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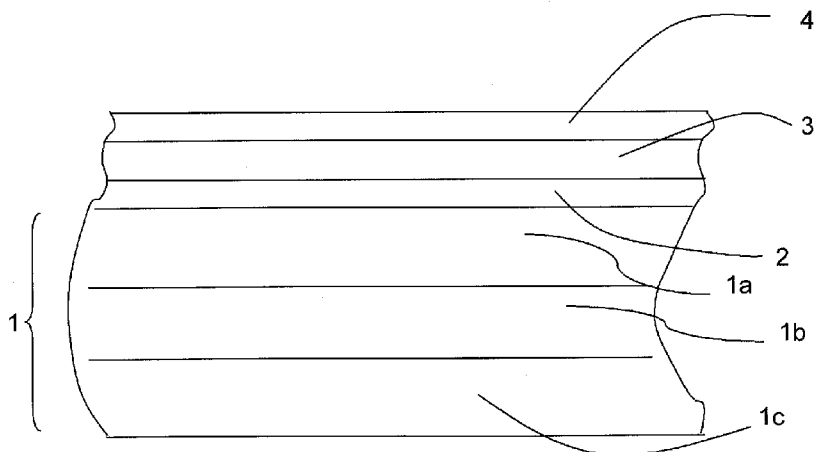
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(54) Title: TISSUE-ADHESIVE MATERIALS

Figure 1



(57) Abstract: A multi-lamellar tissue-adhesive sheet comprises a first, tissue-contacting, layer (1) of material containing tissue-reactive functional groups. A second layer (2) comprising a synthetic polymer is conjoined to the first layer (1). The second layer (2) additionally comprises one or more therapeutic agents.

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Tissue-adhesive materials

Field of the Invention

- 5 This invention relates to a flexible sheet that is suitable for use as a tissue sealant and is intended for topical application to internal and external surfaces of the body for therapeutic purposes. The invention also relates to a process for the preparation of such a sheet, and to methods of using such a sheet.
- 10 In particular, the invention relates to a self-adhesive, biocompatible and hydratable polymeric sheet that delivers therapeutic agents to the tissue surface to which it is applied, and which may be used for therapeutic purposes such as wound healing, joining, sealing and reinforcing weakened tissue. The invention also relates to a process for preparing and methods of using such a
- 15 sheet, and further, to implantable medical devices coated with similar material to that of the sheet.

Background of the Invention

- 20 There is considerable interest in the use, for a number of surgical or other therapeutic applications, of materials that adhere to biological tissues, eg as an alternative to the use of mechanical fasteners such as sutures, staples etc. Formulations of such materials that have hitherto been proposed include viscous solutions or gels that are either manufactured in that form or are
- 25 prepared immediately prior to use by mixing of the ingredients. Such formulations are then applied to the tissue surface using a suitable applicator device such as a syringe.

- Formulations of the type described above suffer from a number of
- 30 disadvantages. If the formulation is of low viscosity, it may spread from the area of application and hence be difficult to apply precisely to the desired area of tissue. If the formulation is more viscous, on the other hand, it may be

difficult to dispense. In either case, the formulation, being prepared in hydrated form, may have a limited lifetime and may be subject to premature curing. It may therefore be necessary for the whole of the formulation to be used at once or discarded. Also, the preparation of formulations immediately
5 prior to use by mixing of ingredients is obviously laborious and time-consuming, and may require the use of additional apparatus. In addition to these drawbacks, the degree of adhesion between tissue surfaces that is provided by such formulations may be less than would be desired.

10 Formulations of tissue adhesive materials have also been applied to suitable supports for application to the tissue surface. The use of therapeutic materials in the form of a sheet, patch or film, for topical administration to either internal or external organs of the body, is well documented for a wide range of medical applications. A disadvantage of products proposed hitherto,
15 however, is that the degree of adhesion to the underlying tissue, and also the cohesive strength, particularly in the longer term, may be inadequate. While the initial adhesion may be satisfactory, the sheet may subsequently become detached from the tissue, often after only a few seconds or minutes, eg as a result of hydration of the sheet following its application. In addition, the
20 flexibility of the product may be insufficient for it to conform readily to the surface to which it is applied, which may also have an adverse effect on its adhesion.

As a result of the inadequate adhesion of these products, it may be necessary
25 to provide further reinforcement, eg through mechanical attachment using sutures, staples or the like. Alternatively, energy (eg light or heat energy) may be applied in order to initiate chemical bonding of the adhesive formulation to the underlying tissue, and hence bonding of the tissue surfaces to each other. Clearly, such approaches introduce further drawbacks. The use of
30 mechanical fastenings such as sutures or staples is often the very thing that the use of such products is intended to replace or avoid. In many instances, the use of such fastenings is either not wholly effective (eg on the lung) or

undesirable, as their introduction gives rise to further areas of tissue weakness. The use of external energy requires the provision and operation of a source of such energy. Such energy sources may be expensive and difficult to operate, particularly in the confines of an operating theatre or similar environment. Also, the use of external energy for attachment can be both time-consuming and (in some cases) requires significant careful judgement on the part of the surgeon, to evaluate when sufficient energy has been delivered to effect attachment without damaging the underlying tissue.

- 5
- 10 A disadvantage of sheet-type products for applications as described above is that they may lack the degree of flexibility that may be necessary or desirable for many applications. This is particularly so for products used in the increasingly important field of endoscopic (keyhole) surgery, which may require the product to be folded or rolled into a compact configuration prior to introduction into the body. Attempts to render such products more flexible, eg by the inclusion of plasticisers, may have the effect of reducing the adhesiveness of the product.
- 15

- Our co-pending international patent application PCT/GB2007/050049
- 20 discloses tissue-adhesive sheets, and related applications of tissue-adhesive material, that overcome or substantially mitigate the above-mentioned and/or other disadvantages of the prior art.

- However, a problem associated with all wounds, which may be encountered
- 25 when using tissue-adhesive sheets of the general type described above, is the risk of infection.

- There is a current interest in the clinical use of wound dressings containing therapeutic agents, in particular anti-infective agents and agents that promote wound healing. This is principally due to advances in impregnation techniques and polymer technologies, along with the increase in prevalence of bacterial resistance to antibiotics. In particular there is renewed interest in
- 30

the use of silver, which has been used for centuries as an anti-infective agent, although the bactericidal mechanisms of silver are still not fully understood. There are now a number of silver-based dressings on the market.

