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(71) Applicant
Woodroof Laboratories Inc., 3100—7 Harvard Street, Santa Ana, California 92704, United States of America (U.S.A.)
(72) Inventor
E. Aubrey Woodroof
(74) Agent
Fitzpatricks

(54) Bio Compatible and Blood Compatible Materials and Methods

(57) Bio- and blood compatible materials are prepared by treating the surface of a substrate to provide reactive primary or secondary amine groups sites which are activated by treatment with a dialdehyde or acrylchloride for coupling to a biological compound in an amount sufficient to provide compatibility. The use of specific substrates, such as a compliant and elastic material, such as a fabric-elastomer membrane matrix, results in a product having advantageous qualities as a thermal burn dressing.

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SPECIFICATION

Bio-compatible and Blood-compatible Materials and Methods

Background of the Invention

This invention relates to bio-compatible and blood-compatible materials their method of preparation and more particularly to an improved biocompatible and compliant material of the type described which may be topically or internally applied or contacted by biologicals, blood, or tissue as for example a burn dressing, a surgical dressing, a cardiovascular graft or implant material, and the like, and to the method of making the same.

There are many instances in medicine in which there is a need for a bio-and blood compatible material for human and animal use and for use in equipment contacted by biologicals or blood, e.g. tubing containers, valves, etc. For example, in extracorporial circulation of blood, i.e. heart lung, artificial kidney, there is a tendency for blood to coagulate on contact with a "foreign surface", see for example U.S. Patents 3,643,123 and 3,810,781. Also, products such as heart valves, materials used in coronary and vascular grafts, and catheters, oxygenator tubing and connectors tend to cause thrombosis of blood.

In addition to the above, materials used as burn dressings and surgical dressings should be bio- and blood compatible. In the case of such dressings, an area in which the present invention finds particular utility, there are additional requirements because of the use of the materials.

As is known in the art, and described in U.S. Patent 3,800,792, treatment of second and third degree burns involves a number of phases, including cleaning and stabilizing the wound area to the development of granulation bed at the wound site. The final phase of treatment is usually the autografting phase which sometimes take place some period of time after development of the granulation bed. The maintenance of the granulation bed is a necessity until such time as autograft is available and successful autografting is completed.

Several different approaches have been used to preserve the wound site, i.e. granulation bed, for example, application of wet dressings which must be changed frequently and tend to add to patient discomfort. Homografts, heterografts and synthetic dressings have also been used.

Accordingly, a wide variety of dressings, characterized as biological and synthetic, have been used in the treatment of burn wounds. Biological dressings include any dressing that has one or more biological components, i.e. protein, carbohydrates, lipids and the like. Presently, homograft and porcine xenograft skin are dressings currently used to maintain the granulation bed.

In burn patients with large areas of burn tissue, the amount of available skin (autograft) is limited and temporary dressings are required for long periods of time to maintain the granulation bed. Homografts (cadevar skin) is the current dressing of choice, when available. Unfortunately, homograft skin has a limited shelf life and is relatively expensive i.e. $85.00 to $95.00 per square foot. Human amniotic membrane has also been used but is less desirable than cadevar skin. Lack of availability and short shelf life are also drawbacks.

Xenograft (porcine) skin is commerically available but is considerably less effective than homografts or autografts. Short shelf life, sterility and limited application are known disadvantages of this material, in addition to an antigenicity problem.

Description of the Prior Art

In addition to the materials previously mentioned, various forms of collagen have been used in the treatment of burns, see U.S. 3,491,760 which describes a "skin" made from two different tanned collagen gel layers.

U.S. Patent 3,471,958 describes a surgical dressing made up of a mat of freeze dried microcrystalline collagen, while British Patent 1,195,062 describes the use of microcrystalline colloidal dispersions and gels of collagen to produce films which are then applied to various fibers such as polyurethane.

A "biolization" process for improving the blood and biocompatibility of prosthetic devices has been described by *Kambic, et al.* and others, see Trans. 3rd Annular Meeting Society for Biomaterials, Vol. 1, p. 42, 1977. Their methods involve deposition of gelatin into a rough textured rubber with subsequent cross-linking and stabilization of the gelatin with .45% gluteraldelyde.

Also of interest is U.S. Patent 2,202,566 which describes collagen fibers in bandages and U.S. Patent 3,113,568 which discloses the use of polyurethane foam in a bandage.

There are numerous references in the literature to various other materials used in burn treatment. For example, collagen membranes have been fabricated from suspensions of bovine skin and evaluated in a rat animal model. The adherence of this material was superior to auto- homo- and xenografts on full and split thickness wounds but inferior to auto- and homografts on granulating wounds, see *Tavis et. al.,* J. Biomed. Mater. Res. 9, 285 (1975) and *Tavis et. al.,* Surg. Forum 25, 39 (1974).

McKnight et. al., developed a laminate of collagen foam with a thin polyurethane sheet, see U.S. Patent 3,800,792. Film prepared from reconstituted collagen has also been used, *Tavis et. al.,* supra, and a commerical grade of such material is available from Tec-Pak Inc. *Gourlay et. al.,* Trans. Amer. Soc.

