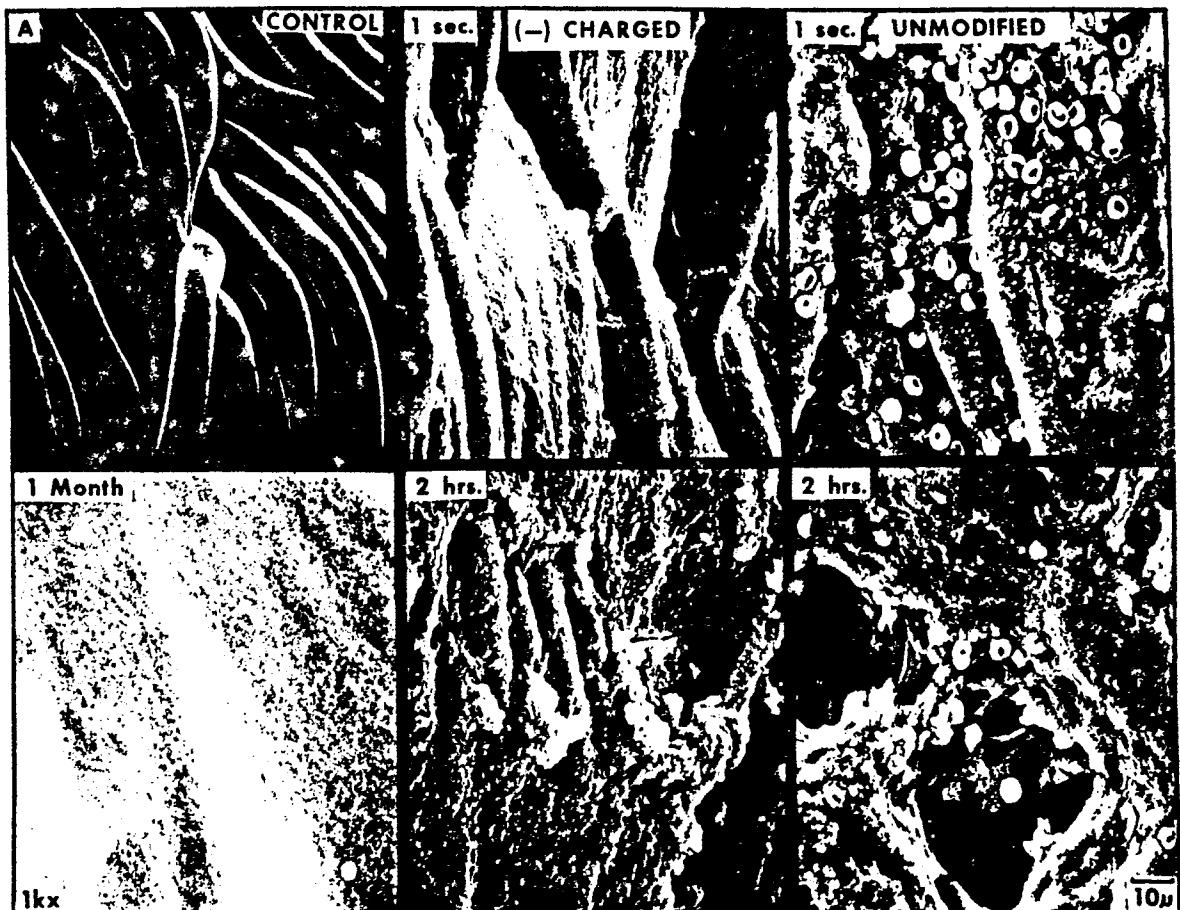


FIG. 1B



FIG. 2A

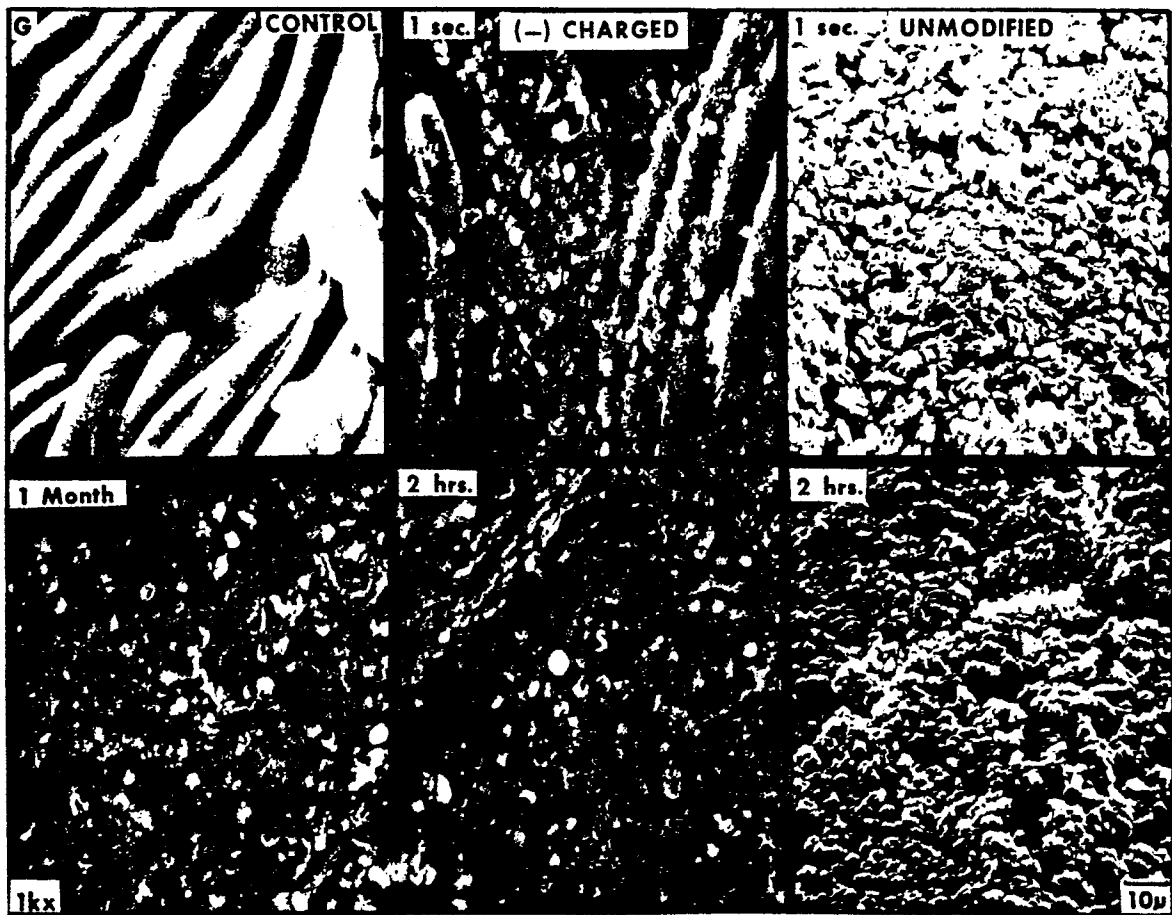


FIG. 2B

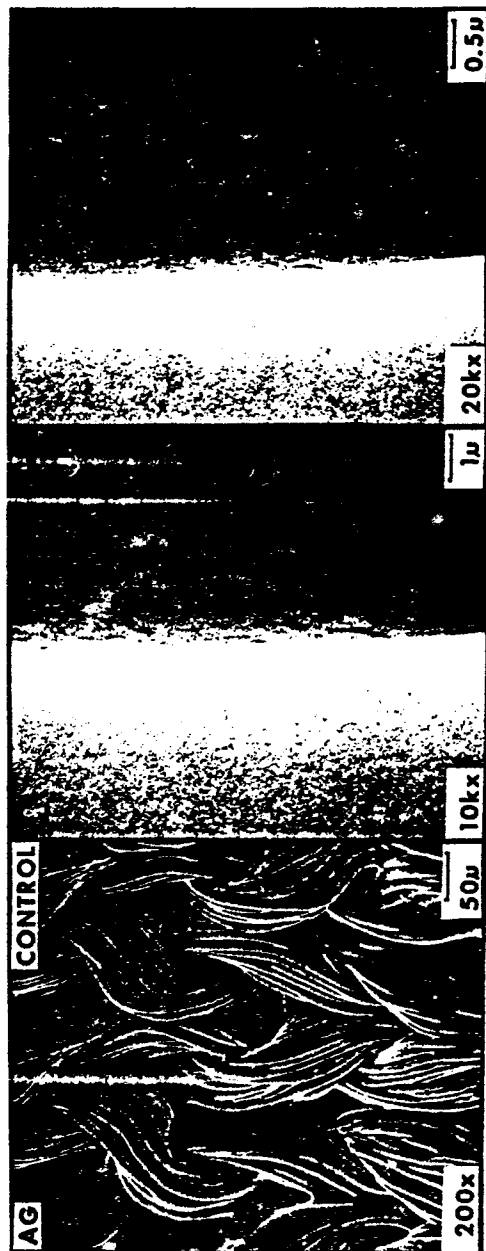


FIG. 3A

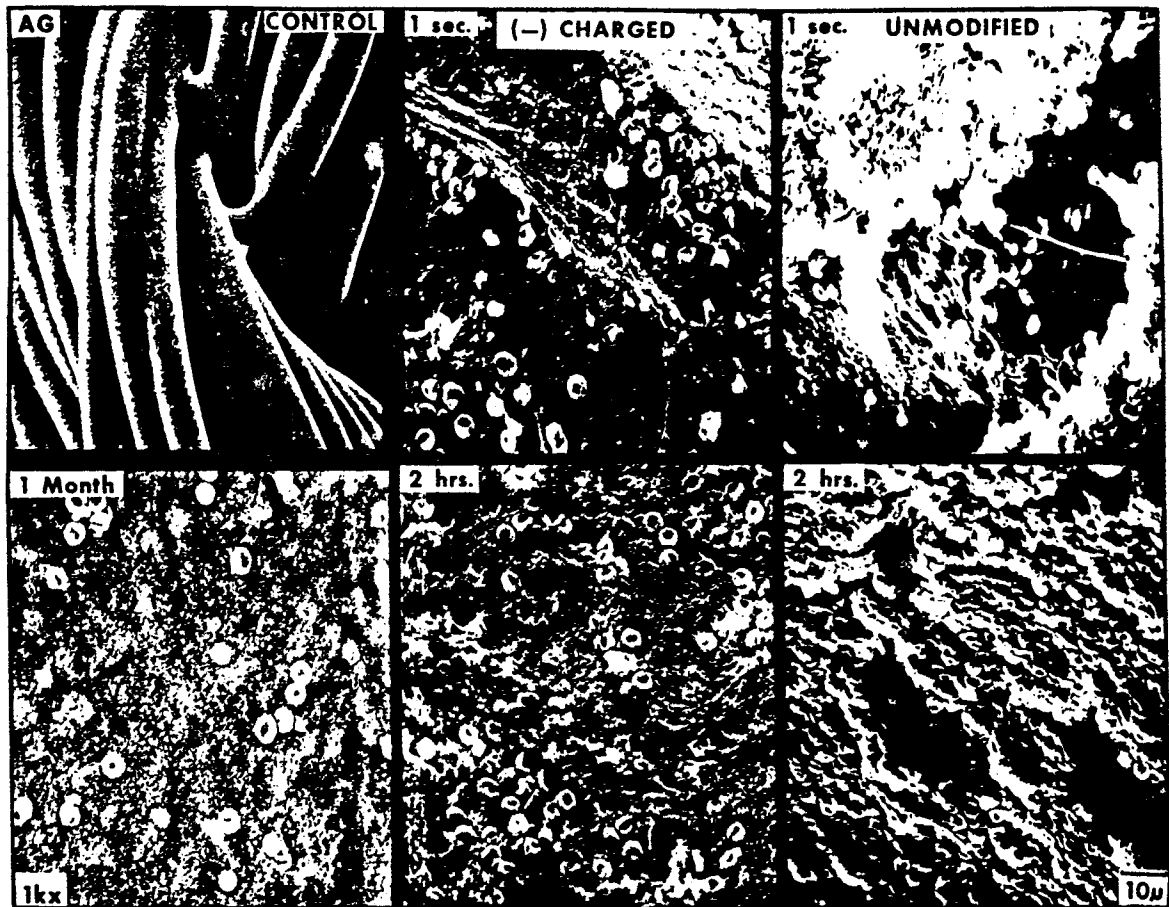


FIG. 3B

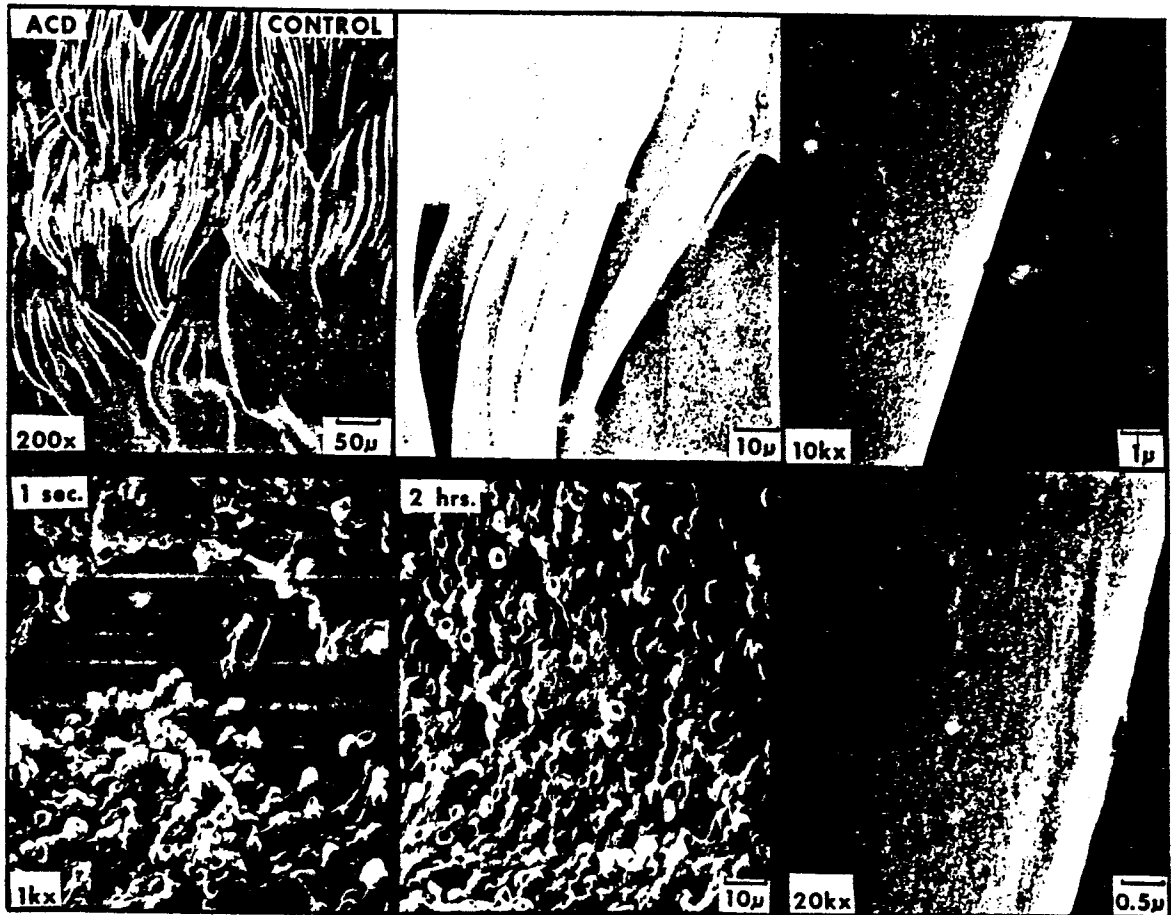


FIG. 4

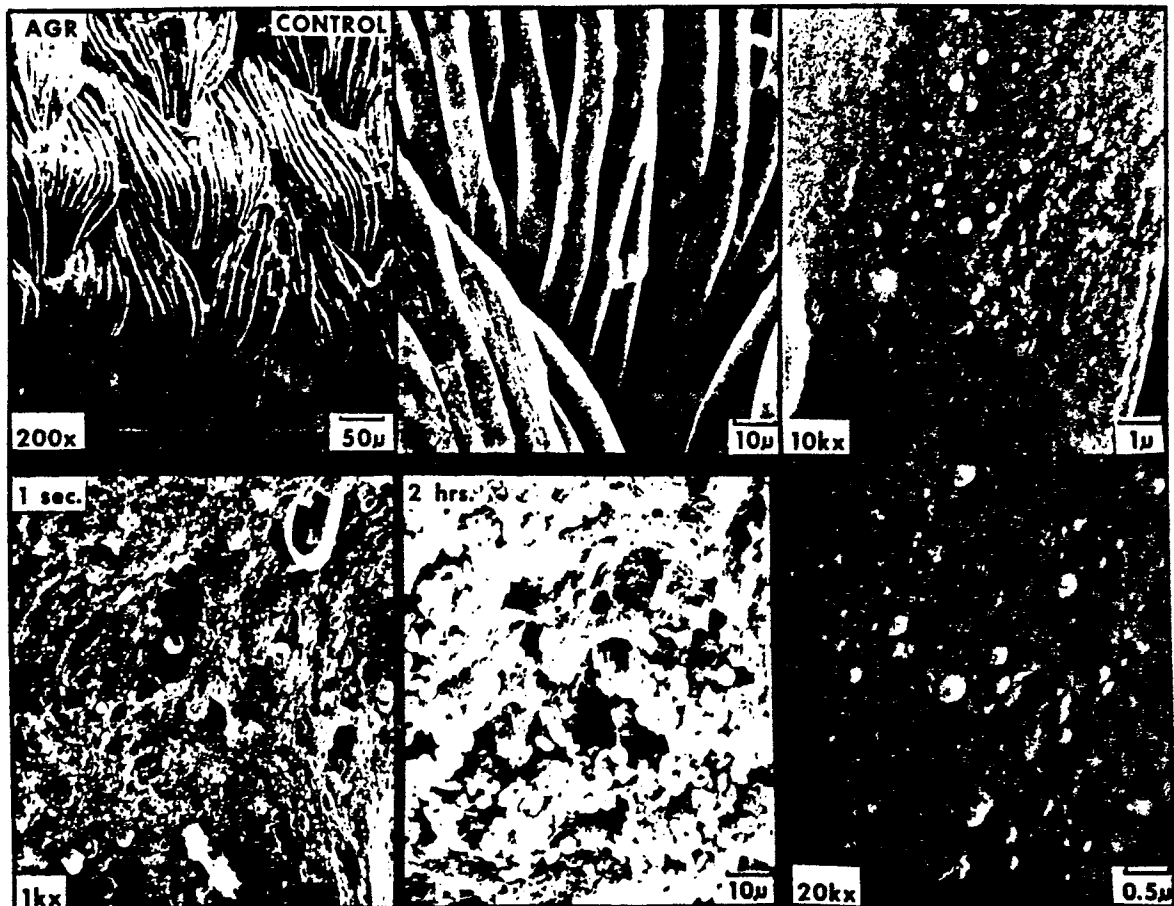


FIG. 5

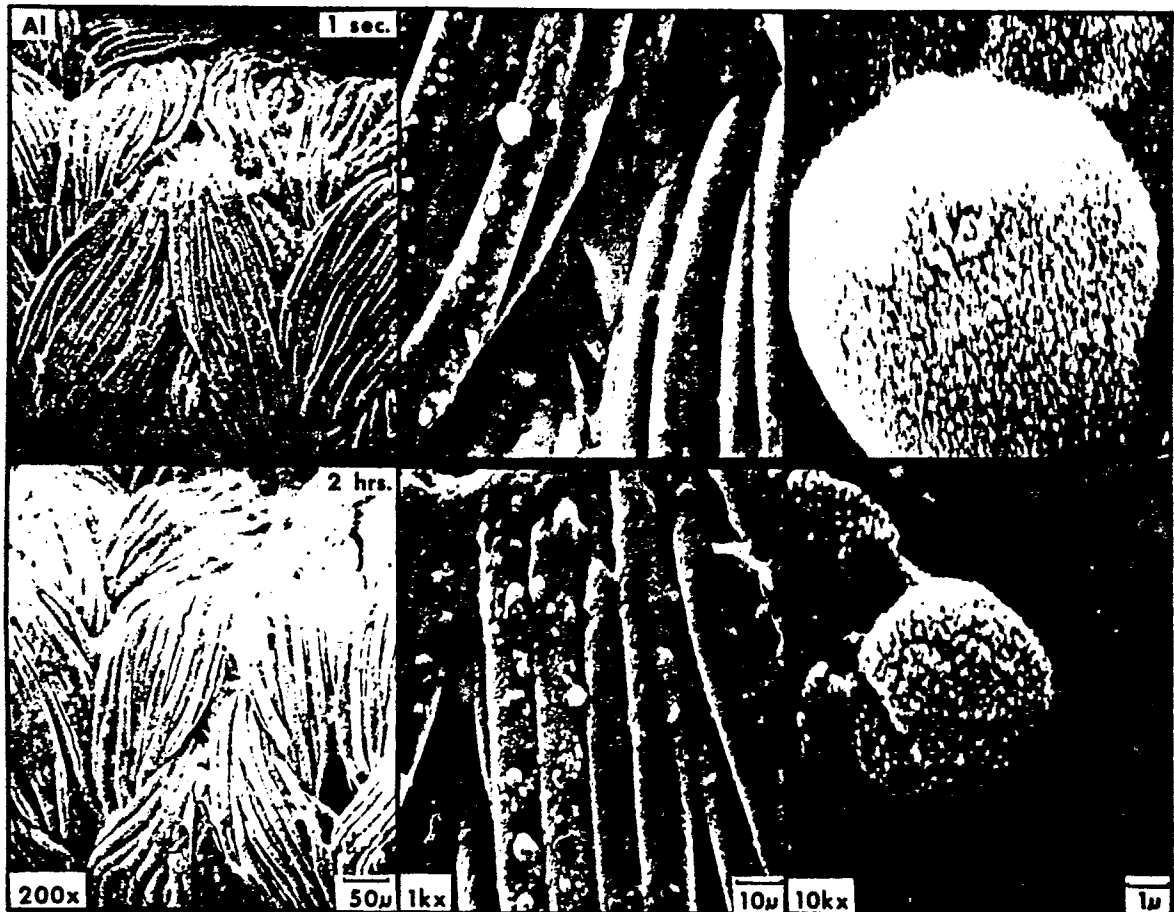


FIG. 6A



FIG. 6B



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. ²)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	