- 5 It has been suggested that a wide range of therapeutic agents may be added to tissue-adhesive sheets of the type described in the prior art above. While therapeutic agents designed for action at or within a specific organ or tissue may be suitable for systemic delivery, the amount of therapeutic agent that must be delivered by this route is generally high, in order to result in the
- 10 delivery of a therapeutically effective amount at the desired site of action. Systemic delivery of high amounts of therapeutic agent increases the likelihood and severity of side effects, and is otherwise disadvantageous, eg for cost reasons. Thus, the use of tissue-adhesive sheets, solely as a means of localised delivery of a therapeutic agent, may be advantageous in many
- 15 applications.

In other applications, the delivery of a therapeutic agent would support and/or improve the use of tissue-adhesive sheets in wound healing, joining, sealing or reinforcing weakened tissue.

20

However, the incorporation of therapeutic agents can adversely affect the physical properties of the sheet, for example the flexibility and/or adhesive strength. Limiting the amount of therapeutic agent that is incorporated in order to preserve the physical properties consequently limits the duration and

25 dose of the drug that can be delivered.

30

Furthermore, it is important that the therapeutic agent is released in a controlled manner at a suitable delivery rate for a particular application, and that delivery is sustained over a period of time that is sufficient to facilitate the desired therapeutic effect. For example, if the therapeutic agent is an anti-infective silver compound, it is desirable for the silver ions to be released into the tissue slowly and continuously, in order that silver ions are always

available for bacterial uptake, and to avoid the possibility of any adverse effects.

There have now been devised further improvements to tissue-adhesive sheets of the type described above, and to related applications of tissue-adhesive material, that overcome or substantially mitigate the above-mentioned and/or other disadvantages of the prior art regarding physical attributes such as flexibility and adhesion, and which also deliver therapeutic agents to the tissue surface, for example to increase the rate of healing and to safeguard against infection.

Brief Summary of the Invention

According to a first aspect of the invention, there is provided a multi-lamellar tissue-adhesive sheet having a first tissue-contacting layer of material containing tissue-reactive functional groups, to which first layer is conjoined a second layer comprising a synthetic polymer, and wherein the second layer additionally comprises one or more therapeutic agents.

The sheet according to the invention is advantageous primarily in that it bonds effectively to tissue, enabling it to be used in a variety of medical applications, and because it provides for the localised slow release of a therapeutic agent at the site of application.

The sheet has been found to offer improved flexibility and yet to retain good adhesiveness. In preferred embodiments, the sheet exhibits good initial adhesion to the tissue to which it is applied (and may thus be described as "self-adhesive"), and furthermore remains well-adhered to the tissue over a longer timescale. Without wishing to be bound by any theory, it is believed that the initial adhesion of the sheet to the tissue is attributable to electronic bonding of the sheet to the tissue, and this is supplemented or replaced by covalent chemical bonding between the tissue-reactive functional groups of

the formulation and the tissue, in particular between amine and/or thiol groups on the tissue surface and the tissue-reactive functional groups of the sheet.

Because the therapeutic agent is located within the second layer of the sheet,
5 it may exhibit more prolonged release from the sheet than if it were contained exclusively within the tissue-contacting layer. The therapeutic agent located within the second layer will generally also not interfere with reaction of the tissue-contacting layer with the underlying tissue, and elution of the therapeutic agent will generally not occur to a substantial degree until the
10 sheet is securely adhered to the tissue.

Initial adhesion of the sheet to the tissue surface is believed to be due to Van der Waals forces and/or hydrogen bonding between the sheet and the tissue surface. On contact with the tissue surface the sheet becomes hydrated,
15 thereby causing reaction between the tissue-reactive functional groups and the underlying tissue surface. Such reactions between the tissue-reactive functional groups and the underlying tissue result in high adhesion between the sheet and the tissue surface. The sheet may absorb physiological fluids (as a consequence of application onto exuding tissue surfaces), and any
20 additional solutions used to hydrate the sheet following application (such fluids can be commonly used solutions used in surgical irrigation), becoming more compliant and adherent to the tissue surfaces, and thereby providing an adhesive sealant with haemostatic (by physical means) and pneumostatic functions.

25 The use of the sheet reduces or eliminates the need for additional means of mechanical attachment to the tissue (eg sutures or staples), or the need to provide external energy in the form of heat or light to bring about adherence of the sheet to the underlying tissue. Another advantage of the sheet according
30 to the invention is that it is applied to the tissue as a preformed article, rather than being prepared by mixing of materials immediately prior to use.

In addition, because the sheet is, until hydrated upon and following contact with the tissue surface, essentially inactive, the sheet is not prone to premature reaction and as a result its shelf-life may be considerable, eg more than six months when stored appropriately at room temperature.

5

By the term "sheet" is meant an article with a thickness that is considerably less than its other dimensions. Such an article may alternatively be described as a patch or a film.

- 10 The lamellar structure facilitates the slow, controlled and sustained release of the therapeutic agent. In general, sheets comprising a multi-lamellar structure have been found to perform better, in terms of adhesion to tissue and/or elasticity and/or maintenance of structural integrity, than sheets with a single structural layer.

15

In preferred embodiments of the invention, the sheet has a third layer conjoined to the second layer, which third layer comprises a tissue-reactive material containing tissue-reactive functional groups, and a fourth layer conjoined to the third layer, which fourth layer comprises a synthetic polymer.

20

The tissue-reactive material of the third layer may be the same as, or similar to, the material of the tissue-contacting first layer. Alternatively, the materials of the first and third layers may be different.

- 25 In general, the sheet according to the invention may comprise alternating layers of material containing tissue-reactive functional groups (eg in the first layer) and layers of synthetic polymer (eg in the second layer). In such cases, the sheet according to the invention therefore typically comprises an even number of layers, with alternating layers of the two types of material. Thus,
- 30 the sheet may be considered to comprise a structural laminate comprising n layers of material containing tissue-reactive functional groups and n layers of synthetic polymer. The individual layers of material containing tissue-reactive

functional groups may all comprise the same material, but do not necessarily do so. Likewise, the individual layers of synthetic polymer may all comprise the same material, but do not necessarily do so.

- 5 The value of $\underline{n}$ may be from 1 (in which case the sheet comprises just two layers) to about 5 or more. Most preferably, $\underline{n}$ has the value 1, 2, 3, 4 or 5. Sheets containing more than two layers have been found to perform better, in terms of adhesion to tissue and/or elasticity and/or maintenance of structural integrity. Thus, in currently preferred embodiments of the sheet according to
10 the invention, $\underline{n}$ is 2 or 3.

By layers that are “conjoined” are meant layers that are bonded together in such a way that the layers remain joined together throughout normal usage of the sheet. More specifically, “conjoined” may involve a certain amount of
15 intermingling of the materials of adjacent layers at the interface between them.

