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(71) Applicant *(for all designated States except US)*: MACNAUGHT PTY. LIMITED [AU/AU]; 47-49 Henderson Street, Turrella, NSW 2205 (AU).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : HILLS, Brian, Andrew [AU/AU]; "West Tree", Dumaresq Road, Dumaresq Via Armidale, NSW 2350 (AU).

(74) Agent: GORDON, Glen, Howard; Arthur S. Cave & Co., Level 10, 10 Barrack Street, Sydney, NSW 2000 (AU).

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(54) Title: A METHOD FOR PRODUCING A BIOCOMPATIBLE SURFACE

(57) Abstract

The invention relates to methods for producing biocompatible surfaces by coating them with phospholipid and binding the phospholipid to the surface by means of ultrasonication.

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A METHOD FOR PRODUCING A BIOCOMPATIBLE SURFACE

TECHNICAL FIELD

The present invention relates to methods for producing biocompatible surfaces by coating them with phospholipid and binding the phospholipid to the surface by means of ultrasonication.

BACKGROUND ART

A major problem experienced with medical equipment and devices that comes into contact with the blood flow, such as cannulae, in dwelling electrodes and catheters, is that blood clots may form on their surfaces. The formation of blood clots on the surfaces of these instruments is dangerous and may prove fatal if the clots are washed off by the blood flow into the vascular system.

It has been reasoned that the best non-thrombogenic surface model is provided by the lining of blood vessels themselves - the endothelial lining. Some blood vessels, such as the aorta and cerebral vessels, have extremely hydrophobic inner surfaces. Electron microscopic studies of the inner surface of these vessels has revealed that they are lined with an oligolamellar lining of phospholipid. It is thought that mimicking the lining of the blood vessels on artificial surfaces should substantially overcome the abovementioned problems.

Early attempts at coating surfaces with phospholipid proved unsuccessful. Surfaces were left in contact with phospholipid or phospholipid/hyaluronic acid suspensions and then tested in blood stirred by a magnetic stirrer to mimic flowing blood. However, blood clots still developed on these surfaces.

US Patent 4 426 330 (Sears) discloses a chemically modified phospholipid for more stable coatings. US Patent 4 438 329 (Chapman) discloses a phospholipid chemically bonded to a polymer for coating surfaces. However, the introduction of new, chemically modified phospholipids into a human body or blood stream may have unforseen

results. Also, the expense and difficulty of preparing these modified phospholipid substances is a disadvantage for their use.

There is therefore an advantage in finding a way to coat surfaces with natural phospholipids on which flowing blood does not coagulate or form clots. A method of coating with phospholipids to produce non-thrombogenic surfaces has therefore been developed.

DISCLOSURE OF INVENTION

It is therefore an object of the present invention to provide a method for producing a biocompatible surface by coating it with phospholipid.

The present invention concerns a method for producing a biocompatible surface, by coating the surface with phospholipid characterised by immersing the surface to be coated in a bath containing a suspension of phospholipid in a liquid in which it is sparingly soluble, and then ultrasonicating the bath to coat the surface with phospholipid.

Another aspect of the invention concerns pre-treating the surface to be coated. The surface is firstly immersed in a phospholipid solution. The solution comprises phospholipid substantially dissolved in a solvent in which it is soluble, such as methanol or chloroform. The surface is then removed from the solution and the solvent allowed to evaporate off. The dry surface is then subjected to the ultrasonication process described above.

Any suitable surface may be coated, such as metal, glass, plastics or ceramics. Any phospholipid may be used and some examples are described in Table 1.