Art. Int. Organs, 21, 28 (1975) have reported the use of a silicone collagen composition, collagen sponge, and non-woven fiber mats.

Park, "Burn Wound Coverings—A review", Biomat, Med. Dev. Art. Org. 6(1), 1—35 (1978) contains a review, with extensive literature citations, of various burn wound coverings, including laminates of velour fabrics such as nylon, dacron (polyester), rayon, Teflon and polypropylene. Velour silastic laminate are reported with the observation that Teflon and polypropylene velours could be easily peeled off the granulation bed. Rayon appeared to adhere well but disappeared after 10 to 14 days leaving only the silastic backing. Dacron and nylon appeared to adhere well.

Nylon velour incorporating polypeptide films and polycaprolactone films were critized because of cracking of the film. Ultra thin silicone fabric composite membranes have been reported by *Kornberg et. al.,* Trans. Amer. Soc. Artif. Int. Organs, Vol. 18, pp. 39—44, 1972.

In the literature reports of some of the above materials, adherence, continued elasticity and flexibility, and water vapor transmission appeared to emerge as important parameters in burn dressings. Thus, as far as burn wound coverings the following characteristics emerge as desirable:

1. The material must adhere to the wound base (comparable to auto- and homograft) to minimize infection and sepsis.

2. It must have adequate flexibility over a period of time in order to cover joints and other areas of body flexion.

3. It must have the proper moisture vapor transmission rate to maintain proper moisture balance at the wound site.

4. It should be capable of being easily stored, sterilized and available for use on short notice for emergency procedures.

5. It must not be toxic, pyrogenic, or antigenic.

6. It should be readily available at reasonable cost.

7. It must be capable of being applied to the wound site so as to completely isolate the site.

8. It must have sufficient strength to be secured by sutures, clips and the like.

In addition to the above, U.S. Patent 3,846,353 describes the processing of silicone rubber with a primary or secondary amine, see also Canadian Patent 774,529.

In addition to the above, there is considerable literature relating to the use of silicone rubber membranes Medical Instrumentation, Vol. 7, No. 4,268,275 September—October 1973; fabric reinforced silicone membranes, Medical Instrumentation, Vol. 9, No. 3,124—128, May—June 1975. U.S. Patent 3,267,727 also describes the formation of ultra thin polymer membranes.

It is also known that various materials may be heparinized in order to impart a non-thrombogenic character to the surface of a material, see for example U.S. Patents 3,634,123; 3,810,781; 3,826,678; and 3,846,353, and Canadian Patent 774,529, supra.

Summary of the Invention

The product and process of the present invention differ from the prior art by providing a composite elastomeric material from a thin film of the thermoplastic (e.g. silicone rubber) and a knitted or woven fabric (e.g. nylon). The thermoplastic component can be layered with high precision (final cured sample thicknessess with a tolerance of $\pm$.00025 inches). The fabric component is placed on the wet thermoplastic component (without wrinkles) and the composite is cured at a temperature of approximately 300°F for 15—60 minutes. To this composite elastomeric matrix one or more biological molecules such as proteins (collagen, gelatin, fibrinogen, egg albumin, human albumin, human gamma globulin, or other animal or plant proteins), carbohydrates (acidic mucopolysaccharides, starch, simple sugars, etc.), lipids (lecithin, choline, unsaturated or saturated free fatty acids, other complex or simple lipids), amino acids (aspartic acid, lysine, glycine, serine, etc.), dipeptides (Glycylglycine, others), larger peptides and the like may be bonded using a number of commerically available reagents to accomplish either hydrophobic or covalent bonds. The process can be thought of as a final product of composition A, B, C. The "A" represents the elastomeric fabric-thermoplastic composite matrix, which provides ideal physical properties (e.g. elasticity, conformability and water vapor transport properties). The "B" represents one or more components used to bond the "C" component (one or more biologicals) to the "A" component (fabric-thermoplastic composite matrix). The completed product A-B-C is used to impart a specific quality or a combination of characteristics of the material (A-B-C) to render them bio- and blood compatible.

The materials of the present invention also exhibit a moisture vapor transmission rate, i.e. the weight of water lost by evaporation through a film membrane at 37°C over a period of 24 hours, of about 10—15 grams per hour per meter squared or about 1—1.5 miligrams per hour per centimeter squared, which is a rate similar to human skin, however, the WVT property of these materials are subject to modification to optomize wound healing.

Where used as a burn dressing, which is the principal but not the sole use of the materials of this invention, the material exhibits a moisture vapor transmission rate in the range indicated and, because of the inclusion of biological components, exhibit good adherence to the burn area. Thus, the materials of the present invention, used as a burn dressing preferably is in the form of a laminate including a thin film of thermoplastic polymer, e.g., silastic rubber, urethane or other elastomeric polymer material, the

film of polymer being of such dimensions and composition as to have a water vapor transmission rate in the range indicated. Physically bonded to the thin polymer film is a thin porous fabric such that the composite is elastic in all directions, i.e. length and width. Covalently coupled to one or both sides of the laminate is one or more biological materials to provide adherence and compatibility to the wound site.