Where the sheet comprises more than one layer of synthetic polymer (eg in the cases described above where the sheet comprises $2\underline{n}$ layers, and $\underline{n}$ is greater than 1), the anti-infective agent may be present in one or more of
20 those layers, eg in all of them.

The layer of synthetic polymer that is furthest from the face of the sheet that, in use, is applied to tissue acts as a barrier layer. In preferred embodiments that layer of synthetic polymer is relatively hydrophobic and the first tissue-
25 contacting layer relatively hydrophilic, so that the anti-infective agent preferentially diffuses to the tissue surface. In such an arrangement, the barrier layer resists diffusion of water and thus resists diffusion of the therapeutic agent. As the therapeutic agent will generally require a certain degree of water solubility to allow it to migrate out of the patch, the main route
30 of diffusion for the therapeutic agent will be via the tissue-contacting layer, ie towards the tissue surface.

In certain embodiments of the invention, the therapeutic agent is an anti-infective agent, which is preferably a compound containing silver atoms or ions, and most preferably a silver salt, eg silver carbonate.

- 5 In another aspect of the invention, there is provided a device suitable for implantation in the human or animal body, which device carries on at least part of the external surface thereof a coating comprising a synthetic polymer and one or more therapeutic agents, at least part of said coating being conjoined to a layer of material comprising tissue-reactive functional groups.

10

- In this aspect of the invention, the coating of synthetic polymer provides a means for attachment to the device of the material comprising tissue-reactive functional groups, the latter material providing a means for anchoring the device in its desired position within the body. This aspect of the invention may therefore be of particular utility in relation to implantable devices that would otherwise be difficult to fix in position within the body, for instance because they are made of a material that is chemically inert and not amenable to reaction with the surrounding tissue or with chemical linking groups. The incorporation of the therapeutic agent in the synthetic polymer coating provides the benefits of a localised therapeutic effect.

20

- In the following detailed description of the invention, reference is made primarily to embodiments of the invention that have the form of sheets. It will be appreciated, however, that analogous comments apply, where appropriate, to embodiments of the invention involving coatings on implantable devices.

25

- In another aspect, the invention also provides a method of joining a tissue surface to another tissue, or of sealing a tissue surface, which method comprises applying to the tissue surface a sheet according to the first aspect of the invention.

30

Detailed Description of the Invention*Abbreviations*

5	AAc	acrylic acid
	AIBN	azo-iso-butyronitrile
	DCC	dicyclohexylcarbodiimide
	DCM	dichloromethane
	DCU	dicyclohexylurea
10	DMF	dimethylformamide
	IPA	<i>iso</i> -propanol
	M_n	number average molecular weight
	M_w	weight average molecular weight
	MeOH	methanol
15	NHS	N-hydroxysuccinimide
	NVP	N-vinyl pyrrolidone
	PLC	poly(lactide-co-caprolactone)
	PLGA	poly(DL-lactide-co-glycolide)
	poly(VP-AAc)	copolymer of vinyl pyrrolidone and acrylic acid
20	poly(VP-AAc(NHS))	copolymer of vinyl pyrrolidone and acrylic acid NHS ester
	poly(VP-AAc-AAc(NHS))	terpolymer of vinyl pyrrolidone, acrylic acid and acrylic acid NHS ester

25 *Nature of the tissue-contacting layer*

The sheet according to the first aspect of the invention has a first tissue-contacting layer that comprises a material containing tissue-reactive functional groups. That material preferably comprises one or more polymers containing

30 tissue-reactive functional groups.

- By "tissue-reactive functional groups" is meant functional groups capable of reacting with other functional groups present in the tissue surface so as to form covalent bonds with the tissue. Tissues generally consist partly of proteins, which commonly contain thiol and primary amine moieties. Many
- 5 functional groups such as imido ester, p-nitrophenyl carbonate, NHS ester, epoxide, isocyanate, acrylate, vinyl sulfone, orthopyridyl-disulfide, maleimide, aldehyde, iodoacetamide, and others, may react with thiols or primary amines, and therefore constitute "tissue-reactive functional groups". As used herein, the term NHS or NHS ester is intended to encompass not only N-
- 10 hydroxysuccinimide itself, but also derivatives thereof in which the succinimidyl ring is substituted. An example of such a derivative is N-hydroxysulfosuccinimidyl and salts thereof, particularly the sodium salt, which may increase the solubility of the tissue-reactive material.
- 15 Tissue-reactive functional groups that may be of utility in the present invention are any functional groups capable of reaction (under the conditions prevalent when the formulation is applied to tissue, ie in an aqueous environment and without the application of significant amounts of heat or other external energy) with functional groups present at the surface of the tissue. The latter class of
- 20 functional group includes thiol and amine groups, and tissue-reactive functional groups therefore include groups reactive to thiol and/or amine groups. Examples are:
- imido ester;
 - p-nitrophenyl carbonate;
 - 25 NHS ester;
 - epoxide;
 - isocyanate;
 - acrylate;
 - vinyl sulfone;
 - 30 orthopyridyl-disulfide;
 - maleimide;
 - aldehyde; and

iodoacetamide.

NHS ester is a particularly preferred tissue-reactive functional group.

- 5 In addition to tissue-reactive functional groups, the polymer(s) that make up the material of the first layer may contain functional groups that, whilst not themselves being reactive to the tissue to which the sheet is applied, do provide good contact adhesion between the sheet and the tissue. Such functional groups are referred to herein as "non-reactive functional groups".
- 10 Examples of non-reactive functional groups include hydroxyl, heterocyclic amines or amides (eg in vinyl pyrrolidone residues) and, particularly, carboxyl groups (eg in acrylic acid residues).

- It is particularly preferred that the tissue-reactive functional groups are
- 15 activated derivatives of non-reactive functional groups. In certain embodiments, all of the non-reactive functional groups may be activated to form tissue-reactive functional groups. In other embodiments, only some of the non-reactive functional groups may be activated to form tissue-reactive functional groups. In the latter case, the strength of initial contact adhesion of
- 20 the sheet to the tissue to which it is applied, and the strength of the longer term adhesion brought about by covalent reaction of the tissue-reactive functional groups with functional groups in the tissue may be varied and controlled by varying the proportion of the non-reactive groups that are in activated form.

- 25 NHS ester is a particularly preferred tissue-reactive functional group, and therefore preferred tissue-reactive polymers are NHS ester-rich polymers. Particularly preferred tissue-reactive polymers are poly(VP-AAc(NHS)) and poly(VP-AAc-AAc(NHS)) terpolymer.