TABLE 1

Phosphoglycerides
phosphatidic acids
cytidylic phosphoglycerides (CDP diglyceride)
choline phosphoglycerides
ethanolamine phosphoglycerides
N-methylethanolamine phosphoglycerides
N,N-dimethylethanolamine phosphoglycerides
N-acylethanolamine phosphoglyceride
serine phosphoglycerides
N-2-(hydroxyethyl)alanine phosphoglyceride
glycerol phosphoglycerides
glycerophosphate phosphoglycerides
phosphatidylglycerol phosphoglyceride (diphosphatidylglycerol)
mono and diacylglycerol phosphoglycerides (lysobisphosphatidic acids)
glucosaminylglycerol phosphoglyceride
O-amino acid esters of glycerol phosphoglycerides
inositol phosphoglyceride
inositol monophosphate phosphoglyceride
inositol diphosphate phosphoglyceride
monomannosyl-hexamannosyl inositol phosphoglycerides
glucose phosphoglyceride
O-diglucosylglycerol phosphoglyceride
Phosphoglycolipids
diacyl (glycerylphosphoryldiglucosyl) glycerol
Phosphodiol lipids
acyl dihydroxyacetone phosphate
alkyl dihydroxyacetone phosphate
Phosphosphingolipids
sphingomyelin (ceramide phosphorylcholine)
ceramide phosphorylethanolamine
ceramide phosphorylglycerol
ceramide phosphorylglycerophosphate
ceramide phosphorylinositol-containing lipids

The preferred phospholipid is phosphatidylcholine, (lecithin).

The ultrasonic treatment is conducted in the normal manner using commercially available ultrasonic equipment.

The suspension of phospholipid used in the bath comprises preferably finely divided phospholipid suspended in a liquid in which it is only sparingly soluble. The suspension is preferably an aqueous suspension.

Phospholipid is added to the suspension in solid form and is suspended by an initial ultrasonication to form liposomes. The phospholipid is preferably suspended at a concentration of 0.1 to 10% w/v, most preferably 1 to 2% w/v. The liquids that may be used to suspend the phospholipid in accordance with the present invention include water and physiological saline solutions.

Any ultrasound device can be used in accordance with the present invention, such as the Model G112SPIT (serial No. 11254) produced by Laboratory Supplies Co. Inc., New York.

The method of coating a surface with phospholipid may also comprise a pre-treatment step, which involves dissolving phospholipid in a solvent; placing the surface to be coated into the phospholipid/solvent solution to allow initial gross deposition of phospholipid onto the surface; removing the coated surface from the phospholipid solvent solution; evaporating off residual solvent on the coated surface; placing the coated surface into the suspension; and ultrasonicating the coated surface as described previously.

Surfaces that may be coated and phospholipids that may be used in accordance with the present invention are as described above. The phospholipid is suspended in the solvent by stirring. Any solvent that dissolves phospholipid may be used in accordance with the present invention, such as chloroform or methanol. Alternatively the pre-treatment by immersion in the dissolved phospholipid may involve the gross deposition of phospholipid from a solution with a low degree of supersaturation. The ultrasonication of the coated surface orientates and consolidates the phospholipid that was deposited on the surface to be coated from the solution.

The slow deposition of the phospholipid from the solution with a low degree of supersaturation onto the surface to be coated enables the phospholipid to be deposited with a better orientation and thus gives an effective coating.

Surprisingly, the methods of the present invention enable the rapid and successful coating of surfaces with phospholipid. As such, in one application the methods may be used to coat the surfaces of medical instruments, thereby making the surfaces non-thrombogenic.

The present invention is particularly applicable for the production of coated catheters for which the surfactant properties of lubrication and release are ideal. It may also be used to produce pacemakers and prosthetic devices which are less likely to be rejected by the body's immune system. The invention may also be applied to ceramic prosthesis to reduce their permeability.

In addition to the anti-friction properties of the phospholipids the present invention has found that surfaces having phospholipid adhered to them seem to be less likely to generate an antibody response than uncoated materials. Similarly, the formation of blood clots is greatly reduced on such coated surfaces. In this respect it is to be noted that by far the most successful anti-coagulent is heparin which can now be grafted to certain surfaces to render then non-thrombogenic. One of the features of grafted heparin is the number of negative charges which they impart, indicating that they might function by providing a site most conducive to the adsorption of the endogenous surfactants, such as phospholipids, which are then the true interface with blood or other body fluids. When such surfaces are removed they are more hydrophobic than before implantation. Hence it would seem better to proceed directly to the phospholipid surface rather than risk desorption of heparin or adjuvant heparin which inhibits coagulation of the blood in general, causing problems to the surgeon.

MODES FOR CARRYING OUT THE INVENTION

The invention will now be described by way of various examples.