Regardless of the form of the substrate, sheet, tube, formed contour and the like, the biological compound is bound by treating the substrate with a primary or secondary amine such that the amino groups are available for further reaction. In one form this is accomplished by incorporating the primary or secondary amine into the substrate such that the amino functional groups extend out of the surface as coupling sites. In another form, the substrate is coated with a primary or secondary amine silating agent in order to provide terminal available amino functional groups, again as coupling sites.

The first form above described is similar in parts to the procedure described in U.S. Patent 3,634,123 and the primary and secondary amines there disclosed may be used in this form of the present invention.

The second form above described offers the advantage of being able to provide available amino groups reactive sites with a variety of substrates both of organic and inorganic character, i.e. substrates other than silastic, urethane, for example other plastics to which the material will adhere to, or to inorganics such as metal or glass.

The procedures thus far described are distinguishable from those of U.S. Patent 3,846,353 which use a long chain alkyl quaternary ammonium salt to ionicly bind heparin to various polymer substrates.

According to the present invention, the available amino functional groups are then activated for bonding to a biological. This is in contrast to U.S. Patent 3,634,123 in which heparin is ionicly linked to the positively charged amine directly, or in contrast to U.S. Patent 3,810,781 which treats the substrate-amine hydrochloride-heparin salt subsequently with a dialdehyde, such as glutaraldehyde, to stabilize the heparin on the substrate surface.

Activation of the amino groups, according to the present invention may be accomplished by one of several ways. In one form dialdehyde, such as glutaraldehyde, is reacted with the primary or secondary amine provided by either of the procedures described, leaving available aldehyde groups average of one per molecule of glutaraldehyde for subsequent reaction with the primary or secondary amines of either proteins, mucopolysaccharides or other amine containing biologicals. In another form, the preferred form, cyanuric chloride is reacted with the primary and secondary amines provided on the substrate as previously described. The available chloride groups of cyanuric chloride may then be used to react with the primary or secondary amines or hydroxyl groups of various biologicals to form covalent bonds.

Other bifunctional reagents that may be used to link substrate amines with biological amines are thiophosgenes, isocyanates, derivitized cyanuric chloride (one cl group removed or alkylated, 1.5-difluoro 2,4-dinitrobenzene, diazobenzidine, toluene-2,4-diisothiocyanates and others.

Thus, a wide variety of new, improved and relatively simple procedures are described for attaching various biologicals on a substrate which, in accordance with this invention, may be used as burn covering having the desirable properties mentioned.

It will be apparent from the following detailed description and specific examples and data that a much improved bio- and blood compatible material has been provided by a relatively simple and reliable procedure. The further advantages and features may be understood with reference to the following description of the invention.

Detailed Description of the Invention

The present invention relates to bio- and blood compatible materials which may take various forms and shapes, for example, rigid and flexible tubes, sheets or formed and contoured shapes, for use in equipment and or in patients. Since burn wound coverings, one form of the present invention, include the characteristics of adhesion to the wound site and elasticity in all directions, although it will be understood by those skilled in the art that the present invention is not limited to such dressings, but has applicability to bio- and blood compatible materials.

In general, the present invention relates to a novel and improved bio- and blood compatible material in which specific characteristics are imparted to a substrate by a novel, simple, affective and inexpensive procedure for covalently coupling to the substrate certain biological materials or combinations thereof. Typical of the biological materials which can be covalently coupled to the substrate are those which include an available primary or secondary amine reactive group which can react with an aldehyde group or arylchloride group (i.e. or cyanuric chloride or its derivative) and those which include an available hydroxyl group which can react with an arylchloride group. Representative materials are proteins, collagen, albumin, gelatin, fibrinogen, animal or plant proteins, complex carbohydrates (i.e.: acidic mucopolysaccharides) simple carbohydrates, lipids, (i.e.: lecithin), peptides and amino acids. Typical of the complex carbohydrates are heparin, hyaluronic acid, chrondrotin sulfate A and C, to mention only a few.

Conceptually the improved and novel products of the present invention, produced by the improved and novel process of this invention, include a suitable substrate treated to provide available

and reactive primary or secondary amine functional reactive sites. The amine functional sites are then activated either by reaction with a dialdehyde, or preferably cyanuric chloride to provide available active aldehyde or arylchloride groups, respectively. Thereafter, one or more biological materials, as previously described, having a hydroxyl, primary or secondary amine, is then coupled to the available free aldehyde or arylchloride group. In this way select biologicals are covalently coupled to the substrate in an amount and in a form sufficiently stable to provide bio- and blood compatibility to the substrate.

The useable substrates may be a wide variety of materials depending upon the procedure and to provide available primary and secondary amine functional reactive sites. For example, a reactive silicone containing a primary or secondary amine may be used as a primer and coated on the substrate to provide the reactive amine group. Such a procedure is described in Canadian Patent 774,529, however, the amine is then alkylated to form a positively charged quaternary ammonium salt which is then used to ionicly bind heparin to the surface of the substrate.