- 30 Sufficiency of the degree of initial adhesion of a sheet to the tissue, by the bioadhesive polymer(s), can be quantitatively determined *in vitro*, for example

- by performing an adhesion strength test. This test is performed by allowing the sheet to adhere to a suitable substrate (secured in a fixed position), while the sheet itself is physically attached at a separate point to the load of a tensile testing apparatus, positioned so that, prior to the test, the sheet is not under load. The load cell is moveable along an axis substantially perpendicular to that along which the substrate is positioned. The test involves movement of the load cell away from the substrate, at a constant predetermined rate, until the sheet detaches from the substrate. The output of the test is a quantitative measure of the energy of adhesion for that sheet – ie the cumulative amount of energy required to break the interaction between the sheet and the substrate to which it is adhered. A suitable cumulative energy of adhesion for the sheet according to the invention would be not less than 0.5mJ.
- 15 In certain embodiments of the invention, a preferred tissue-reactive polymer is poly(VP-AAc-AAc(NHS)) terpolymer. The carboxyl groups on poly(VP-AAc) may be converted to NHS esters by reaction with NHS in the presence of DCC (see Example 2). If the acid content of the poly(VP-AAc) is determined (in moles), the proportion of acid groups converted to tissue-reactive groups may be controlled by adding the desired mole percent of NHS. As used herein, the notation $\text{poly}(\text{VP}_x\text{-AAc}_y\text{-AAc(NHS)}_z)$ indicates the molar percentages of the three different monomer residues or derivatives present in the terpolymer. For instance, $\text{poly}(\text{VP}_{50}\text{-AAc}_{25}\text{-AAc(NHS)}_{25})$ represents a terpolymer containing 50% vinyl pyrrolidone residues, 25% acrylic acid residues, and 25% NHS-activated acrylic acid residues.

The tissue-reactive polymer most preferably has a weight average molecular weight M_w that is greater than 100,000, more preferably greater than 120,000, 140,000, 160,000, 180,000 or 200,000. The M_w of the tissue-reactive polymer may be greater than 300,000, or greater than 400,000. The M_w may be less than 500,000, but may be greater. Tissue-reactive polymers with relatively high weight average molecular weight M_w , ie M_w greater than 100,000, more

preferably greater than 120,000, 140,000, 160,000, 180,000 or 200,000, are found to confer particularly useful properties on the sheet according to the invention.

- 5 The properties of the tissue-adhesive sheet may be optimised by inclusion of other polymers and additives.

Nature of therapeutic agents

- 10 Any of a variety of therapeutic agents can be delivered by the tissue-adhesive sheets and methods of the present invention. By "therapeutic agent" is meant any pharmaceutically active substance or its prodrug, or a salt or solvate of a pharmaceutically active substance.
- 15 By "prodrug" is meant any structural derivative of a therapeutic agent which is chemically transformed within the body to exert its pharmacological or therapeutic action. For example, an ester of a therapeutic compound containing a hydroxy group may be convertible by hydrolysis *in vivo* to the parent molecule. Alternatively, an ester of a therapeutic compound containing
- 20 a carboxy group may be convertible by hydrolysis *in vivo* to the parent molecule.

- Therapeutic agents provided here are exemplary, and are not meant to be limiting in any way, and include analgesics (eg endorphins), antimicrobials (eg
- 25 gentamicin, bacitracin, aciclovir), antineoplastics (eg doxorubicin, bleomycin), anti-inflammatory agents (eg celecoxib), angiogenic agents (eg vascular epithelial growth factor, fibroblast growth factor), anti-angiogenic agents (eg endostatin), growth promoters (eg vascular epithelial growth factor, fibroblast growth factor) and therapeutic monoclonal antibodies (eg basiliximab,
- 30 trastuzumab).

The local, sustained delivery of anti-cancer agents is of particular interest, eg for residual/non-resectable colorectal and pancreatic cancers. Exemplary anti-cancer agents include doxorubicin.

- 5 The incorporation of therapeutic agents to improve and/or increase the rate of wound healing and reduce infections, for both topical and internal applications, is also of particular interest.

Preferred therapeutic agents for use in the present invention include, but are
10 not limited to, anti-infective agents.

By “anti-infective agent” is meant any agent that is capable of acting against infections, by killing infective micro-organisms and/or inhibiting the spread of an infective micro-organism. Infective micro-organisms include bacteria,
15 parasites, yeast, moulds, fungi, viruses, prions and viroids.

Anti-infective agents suitable for use in the sheets of the present invention may be drugs, such as antibiotics or antifungals.

- 20 Examples of antimicrobial or antibacterial compounds that may be incorporated into the sheets of the invention are triclosan, neomycin, clindamycin, polymyxin, bacitracin, benzoyl peroxide, tetracyclines such as doxycycline or minocycline, sulfa drugs such as sulfacetamide, penicillins, cephalosporins such as cephalexin, and quinolones such as lomefloxacin,
25 olfoxacin or trovafloxacin.

Antiviral compounds that may be incorporated include acyclovir, tamvir, and penciclovir.

- 30 Antifungal compounds include farnesol, clotrimazole, ketoconazole, econazole, fluconazole, calcium or zinc undecylenate, undecylenic acid,

butenafine hydrochloride, ciclopirox olamine, miconazole nitrate, nystatin, sulconazole, and terbinafine hydrochloride.

However, due to increasing concerns about antibiotic and antifungal
5 drug-resistance, the use of alternative anti-infective agents may be preferred.

Preferably the anti-infective agent comprises a metal with antimicrobial properties. Thus, the anti-infective agent may comprise a finely divided metal, or a compound containing metal ions.

10

Metal ions that have infective properties include silver, gold and copper.

Preferably, the anti-infective agents utilised in the present invention are compounds containing silver, gold or copper ions, most preferably silver ions.

15

Silver ions safely kill infective micro-organisms such as bacteria, yeasts, moulds and fungi. The incorporation of compounds containing silver ions in the tissue-adhesive sheets of the present invention may be used to treat existing infection. However, more commonly the incorporation of silver ions
20 may be used as a preventative or precautionary measure, in particular to safeguard against the occurrence of post-operative infections caused by adventitious micro-organisms at the site of application.

25

The efficacy of tissue-adhesive sheets of the present invention in which an anti-infective agent is incorporated in the second layer can be assessed in vitro by performing agar diffusion assays. The test is performed by adding the sheet to the surface of a suitable agar plate inoculated with the micro-organism of choice, prior to submersion and also following submersion in DPBS for a set period of time. The output of the test is a zone of inhibition
30 around the surface of the sheet, indicating growth inhibition of the micro-organism due to the presence of the anti-infective agent.