EXAMPLE 1

Clean glass rods were placed in a bath of water containing 2% egg lecithin and ultrasonicated in the apparatus for 75 minutes. The glass rods were then allowed to drain for 135 minutes and suspended in 125 ml of blood in a beaker. The blood was kept flowing past the rods by a magnetic stirrer. Uncoated, clean glass rods were used as controls in the same blood at the same speed. At intervals of 30 seconds both sets of rods were checked for build up of coagulated material. Within 30 minutes there was appreciable build up of clotted blood on the control rods whereas those rods coated with phospholipid were clean.

COMPARATIVE EXAMPLE 2

The experiment was repeated by solvent depositing the egg lecithin from a solution (2% in methanol) which was allowed to evaporate for two and a half hours. Although the coated rods initiated less clotting than the uncoated control rods, the incidence of clotting was still markedly more than seen for the rods coated in Example 1.

COMPARATIVE EXAMPLE 3

The experiment was performed by solvent depositing soya lecithin in chloroform. Whilst these rods showed less clotting than the uncoated control rod, they were found to be less effective than the rods of comparative example 1 and markedly less effective than the rods coated using ultrasound.

EXAMPLE 4

The Experiments 2 and 3 were repeated, but the surfaces were allowed to dry before being placed in the bath and ultrasonicated as described in Example 1. The glass rods resulting were placed in blood as described in Example 1. The rods thus treated were clean of blood, amd the incidence of clotting was less than in Example 2 or 3.

It will be obvious to those skilled in the art that

numerous variations and modifications could be made to the method of the present invention as described, with reference to the examples, without departing from the overall scope or spirit of the invention.

CLAIMS

1. A method for producing a biocompatible surface, by coating the surface with phospholipid, characterised by immersing the surface to be coated in a bath containing a suspension of phospholipid in a liquid in which it is sparingly soluble, and then ultrasonicating the bath to coat the surface with phospholipid.

2. The method according to claim 1 further characterised in that before immersing the surface in the bath the surface is pre-treated by being immersed in a solution of phospholipid dissolved in a solvent, removed from the solution and allowed to dry.

3. The method according to claim 1 wherein the bath contains an aqueous suspension of phospholipid.

4. The method according to claim 1 wherein the phospholipid in lecithin.

5. The method according to claim 2 wherein the solvent is methanol or chloroform.

6. The method of claim 1 wherein the surface is that of a catheter, prosthetic device or a heart pacemaker.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 90/00292

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] A61L 33/00

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC	A61L 33/00, A61 - Keywords: Phospholipid, Lecithin, Biocompatible Non-thrombogenic, Anti-thrombogenic

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

MEDLINE, BIOSIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, [11] with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
P,Y	Biochem. Soc. Trans., Vol. 17, No. 6, published 1989, D. Chapman and P.I. Haris "Biomembrane structures. Fourier transform infrared spectroscopy and biomembrane technology", pages 951-3	(1-4)
P,Y	Prog. Clin. Biol. Res., Vol. 292, published 1989, D. Chapman et al. "Biomembranes: basic science and future technology" pages 3-12	(1-4)
Y	US,A, 4426330 (SEARS) 17 January 1984 (17.01.84) Column 3 lines 7-45 and 54-68, Column 4 lines 1-18	(1-4)
Y	US,A, 4803075 (WALLACE et al) 7 February 1989 (07.02.89) Column 2 lines 42-68, Column 3 lines 20-68, Column 4 lines 1-7	(1-4)
Y	US,A, 4725442 (HAYNES) 16 February 1988 (16.02.88) (continued)	(1-4)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 October 1990 (17.10.90)	25 October 1990
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	RON EVANS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	US,A, 4348329 (CHAPMAN) 7 September 1982 (07.09.82)	(1-4)
Y	WO,A1, 87/02684 (BIOCOMPATIBLES LTD) 7 May 1987 (07.05.87)	(1-4)

V. [ ] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.[ ] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2.[ ] Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.[ ] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1.[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 90/00292

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
US 4426330	EP 72111	AT 38039	CA 1240692
	DE 3474667	EP 118316	JP 59204198
US 4803075	AU 74671/87	EP 251695	JP 63119772
US 4725442	CA 1242645	EP 153926	JP 60501557
	WO 8500011		
US 4348329	DE 3070993	EP 32622	JP 1158013
WO 8702684	DK 3398/87	EP 247114	GB 8527071

END OF ANNEX