Thus, typical substrates are glass, and the elastomers, silicone rubbers and thermoplastics used in medical applications. Representatives of such materials are:

silicone rubbers and elastomer polysiloxanes, natural rubber, polybutadiene, styrene-butadiene, butyl rubber,
for example;

thermoplastics such as polyethylene, polypropylene polystyrene, polyvinylchloride, polyvinyl acetate, ethacrylate and methacrylate polymers and copolymers and the like.

For burn wound dressings it is preferred to use silicone rubbers of membrane thickness as will be described.

A useable primer is an aminofunctional silane coupling agent such as gamma (Beta-aminoethyl) aminopropyltrimethoxysilane, available as Dow Corning Z-6020. This primer also bonds well to materials such as nylon, dacron and the like, the latter may optionally be components of the substrate, as will be apparent with the description of burn wound dressings.

A typical aminofunctional silating primer and its application are as follows:

Four milliliters of the aminofunctional silating agent was added to 4 milliliters of absolute methyl alcohol along with 0.32 milliliters of distilled water and the resultant material was allowed to sit overnight at room temperature. To the primer solution 69 milliliters of absolute methyl alcohol was added to make a dipping solution into which silicone rubber membranes were dipped for 3—5 seconds. The membranes were then dryed in an oven at 100—110°C for 40 to 60 minutes. The result is a substrate having primary amino functional groups thereon for further reaction as will be described.

Primary or secondary amine functional groups may also be attached to a substrate by physically entrappung an alkyl amine in the substrate (i.e. dodecylamine or other organic primary or secondary amines). For example, using a silicone rubber, a solvent in which the amine will dissolve and which causes the rubber to swell, a substrate may be formed with amine functional groups trapped therein and thereon. In a typical example, 4% by volume of dodecylamine (by volume) was dissolved in a 60:40 solvent mixture of toluene and isopropyl alcohol. The substrate was immersed in the amine solution, at room temperature, for 5—8 seconds and then allowed to dry at room temperature for an additional 10—20 minutes. The result is a substrate wherein primary or secondary amine functional groups are attached and available for further reaction.

In the case of burn wound dressings, which represents a preferred form of this invention, the substrate preferably is in the form of a thin rubber membrane-fabric composition which is stretchable in all directions and which has a water vapor transmission rate of betwee 6 to 12 grams per hour per meter square foot. Also, the burn covering substrate should be sufficiently strong so that in normal handling and use it does not tear.

A typical burn covering substrate material may be prepared as follows:

A dispersion of 13% dimethylsiloxane elastomer is layered with a precision layering tool at a uniform thickness of .006, .008, .010, .012, .014, .016, .018 or .020 inches thick plus or minus .0003 inches over a mylar (polyethylene terephthalate) support member. A typical silicone rubber which may be used is that available from Dow Corning as Q7-2213. After deposition of the dispersion, a firmly knitted fabric (dacron or nylon), of 25 denier or less is laid over the wet silicone rubber. The fabric is preferably one which is stretchable to 100% or more elongation in all directions. The uncovered composite is allowed to sit at room temperature for 15 minutes and then transferred and cured in an oven at 150°C for about one hour. The cured composite, still on the support is then stored until processing for binding biologicals thereto.

After curing, the thickness of the silicone rubber is .0006, .0008, .00010, .0012, .0014, .0016, .0018, or .0020 inches plus or minus .0003 inches. Thickness of .0008, .0010 and .0012 inches are preferred for burn dressing membranes. Prior to chemical modification the cured composite may be removed from the mylar support, without the use of release agents, by immersing the support and composite in a 60:40 toluene and isopropyl alcohol solution for 10 minutes at room temperature. The composite is gently pulled off the support and allowed to air dry prior to modification.

The above described composite is a substrate, stretchable in all directions, and having a water vapor transport rate in the range noted and adaptable to modification. The fabric is located at the face

of one side of the membrane, i.e. partly imbedded in the membrane with portions of the fabric exposed. In use, the fabric side is applied over the woundside so that the fabric faces the wound.

Attachment of biologicals to the burn dressing involves first attachment of primary or secondary amine groups on one or both sides of the composite. This may be done by either of the procedures already described. Regardless of the procedure, it is noted that amine groups are present on the exposed fabric surface side as well as on the exposed membrane surface side.

The next step in accordance with this invention, regardless of the nature of the substrate, is to activate the amine functional groups. The preferred procedure in accordance with this invention is as follows:

A saturated solution of cyanuric chloride in acetone is prepared and chilled to 0°C. The substrate (silicone rubber-fabric composite with attached amine functional groups) is then immersed in the chilled solution for 10 seconds. As a result, a bond through the amine to cyanuric chloride at the site or one of the chlorides is formed so that there is now available two chloride groups on the cyanuric chloride available for further reaction.