In the most preferred embodiments, the anti-infective is a silver salt, eg silver carbonate. Other silver compounds that may be used include silver nitrate, silver acetate, silver sulfadiazine and silver oxide.

- 5 The silver salt may be present at low concentrations levels in the range of about 0.001% to 10% by weight of the tissue-adhesive sheet, preferably about 0.01% to 5% by weight of the tissue-adhesive sheet, more preferably about 0.1% to 2% by weight of the tissue-adhesive sheet, and most preferably at about 1% by weight of the tissue-adhesive sheet.

10

The silver salt may be present in the range of about 0.01% to 90% by weight of the second layer, preferably about 0.1% to 50% by weight of the second layer, more preferably about 1% to 20% by weight of the second layer, and most preferably at about 10% by weight of the second layer of the tissue-adhesive sheet.

15

In the currently most preferred embodiments of the sheet, the first tissue-contacting layer containing tissue-reactive functional groups and other layer(s), if any, that contain a tissue-reactive material containing tissue-functional groups all comprise poly(VP-AAc-AAc(NHS)) terpolymer. The second layer, of synthetic polymer, comprises polycaprolactone and silver carbonate, and the any other layer(s) of synthetic polymer comprise polycaprolactone, optionally also containing silver carbonate.

20

- 25 The controlled and sustained release of anti-infective silver ions at the site of application is thought to be achieved because the silver carbonate entrapped in the second layer is slowly dissolved by the ingress of water as the patch itself hydrates and, over time, degrades. Silver carbonate has limited water solubility (0.033 g/L), therefore its rate of release is not immediate, however
- 30 as the material dissolves the silver ions diffuse out of the patch and further solvation occurs.

The silver ions preferentially diffuse to the tissue surface because the barrier layer, eg of polycaprolactone, is relatively hydrophobic and the poly(VP-AAc-AAc(NHS)) terpolymer is relatively hydrophilic.

- 5 Analogous mechanisms may be applicable to other embodiments of the present invention. Clearly, the rate of release of the therapeutic agent can be controlled by appropriate design of the sheet.

10 The barrier layer of synthetic polymer may also include one or more therapeutic agents.

Nature of synthetic polymer

15 The sheet according to the first aspect of the invention includes a second layer comprising a synthetic polymer. The second layer may consist entirely or substantially entirely of synthetic polymer and therapeutic agent.

20 A variety of suitable synthetic polymers may be used to form the second layer, provided that they exhibit suitable properties together with suitability for medical applications, in particular absence of toxicity, biocompatibility and, usually, biodegradability.

25 Most commonly, the second layer comprises just one synthetic polymer. Alternatively, the second layer may be formed from more than one synthetic polymer.

30 The therapeutic agent must be able to diffuse out of the second layer. It is preferred that the synthetic polymer is permeable to the therapeutic agent when the sheet is hydrated. Alternatively, the release of therapeutic agent may depend entirely on degradation of the sheet over time.

- It is also preferred that there is no significant binding between the therapeutic agent and the synthetic polymer. For example, synthetic polymers containing tissue-functional groups, which are suitable for the first tissue-contacting layer, are not likely to be suitable for the second layer. The reactivity of the tissue-
- 5 reactive groups is likely to result in chemical bonding between the therapeutic agent and the polymer, such that the therapeutic agent would be bound and entrapped in the second layer during manufacture, and could not be sufficiently dissolved upon hydration of the sheet to diffuse out of the sheet. Similarly, if a therapeutic agent were included in the first tissue-contacting
- 10 layer, a) the agent is likely to be retained in the material containing tissue-reactive functional groups, and b) the adhesive strength of the sheet is likely to be impaired, because some (or all) of the tissue-reactive groups are taken up with bonding to the therapeutic agent.
- 15 It is of note that the issue of tissue-reactive groups bonding with the therapeutic agent is relevant principally to the manufacture of the tissue-adhesive sheet. When sheets of the present invention are hydrated and the therapeutic agent is dissolved, it diffuses out of, or otherwise permeates, the second layer and the first layer of material containing tissue-reactive
- 20 functional groups to be delivered to the tissue surface, without significant reaction with the tissue-reactive groups in the first layer.

Thus, preferred synthetic polymers for use in the invention are generally inert, in that they do not form chemical bonds with the therapeutic agent during

25 manufacture.

Indeed, a particular advantage of the tissue-adhesive sheets of the present invention is that therapeutic agents having broader range of chemistries may be incorporated, compared to the range of therapeutic agents that may be

30 suitable for use in other tissue-adhesive sheets described in the prior art. In particular, the sheets of the present invention allow delivery of therapeutic

agents having cross-linkable groups, which agents would otherwise become entrapped by cross-linking to tissue-adhesive materials.

5 A particularly preferred group of synthetic polymers that may be suitable for use in the invention are biodegradable polyesters. Specific examples of such polymers are polylactic acid and polyglycolic acid, and copolymers and blends thereof. Other examples include polycaprolactones and polyhydroxyalkanoates, such as polyhydroxybutyrate, polyhydroxyvalerate and polyhydroxyhexanoate.

10

The currently most preferred polyester polymers for use in the invention are polycaprolactone and poly(lactide-co-glycolide) [also referred to as poly(lactic-co-glycolic acid)] copolymers, which are generally biodegradable and biocompatible, and are soluble in a wide range of organic solvents.

15

The polycaprolactone that is used may be polycaprolactone homopolymer, or may be a copolymer of caprolactone with lactic acid and/or glycolic acid. The presently most preferred polycaprolactone for use in the invention is PLC. A suitable form of PLC for use in the invention is available from Purac BV, and
20 comprises 70 mol% lactide and 30 mol% caprolactone, with an inherent viscosity of 1.2-1.8 dL/g (chloroform, 25°C, 0.1 g/dL) and a glass transition point (T_g) of 20°C.

In the currently most preferred embodiments of the sheet, particularly
25 preferred embodiments are those that comprise alternating layers of biodegradable polyester material and material containing reactive functional groups, wherein at least one layer of biodegradable polyester additionally comprises one or more anti-infective agents.

30 In such embodiments, the layer that is furthest from the surface of the sheet that, in use, is applied to the tissue is preferably substantially non-adhesive to tissue. Such sheets will therefore generally adhere only to the target tissue

(to which the tissue-contacting layer, containing tissue-reactive functional groups, is applied), and not to surrounding tissues (eg the pleural or peritoneal wall), in the manner of a "patch".