<u>US - A - 3 914 802</u> (F.G. REICK) * column 1, lines 4-6 and line 65 - column 2, line 4; column 2, lines 19-27 and line 55 - column 3, line 16; column 3, line 60 - column 5, line 32; claims 1,4,5 * --	1,8-10,20	A 61 F 1/22 1/24
	<u>US - A - 3 744 062</u> (V. PARSONNET) * column 2, lines 4-28 and line 57 - column 3, line 13 * --	1,5,6,8-10,13,14,20	
	<u>US - A - 3 609 768</u> (W.A. AYRES) * claims 1-7 * --	1	TECHNICAL FIELDS SEARCHED (Int.Cl. ²)
	<u>US - A - 4 038 702</u> (P.N. SAWYER) * claim * --	1	A 61 F 1/24 1/00 1/22
	<u>US - A - 3 512 183</u> (W.V. SHARP et al.) * column 2, lines 20-67; examples II a III; claims * --	1	
	MEDICAL PROGRESS TECHNOLOGY, vol. 5, nr. 2 (1977) Springer Verlag, Berlin S.D. BRUCK: "Biological Evaluation of Biomaterials for Cardiovascular Applications: Some Current Results" pages 51-56 (Invited paper presented at the Workshop on Biomaterials, 11th International Conference on Medical and Biological