While the use of cyanuric chloride is preferred because it is more reactive, it is also possible to activate the amine functional groups by reaction with a dialdehyde, such as glutardialdehyde. U.S. Patent 3,810,781 described the use of this material to stabilize heparin ionicly bound to a substrate containing a positively charged amine, i.e. the heparinized surface is subsequently treated with the dialdehyde. In contrast, the present invention reacts the amine with the dialdehyde to provide a reactive aldehyde group covalently bound to amine-substrate surface.

Thus, a typical procedure involves incubating a substrate with the primary or secondary amine thereon, formed as described by either of the previous procedures, in a 0.5% solution of glutaraldehyde solution, 1/15 M disodium hydrogen phosphate, pH 8.2—8.3 for 2—3 hours at room temperature. The result is a substrate in which the primary or secondary amine has reacted with one of the aldehydes of glutaraldehyde to form a Schiff Base covalent bond leaving the other primary or secondary amine containing compounds.

Linkage of the biological may be accomplished one of several ways, depending upon the nature of the biological and the type of amine activation. For example, in the case of proteins, a 0.5—2.0% solution of the protein in 1/15 M disodium hydrogen phosphate solution is prepared, pH 8.2—8.3 and the activated substrate is taken directly from the glutaraldehyde activating solution submerged and incubated in the protein solution for 2—8 hours at 25—55°C. The amine-silicone-fabric composite material can also be activated by cyanuric chloride and biologicals bound by being taken from the saturated cyanuric chloride solution and incubated in a protein solution as described. Essentially the same procedure is used for attachment of mucopolysaccharides to the substrate surface (e.g.: a 0.5% buffered solution thereof as described, and incubated as described).

Depending upon the route taken the biological is covalently bounded through the primary or secondary amine groups of the biological, through the aldehyde to the amine to the substrate. In the case of cyanuric chloride activation, the primary or secondary amine or hydroxyl groups of the biological is covalently coupled through the cyanuric chloride to the amine to the substrate.

By way of illustration of the products of the present invention and the unique character of the biologically activated materials which are bio- and blood compatible, various products were prepared and evaluated as burn dressings in terms of adhesion to animals. The test involved removing the skin of the test animal (rats) and testing the adherence of various products to the subdermal facial tissue. Circular test coupons of 6 mm in dimeter were prepared from each material type and the discs were applied to ten different locations on each of the test animals. After 5 hours, the force in grams necessary to remove each of the discs was measured by a tensiometer and adherence was recorded in grams/cm^2. The test was repeated for a 72 hour adhesion period and the data again collected. (Details of the test methodology is described by *Tavis et. al.,* Annuals of Surgery, Vol. 184, No. 5 pg. 594—600, 1976).

As a basis for comparison, products of the present invention were compared with homograft, pigskin and modified bovine collagen membrane, the material with the highest adherence was assigned a value of 100 and other materials were normalized on the basis of their adherence value. All samples were ranked in overall adherence both at 5 hours and 72 hours. The following codes were used to describe each of the products of this invention and the materials evaluated for adherence.

The material code is a series of three letters, x x x, the first letter describing the substrate; the second letter, the activating agents/bonding agent(s); and the third, the biological component bonded to the surface.

The activating agent(s)/bonding agent(s) code is as follows:

A. Dodecyl amine, glutaraldehyde treatment of amine.
B. Silated by coating, glutaraldehyde treatment of amine.
C. Dodecyl amine, cyanuric chloride activation.
D. Silated by coating, cyanuric chloride activation.
E. Dodecyl amine.
F. Silated by coating.
G. No activating agents.

The substrate (fabric silicone rubber composite) code is as follows:
A. Edwards membrane-cotton gauze/silicone rubber (see infra).
B. 18/3 nylon fabric 300%x50% elongation.
C. 18/3 nylon fabric 150%x240% elongation.
D. Silicone rubber without fabric.

The code for the biological component bonded to the activated surface, is as follows:

A. Heparin
B. Chondroitin sulfate C
C. Egg albumin
D. Collagen (tropocollagen-rat skin)
E. Lysine
F. Fibrinogen
G. Hemiglobin
H. Aspartic Acid
I. Alanine
J. Glutamic Acid
K. Glycine
L. Glyclglycine
M. Human albumin
N. Gelatin (Porcine Skin)
O. Nothing
P. Lecithin

Overall, the test involved evaluation of a multiplicity of materials, including those presently in use, for the purpose of establishing adherence of the products of the present invention for use as burn dressings. Those materials such as the Edward Membrane formerly made by Edwards Laboratories a division of Amer. Hosp. Supply Corp. and Pigskin, homograft and Collagen membrane formerly made by Edwards Laboratories offer a basis for comparing the adherence of the products of this invention with those recognized in the field as being of use as burn dressings. The Edwards membrane is a composite of a silicone rubber polymer backing and a non-elastic cotton gauze facing having a substantial thickness variation (.0005—.0020 inches thick) as compared to the thin membrane material substrates of the present invention. Further the Edwards membrane is not as stretchable as the substrates of the present invention, the latter preferable having greater than 100% elongation in all directions. Moreover, the Edwards membrane is not biologically activated, although as a basis of comparison, this membrane was used as a substrate and activated in accordance with this invention. Collagen membrane has a cotton gauze component; the same as the Edwards material.