- 5 Other examples of synthetic polymers that may be suitable are aminated polymers such as aminated PEGs (including those sold under the trade name JEFFAMINE) and polyallylamines.

Property Enhancing Additives

10

Although in general the sheet according to the first aspect of the invention has adequate flexibility, it may nonetheless be desirable to further improve the flexibility, elasticity and/or wet-strength of the sheet by the addition of one or more plasticizers and elastomers to the structural layer or laminate and/or the
15 tissue-contacting layer. In particular, low molecular weight species such as glycerol and low molecular weight PEG may be incorporated into the formulations to increase flexibility. Examples of suitable elastomers that may be incorporated into the product include poly(caprolactones), poly(urethanes) and poly(silicones).

20

Such materials may increase the flexibility and/or elasticity of the sheet when added at levels of up to 30% by weight of the ingredients that make up the sheet.

- 25 However, the inclusion of high levels of such materials may have a detrimental effect on the adhesive performance of the sheet. To offset this disadvantage, additives may be functionalised to include tissue-reactive functional groups that may participate in tissue-adhesion.

Buffers

The reaction between tissue-reactive functional groups on the sheet and functional groups on the surface of the tissue may vary with pH. It may
5 therefore be preferable to buffer the tissue surface immediately prior to application or, more preferably, to include a buffer in the formulation used to prepare the sheet, in particular the tissue-contacting layer of the sheet. The mean work of adhesion of certain sheets according to the invention to explanted porcine liver may be improved by buffering the tissue surface with
10 pH 10.5 phosphate/carbonate buffer.

Conjoining of components of the sheet during manufacture

The layers of the tissue-adhesive sheet according to the present invention
15 may be conjoined during the process of manufacture. Such conjoining may increase the physical strength of the sheet and may optimise the properties of the sheet, in particular in terms of the time required for biodegradation of the sheet after it has been applied.

20 Conjoining may be brought about by various means, including casting of component layers from common solvents.

Physical form of the sheet

25 The sheet may typically have an overall thickness of from 0.01 to 1mm, typically 0.01 to 0.5mm, and more commonly 0.015 to 0.2 mm or 0.015 to 0.1mm, eg 0.015 to 0.05mm.

In currently preferred embodiments, the thickness of the tissue-contacting
30 layer may be such that it has a thickness that accounts for more than 20% of the overall thickness of the sheet, or more than 30%.

The sheet may be produced with, or subsequently cut to, dimensions of from a few square millimetres up to several tens of square centimetres.

Manufacture of the sheet

5

Most conveniently, the sheet according to the invention may be prepared by stepwise formation of the individual layers that make up the sheet.

10

The layer comprising a synthetic polymer that is distal to the tissue-contacting layer may be prepared first, eg by casting a solution of the material that makes up that layer in a suitable solvent, either on a suitable plate or mould or onto a suitable release paper, eg a silicone-coated release paper. The cast solution is then dried or allowed to dry, optionally under conditions of elevated temperature and/or reduced pressure.

15

In embodiments that comprise only the tissue-contacting first layer and the second layer, the second layer is thus formed first, followed by the tissue-contacting first layer.

20

In the presently preferred multi-layer embodiments, the barrier layer (ie the layer that is furthest from the surface that is applied, in use, to the tissue) is formed first, followed successively by the other layers, in each case with drying to remove solvent, preferably under reduced pressure.

25

Finally, the (first) tissue contacting layer may be cast onto the (second) layer that comprises a synthetic polymer and one or more therapeutic agents. Once again, this may be followed by drying to remove solvent, preferably under reduced pressure.

30

Each layer of the sheet may be cast in a single operation. Alternatively, particularly for layers that are relatively thick (ie that are thick relative to other

layers of the sheet), a layer may be built up by successive casting of thinner sub-layers.

5 The one or more anti-infective agents are most conveniently incorporated into the second layer by inclusion of the anti-infective agent in the solution of synthetic polymer from which the second layer is cast.

10 Once manufactured, and prior to use, the sheet according to the invention will typically have a water content of less than 10% w/w, and more commonly less than 5% w/w.

During manufacture an image or alphanumeric marking may be printed onto the surface of the individual layers of the sheet. This marking may be used to distinguish the tissue adhesive surface from the non adhesive surface,
15 alternatively it may be used to denote the identity of the product or manufacturer. A chromophore that may be used as a marking agent includes methylene blue. For example, in four-layer embodiments a layer of methylene blue may be printed or otherwise applied after formation of the third layer and before second layer is formed, thus providing an image or other marking
20 between the second and third layers.

Typically, implantable devices according to the invention may be prepared by any convenient method of applying the coating to the device. For example, the layers of the coating may be cast on the device in an analogous manner to
25 the way in which the materials of the layers are cast, as described above. Alternatively, the coating may be applied by dipping of a device in liquid formulations or by spraying the device with liquid formulations.

Therapeutic applications of the sheet

30

The sheet according to the invention is suitable for application to both internal and external surfaces of the body, ie it may be applied topically to the exterior

of the body (eg to the skin) or to internal surfaces such as surfaces of internal organs exposed during surgical procedures, including conventional and minimally invasive surgery.

- 5 The sheet according to the invention is particularly suitable for surgical applications in the following areas:

Thoracic / cardiovascular

General surgery

- 10 ENT

Urology

Oral / maxillofacial

Orthopaedic

Neurological

- 15 Gastroenterology

Ophthalmology

Gynaecology / obstetrics

- 20 In each case the sheet provides a localised anti-infective effect by the slow, controlled release of an anti-infective agent at the site of application. Possible uses are described in more detail below.

Wound healing

- 25 The degradable nature of the sheet and localised anti-infective effect mean that it may support and promote wound healing during both internal and topical procedures. Once the sheet begins to degrade, fibroblasts will move in and begin to deposit components of the extracellular matrix. The sheet can therefore be used as an internal or external dressing. In addition to the one or more anti-infective agents, factors such as growth factors and cAMP
- 30 that are known to promote the proliferation of skin cells may be added to the formulation to assist in the healing process. A sheet may be designed to

control the transmission of moisture and this, in combination with the localised anti-infective effect, may be particularly useful in the treatment of burns.

Skin closure

- 5 The sheet may be applied topically to promote wound closure (as an alternative to sutures). This may have beneficial effects in that it may reduce scarring, and the formulation and sheet may thus be useful for cosmetic purposes during minor surgery (eg in Accident & Emergency Departments). Self-adhesive properties of the sheet may make it easy to apply quickly.