Engineering, Ottawa, Canada, Aug. 2-6, 1976) * page 53, C. "Polyacrylamide Hydrogels" * --	1,5	CATEGORY OF CITED DOCUMENTS X: particularly relevant A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: conflicting application D: document cited in the application L: citation for other reasons
	./.		&: member of the same patent family, corresponding document
✓	The present search report has been drawn up for all claims		
Place of search The Hague		Date of completion of the search 29-11-1978	Examiner WILLEKENS



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. ²)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D	<u>US - A - 3 927 422</u> (P.N. SAWYER) * claims 1-8, 11-13 * & FR - A - 2 335 198 & DE - A - 2 557 947 --	1, 5, 6	
P	<u>US - A - 4 082 507</u> (P.N. SAWYER) * example 6; column 9, lines 4-11; claims 1-6, 10, 11 to 15 * & FR - A - 2 378 504 & DE - A - 2 720 544 --	1-3, 5, 6, 10- 14, 16	
P	<u>CH - A - 593 676</u> (INTERMEDICAT GmbH) * example 1; claims * --	1-3, 5, 6, 10- 14	TECHNICAL FIELDS SEARCHED (Int. Cl. ²)
A	<u>US - A - 3 096 560</u> (W.J. LIEBIG) * column 2, lines 34-60; claim 1 * --	1, 8, 9	
A	<u>US - A - 3 276 448</u> (R.L. KRONEN- THAL) * column 2, lines 1-9, 26-49; example 1; claims 1-3 * --	1, 5, 6, 8, 9, 10, 13, 14, 20	
A	<u>FR - A - 2 029 155</u> (I MOSKOVSKY MEDITSINSKY INSTITUT) * example 1; claims * --	1, 5, 6	
A	<u>GB - A - 1 008 193</u> (ETHICON) * example V; claims 1-6, 9 * --	1, 5, 6, 8-10, 13, 14, 20	
A	<u>GB - A - 1 064 850</u> (ETHICON) * example 1; claims 1-3 * --	1, 5, 6, 8-10, 13, 14, 20	
	.	.	



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	<u>DE - A - 1 494 939</u> (E. BUDDECKE) * claims 1,2; page 4, lines 14-19; page 12, lines 13-17 * --	1,5	
A	<u>GB - A - 1 097 787</u> (ETHICON) * claims 1,2,5,9 * --	1	
A	<u>US - A - 3 563 925</u> (K. KLIMENT et al.) * claims; examples 1,2 * -----	1,5	
			TECHNICAL FIELDS SEARCHED (Int. Cl.)