In the test, with the exception of homograft and pigskin, which do not include fabric, all substrates were applied with the fabric side in contact with the dorsal facial surface of the test animal. The circular coupons were applied principally to the backs of the test rats to prevent them from being reached and possibly eaten by the test animals. In some instances the patches were scraped loose when the animals contacted the cage walls in their normal movements in the cages. Where this occurred, a value of zero was used and totaled and averaged in the data.

Table I
Adherence Data Summary
5 hr. Adherence Test

Rank	*Sample #*	*Material*	*Adherence (gm/cm²)*	*% Maximum Adherence*	*#Adhering of 10 Samples Placed*
1	25	Homograft	167	100	8
2	23	Collagen M.	133	80	7
3	24	Pigskin	116	69	9
4	2	ACC	99	59	9
5	19	BDC	95	57	6
6	14	ADK	86	51	8
7	1	ADC	82	49	8
8	13	ADE	68	41	9
9	11	ADD	63	38	9
10	8	ACK	59	35	9
11	18	BCC	54	32	6
12	9	ACG	52	31	9
13	12	ADB	51	31	8
14	17	ABC	49	29	7
15	10	ADR	49	29	8
16	20	BCD	47	28	7
17	15	ADG	44	26	9
18	22	BDC_2	44	26	9
19	21	DBB	42	25	8
20	16	AAC	31	19	6
21	5	ACD	30	18	9
22	4	ACF	30	18	10
23	3	ADC	28	17	9
24	7	ACE	26	16	8
25	6	ACB	22	13	9
MEAN±STD. DEV.			62.7±36	28±25	8.2±1.1

Table II
Adherence Data Summary
72 hr. Adherence Test

Rank	Sample #	Material	Adherence (gm/cm^2)	% Maximum Adherence	# Adhering of 16 Samples Placed
1	13	ADE	524	100	10
2	25	Homograft	512	98	6
3	14	ADK	499	95	10
4	6	ACB	498	95	11
5	17	ABC	483	92	11
6	23	Collagen M.	472	90	6
7	12	ADB	457	87	10
8	15	ADG	455	87	8
9	18	BCC	455	87	14
10	20	BCD	499	86	13
11	16	AAC	435	83	10
12	24	Pigskin	424	81	5
13	3	ADC	420	80	9
14	5	ACD	419	80	10
15	2	ACC	399	76	12
16	21	BDD	395	75	6
17	11	ADD	392	75	13
18	9	ACG	379	72	10
19	10	ADF	372	71	11
20	8	ACK	339	65	10
21	19	BDC	335	64	12
22	1	ADC_2	328	61	10
23	22	BDC_2	272	52	9
24	7	ACE	246	47	8
25	4	ACF	244	47	6
MEAN±STD. DEV.			*408*±79	78±15	9.6±2.4

Table III
Adherence Data Summary
5 hr. Adherence Test

Rank	Sample #	Material	Adherence (gm/cm^2)	% Maximum Adherence	# Adhering 10 Samples Placed
1	22	BDH	389	100	9
2	24	Collagen M.	378	97	10
3	16	BDK	297	76	10
4	6	BDO	287	74	8
5	10	BDF	283	73	8
6	25	Homograft	269	69	10
7	13	BCM	261	67	10
8	21	BCH	244	63	10
9	4	BFO	230	59	9
10	18	BDL	230	59	10
11	17	BCL	216	56	9
12	2	AOO	191	49	9
13	12	BDC	180	46	10
14	3	BEO	177	46	2
15	19	BCE	177	46	10
16	1	BOO	173	44	9
17	14	BDM	163	42	10
18	5	BCO	158	41	10
19	9	BCF	149	38	7
20	15	BCK	145	37	10
21	20	BDE	145	37	10
22	11	BCC	134	34	10
23	7	BCD	120	31	9
24	8	BDD	113	29	10
25	23	Pigskin	85	22	8
MEAN±STD. DEV.			208±78	53±20	9±2

Table IV
Adherence Data Summary
72 hr. Adherence Test

Rank	# Sample	Material	Adherence (gm/cm²)	% Maximum Adherence	# Adhering of 16 Placed Samples
1	4	BFO	601	100	8
2	9	BCF	584	97	2
3	6	BDO	548	91	7
4	18	BDL	527	88	3
5	19	BCE	520	87	11
6	15	BCK	509	85	6
7	13	BCM	502	84	10
8	21	BCH	495	82	11
9	5	BCO	467	78	7
10	8	BDD	463	77	7
11	7	BCD	460	77	7
12	2	AOO	431	72	5
13	11	BCC	431	72	10
14	24	Collagen M.	417	69	11
15	14	BDM	410	68	9
16	12	BDC	389	65	12
17	10	BDF	382	64	4
18	20	BDE	378	63	11
19	23	Pigskin	375	62	12
20	17	BCL	357	59	5
21	16	BDK	347	58	8
22	25	Homograft	336	56	8
23	1	BOO	332	55	2
24	22	BDH	308	51	8
25	3	BED	304	51	5
MEAN±STD. DEV.			435±85	72±14	7.6±3