10

Hernia repair

The sheet may be used to provide reinforcement in hernia repair procedures. The self-adhesive attachment overcomes the potential issues faced by conventional surgical reinforcing mesh products, which require suturing or

15 stapling in an already weakened area. The sheet for such a procedure may be engineered to have short or long term durability, depending on the degree of tissue repair required. The sheet may also be able to withstand the application of staples.

- 20 The invention may also find application in the provision of an adhesive coating to hernia mesh devices, which coating also provides a localised anti-infective effect.

Anastomosis

- 25 The sheet provides a means for rapid sealing of, and prevention of leaks in, joined tubular structures such as blood vessels, and vascular and bladder grafts, and the GI tract. The ability of the sheet to support tissue repair may be of particular value if used in nerve repair.

Sealing large areas of tissue

30 The good sealing and handling properties of the sheet, combined with its self-adhesive properties and ability to cover a large surface area, mean that it may

be of particular use in sealing resected tissue surfaces – in particular those where diffuse bleeding is an issue (eg the liver). The sheet also provides an ideal support matrix for tissue repair at such sites. This could also be applicable to limiting leakage of cerebro-spinal fluid following neurological surgery.

Sealing air leaks

In addition to the patch properties described above, the high tensile strength and good inherent elasticity of the sheet (after hydration and reaction of the tissue-reactive functional groups), make it particularly suitable for sealing air leaks in the lung, particularly following lung resection. Again, after effecting a seal, the sheet provides an ideal support matrix for tissue repair at such sites.

Sealing cerebrospinal fluid (CSF) leaks

The patch properties described above make it suitable for preventing leakage of CSF. Current methods such as fibrin sealants, haemostatic agents, and other preparations are not specifically approved for dural sealing. After effecting a seal, the sheet provides an ideal support matrix for tissue repair at such sites.

Haemostasis

The sheet may be applied to a bleeding area, acting as a physical barrier. The tissue-reactive material in the sheet may immobilise proteins and thereby promote haemostasis.

Prevention of post-surgical adhesions

Post-surgical adhesion, the formation of undesired connective tissue between adjacent tissues, is a serious problem which can give rise to major post-surgical complications. It is a particular problem in bowel surgery where it can cause, for instance, twisting of the bowel, which may then necessitate further surgical intervention. The application of sheet material in accordance with the invention to tissues exposed in a surgical procedure can be effective in

preventing post-surgical adhesions between that tissue and neighbouring tissues.

*Minimally invasive procedures (including natural orifice transluminal
5 endoscopic surgery (NOTES)*

The use of minimally invasive techniques for taking tissue samples by biopsy, inserting devices, delivery of therapeutic agents and performing surgical procedures is rapidly developing as an alternative choice to traditional “open” surgery. Minimally invasive procedures typically result in less pain, scarring,
10 quicker recovery time and fewer post-operative complications for patients, as well as a reduction in health care costs. Procedures are undertaken using specially designed instruments which are inserted through small keyhole-sized surgical incisions or natural body orifices such as mouth, urethra, anus, vagina etc. The sheet may be introduced into the body via existing and
15 specially designed minimally invasive surgery instruments and trocar systems, and the sheet may be shaped or prepared to an appropriate size and configuration, including buttresses for use with stapling devices. The flexibility of the sheet enables it to be formed into a configuration of reduced size, for instance by folding or rolling the sheet, and hence facilitates the use of the
20 sheet in minimally invasive surgery procedures and/or other procedures where access is restricted. The good initial contact adhesion of the sheet to the tissue to which it is applied may also be particularly useful in such procedures.

25 Brief Description of Drawings

Figure 1 shows schematically, and not to scale, the structure of a first embodiment of a tissue-adhesive sheet according to the invention.

30 Figure 2 illustrates the synthesis of poly(VP₅₀-AAc₂₅-AAc(NHS)₂₅).

Figure 3 shows the zone of inhibition measured in agar diffusion tests.

Figure 4 is a plot showing the anti-infective effect as a function of time and loading of silver carbonate.

- 5 Figure 5 is a view similar to Figure 1, of a second embodiment of a tissue-adhesive sheet according to the invention.

Detailed Description of Preferred Embodiments

- 10 The invention will now be described in greater detail, by way of illustration only, with reference to the following Examples.

Example 1 describes the manufacture of a first embodiment of a tissue-adhesive sheet according to the invention. Example 2 describes the synthesis
15 of material containing tissue-reactive groups that is used in the sheets of Example 1. Example 3 describes the agar diffusion testing against *S. aureus* ATCC for tissue-adhesive sheets manufactured according to Example 1. Example 4 gives the results of agar diffusion testing against a range of other micro-organisms. Example 5 describes the manufacture of a second
20 embodiment of a tissue-adhesive sheet according to the invention.

Example 1

Preparation of multi-lamellar sheet

- 25 A first embodiment of a multi-lamellar tissue-adhesive sheet according to the invention is shown schematically in Figure 1. The sheet comprises four layers:
- a) a first, tissue-contacting layer 1 of poly(VP-AAc-AAc(NHS));
 - b) a second layer 2 of PLGA that also incorporates silver carbonate;
 - 30 c) a third layer 3 of poly(VP-AAc-AAc(NHS)); and
 - d) a fourth (barrier) layer 4 of PLGA.

The second and fourth layers 2,4, which comprise PLGA, each have a thickness of approximately $4\mu\text{m}$, and the intervening third layer 3 has a thickness of approximately $3\mu\text{m}$. The tissue-contacting first layer 1 has a thickness of approximately $22.5\mu\text{m}$, and consists of three sub-layers 1a-c of approximately equal thickness. A logo or other pattern is printed, using a solution of methylene blue, on the surface of the third layer 3 that is conjoined to the second layer 2.

The sheet is prepared as follows:

10

1.1 Preparation of solutions

Four solutions are prepared as follows:

Solution A is 10g PLGA dissolved in 100ml DCM.

15 Solution B is 7.5g poly(VP-AAc-AAc(NHS)) dissolved in 100ml DCM/MeOH 15/4.

Solution C is 2.5g methylene blue dissolved in 50ml water.

Solution D comprises 10g PLGA and 1g silver carbonate in 100ml DCM.

20 The layers are cast in reverse order, as follows:

1.2 Casting of layer 4

Solution A is cast onto silicone backed release paper using a device referred to as a K bar. The film is dried for 30 minutes at 20°C /atmospheric pressure.

25 The film is not removed from the release paper.

1.3 Casting of layer 3

Solution B is cast onto Layer 1 using a K bar. The film is dried for 30 minutes at 20°C /atmospheric pressure. The film is not removed from the release

30 paper.