Table V

Material	Number of Animals (Rats)	Average Starting Weight (gms)	Percent Mortality (3 Wks)	Average Weight Gain (gms/day) and Correlation with Time (r) (gms/Day)	(r)	Relative Synthetic Skin Performance (Adherence and Conformance/100 max. Value) WK-1	WK-2	WK-3
BDK	6	298	33	.88	.65	52	29	23
BFO	5	298	20	−.38	−.44	53	20	20
BDE	8	290	37.5	1.0	.95	60	35	28
BDH	5	298	60	1.1	.86	54	20	0
BDC	6	276	16.7	1.35	.96	61	33	25
BDL	6	269	16.7	1.39	.97	60	32	28
BCC	7	278	57	1.41	.82	81	53	50
BCH	5	329	0 (2 wks)	1.85	.99	76	45	No Data
MEAN± STD. DEV.	6±1.1	292±18.8	30±21	1.1±.66	.72±48	62±11	35±10	25±15

The data in Table V represents the results of a test in which the best materials, based on adherence data were placed on 20% full thickness dorsal defects of rats. Evaluations were made each week and values assigned for adherence and conformity, as follows:

Membrane Adherence	*Membrane Conformity*
1. No adherence	1. No observation—non-adherent
2. Minimal	2. Minimal adherence with ridges and ripples
3. Moderate	3. Moderately adherent with ridges and ripples
4. Mostly adherent	4. Mostly adherent with ridges and ripples
5. Very adherent	5. High conformity and adherence.

On the basis of the above data and further testing it was found that the bio- and blood compatible materials of the present invention when structured for use as a burn dressing optimally includes a substrate which is substantially stretchable, compliant, and conformable. A preferred material is, accordingly, material C (18/3 nylon fabric 150%×240% elongation) primarily because of the ability of the substrate to stretch and conform to the area of application.

In combination therewith, it is preferred to use a bonding agent such as dodecyl amine which is cyanuric chloride (C) activated with gelatin (porcine skin) coupled thereto. Also useable is a coated silating material (D).

By way of example, a comparison of the preferred burn dressing with the accepted standard demonstrate the performance of the preferred material:

Material	*Performance*	*No. of Animals*
Pigskin	87.2±11.4	14
CCM	97.6± 4.6	12
CDN	91.2± 9.6	16

The above data are significant in establishing the superiority of these fabricated materials over the others and those naturally available when used as burn dressings. It is to be understood, however, that other combinations of components may be preferred based on other ultimate uses of the materials of this invention.

Although coupling of the biological has been described, under proper conditions other techniques, known in the art may be used, see for example "Immobilized Enzymes for Industrial Reactors", Messing, Academic Press, N.Y. 1975, pages 99—123. The preferred system, however, is that as set forth herein.

The above described materials, especially those of Table V and CCN and CDN represent the better of the group prepared for use as burn dressings, the test data on rats establishing the ability of the better materials to remain adherent with nearly complete connective tissue ingrowth and without superation for periods up to one month.

The materials are easily packaged, easily sterilized or other appropriate procedures and possess a relatively long shelf life.

It will also be apparent from the foregoing description that the products are relatively easily fabricated since the effective chemical reactants are easily attached by appropriate chemical bonds to various substrates. Since the reactions are relatively fast and controllable, selected surface portions or all surface portions may be treated to provide one or more zones of various desired biologicals.

Further, preliminary data on several of the specific biologicals indicates that the materials are non-extractable, free of bio- and blood extractable contaminants and non-antigenic.

In the case of burn dressings, an important practiced application of the present invention, the novel substrate in membrane form which is stretchable and compliant and thus easily fits a variety of body contours, offers unique advantages over prior materials. Also, the effective attachment of effective biologicals provides for adherence to the wound site over extended periods, with proper water vapor transport through the membrane, important characteristics in burn therapy for dressings of this type.

It will, accordingly, be apparent to those skilled in the art that various alterations, changes and modifications may be made with respect to the products and procedures herein described without departing from the scope of the present invention as set forth in the appended claims.

Claims

1. A bio- and blood compatible material for human and animal use comprising:

a substrate having functional groups extending from at least a portion of the surface thereof, said functional groups being selected from the group consisting of primary and secondary amino groups,

said amino groups having attached thereto a reactive group selected from the group consisting of aldehyde and arylhalide groups,

a biological coupled to said substrate by reaction between the primary or secondary amine or

hydroxyl of said biological and the aldehyde and arylhalide groups attached to said primary and secondary amines which extend from said surface, and

said biological operating to provide bio- and blood compatible qualities to said surface portion.

2. A material as set forth in Claim 1 wherein said substrate is rigid.

3. A material as set forth in Claim 1 wherein said substrate is flexible.

4. A material as set forth in Claim 1 wherein said biological is a material selected from the group consisting of proteins, carbohydrates, lipids, amino acids, and peptides.

5. A material as set forth in Claim 3 wherein the biological is a material selected from the group consisting of heparin, chrondroitin sulfate C, egg albumin, collagen, lysine, fibrinogen, hemiglobin, aspartic acid, alanine, glutamic acid, glycine, glyclglycine, human albumin, gelatin, and lecithin.

6. A material as set forth in Claim 1 wherein said amino group has an arylhalide group attached thereto.

7. A material as set forth in Claim 1 wherein said biological is gelatin.

8. A material as set forth in Claim 1 wherein said substrate is a tubular member.

9. A material as set forth in Claim 1 wherein said substrate is a composite of a nylon mesh stretchable in all directions and compliant and includes a silicone rubber facing,

said biological being coupled to the surface of the silicone rubber facing which is in contact with said mesh.

10. A material for use as a dressing for thermal burns comprising:

a composite elastomeric substrate which includes a fabric backing and a facing of a thin thermoplastic material,

said composite elastomeric substrate being stretchable in all directions and compliant,

said thermoplastic having functional groups selected from the group consisting of primary and secondary amino groups extending from at least a portion of the surface thereof,

said amino groups having attached thereto a reactive group selected from the group consisting of aldehyde and arylchloride groups,

a biological coupled to said thermoplastic by reaction between the primary or secondary amine, or hydroxyl group of said biological and the aldehyde and arylchloride groups attached to said primary and secondary amino groups extending from at least a portion of said surface, and

said biological imparting bio- and blood compatible characteristics to said portion of said surface.

11. A material as set forth in Claim 10 wherein said composite elastomeric substrate has a water vapor transmission rate, as measured at 37°C for 24 hours, in the range of 10 to 15 grams per hour per meter squared.

12. A material as set forth in Claim 10 wherein the thermoplastic material has a thickness in the range of .0006 to 0.020 inches.

13. A material as set forth in Claim 10 wherein said thermoplastic has a thickness in the range of 0.0008 and 0.0012 inches.

14. A material as set forth in Claim 10 wherein said thermoplastic is selected from the group consisting of silicone rubber, elastomer polysiloxanes, natural rubber, polybutadiene, styrene-butadiene, butyl rubber, polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyvinyl acetate, and ethacrylate and methacrylate polymers and copolymers.

15. A material as set forth in Claim 10 wherein said biological is selected from the group consisting of heparin, chrondroitin sulfate C, egg albumin, collagen, lysine, alanine, glutamic acid, glycine, glyclglycine, human albumin, gelatin and lecithin.

16. A material as set forth in Claim 10 wherein said composite elastomeric substrate has an elongation in each direction of at least 50%.

17. A material as set forth in Claim 16 wherein said fabric is an 18/3 nylon mesh.

18. A material as set forth in Claim 10 wherein said material is sterile.

19. A material as set forth in Claim 10 wherein said biological is gelatin.

20. A material as set forth in Claim 10 wherein said biological is human albumin.

21. A material for use as a dressing for thermal burns comprising:

a sterilized composite matrix substrate,

said substrate including a mesh fabric backing which has an elongation in each direction of at least 50%,

a silicone rubber membrane member attached to said fabric,

said silicone rubber membrane having a thickness in the range of between 0.008 and 0.0012 inches,

and a biological chemically coupled to at least a portion of the surface of said substrate in an amount sufficient to provide bio- and blood compatible characteristics thereto.

22. A material as set forth in Claim 21 wherein said biological is human albumin.

23. A material as set forth in Claim 21 wherein said biological is gelatin.

24. A material as set forth in Claim 21 wherein said matrix substrate has a water vapor transmission rate, as measured at 37°C for 24 hours, in the range of between 10 and 15 grams per meter squared.

25. A method of providing bio- and blood compatible characteristics to a substrate comprising:

the steps of treating at least a portion of the surface of said substrate to provide reactive primary or secondary amine functional reactive sites,

activating said primary or secondary amine functional reactive sites by treatment with a dialdehyde or a arylchloride to provide reactive aldehyde chloride groups, and

coupling to said substrate a biological having a hydroxyl or primary or secondary amine group by reaction of said group of said biological with said reactive aldehyde or arylchloride groups.

26. A method as set forth in Claim 25 wherein said substrate is a fabric elastomer matrix and wherein said biological is coupled to the surface of said elastomer contacted by said fabric.

27. A method as set forth in Claim 26 wherein said biological is gelatin.

28. A method as set forth in Claim 26 wherein said biological is human albumin.

29. A method as set forth in Claim 26 wherein said substrate is a nylon mesh fabric having an elongation of at least 50% in each direction and said elastomer being a silicone rubber membrane of a thickness between .0006 and .020 inches.

30. A method as set forth in Claim 29 further including the step of sterilizing the resultant product.

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