1.4 Application of logo

Solution C is printed onto the surface of Layer 3 to form a trade/visualisation logo (not visible in Figure 1).

5 1.5 Casting of layer 2

Solution D is cast onto Layer 3 using a K bar. The film is dried for 30 minutes at 20°C/atmospheric pressure. The film is not removed from the release paper.

10 1.6 Casting of layers 1a-c

Solution B is cast onto Layer 3 using a K bar to form layer 1a. The film is dried for 30 minutes at 20°C/atmospheric pressure. The film is not removed from the release paper.

15 Solution B is cast onto Layer 1a using a K bar to form layer 1b. The film is dried for 30 minutes at 20°C/atmospheric pressure. The film is not removed from the release paper.

20 Solution B is cast onto Layer 1b using a K bar to form layer 1c. The film is dried for 16 hours at 20°C/reduced pressure. The film is not removed from the release paper.

1.7 Cutting out

25 The product is cut to size using specially designed cutters and peeled away from the release paper.

1.8 Final drying

The product is dried for 24 hours at 20°C/reduced pressure

Example 2

Synthesis of poly(VP₅₀-AAc₂₅-AAc(NHS)₂₅) terpolymer

The reaction is shown schematically in Figure 2.

5

2000ml of deoxygenated DMSO is heated to 80°C. 121.3g (1.09 moles) of NVP and 78.7g (1.09 moles) of AAc are added to the DMSO followed by 0.04g (2.44×10^{-4} moles) of AIBN. The reaction is left at 80°C for 17-19 hours and then allowed to cool to room temperature. 125.6g (1.09 moles) of NHS is dissolved in the polymer solution followed by the addition of 112.6g (0.545 moles) of DCC dissolved in 225ml of DMF. The reaction is left stirring at room temperature for 96 hours. The reaction by-product, DCU, is removed by filtration under reduced pressure using a sintered glass filter. The polymer is isolated by mixing with 2000ml of IPA followed by precipitation from 13000ml of diethyl ether followed by filtration. The polymer is washed three times in 2500ml of diethyl ether and then dried at 40°C under reduced pressure.

10

15

The polymer is purified further to remove trace amounts of contaminants by a Soxhlet extraction using IPA.

20

The Soxhlet extracted polymer is purified further by preparing a 6% w/v solution in DCM/MeOH (15/4 v/v) and then precipitation from a 50-fold excess of diethyl ether, followed by subsequent washing in diethyl ether. The purified polymer is dried at 40°C under reduced pressure.

25

The polymer comprises approximately 50% vinyl pyrrolidone units, approximately 25% acrylic acid units, and approximately 25% NHS-activated acrylic acid units.

30

Approximate molecular weights M_w = approximately 200,000.

Example 3

Agar diffusion testing

3.1 General method

5

n = 6 discs (each at 6mm diameter) of four-layer tissue-adhesive sheets prepared according to Example 1, containing 1%, 2% and 5% silver carbonate, were prepared and individually added to a 15ml centrifuge tube. To each tube, 2ml DPBS was added. As controls, blank paper discs (n = 3) and Gentamicin (10µg, n = 3) discs (both supplied by Oxoid) were also added to 15ml centrifuge tubes, to which DPBS (2ml) was added. All centrifuge tubes were incubated at 37°C for a period of 7 days, 14 days or 22 days.

10

15

Following incubation, DPBS was drained from each centrifuge tube and the disc removed using sterile tweezers. Each disc was blot-dried before being stored in a Petri-dish until required. Agar diffusion tests were then conducted.

20

An Oxoid Culti-loop of *Staphylococcus aureus* ATCC 25923 was used to streak a Tryptone Soy Agar (TSA) plate in accordance with the manufacturer's instructions, which was subsequently incubated at 36°C overnight. The resultant colonies were used to directly prepare a suspension in 4.5ml purified water equal in turbidity to a 0.5 McFarland standard ($\sim 1 \times 10^8$ cfu/ml). A 1:10 dilution of this suspension was then prepared by added 0.5ml of the bacterial suspension to 4.5ml purified water.

25

30

A sterile swab was used to spread the inoculum over the surface of a Mueller Hinton 2 agar plate, within 15 minutes of this inoculum being prepared. Discs were then positioned, going clockwise, as follows: Gentamicin (10µg) (not submerged in DPBS, this was used as an additional control to ensure the assay was functioning adequately), Gentamicin (10µg) (submerged in DPBS), blank paper disc, tissue-adhesive sheet of Example 1 without silver carbonate (control), sheet of Example 1 with 1% silver carbonate, sheet of Example 1

with 2% silver carbonate and sheet of Example 1 with 5% silver carbonate. Discs were applied to the surface of Mueller Hinton 2 agar within 15 minutes of inoculation. This procedure was carried out in triplicate.

- 5 Plates were incubated at 36°C for approximately 20 hours (Mueller Hinton 2 agar plates were placed in the relevant incubator within 15 minutes of the test discs being applied to the surface of the agar).

10 After the incubation period, the zones of inhibition were measured (see Figure 3). Due to the difficulty in obtaining perfect samples following submersion in DPBS, the width of the zones of inhibition is quoted in the results, ie from one edge of the test sample to the outside of the zone of inhibition.

- 15 Agar diffusion tests were also conducted, in the same manner as discussed above, on dry samples at t=0.

3.2 Results

- 20 The data for agar diffusion testing of tissue-adhesive sheets according to the present invention is presented in Tables 1-4 and Figure 4. A localised anti-infective effect is demonstrated by the growth inhibition of several different bacterial species.

- 25 At t=0 clear zones of inhibition were obtained around the discs containing 1%, 2% and 5% silver carbonate, which illustrates that silver was being released. At t=7, 14 and 22 days, clear zones of inhibition were still observed, although some reduction in efficacy was evident.

- 30 At all time points the zone of inhibition for the 2% and 5% silver carbonate discs exceeded that of the 1% disc, albeit slightly and not statistically significant (t-test, $p=0.01$). This suggests that the concentration of silver being

released from the discs containing 2% or 5% silver carbonate was at a greater concentration over a longer period of time in comparison to the discs containing 1% silver carbonate.

- 5 The results confirm that silver carbonate can be incorporated into the second layer of a tissue-adhesive sheet according to the present invention, to yield an effective, localised anti-infective product.

Table 1

- 10 Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing silver carbonate (and control discs) against *S. aureus* ATCC 25923, at t=0

	Zone of inhibition width (mm)			
	Replicate 1	Replicate 2	Replicate 3	Mean
Blank	0	0	0	0
10µg Gentamicin	11.0	10.5	11.0	10.8 ± 0.3
0% Ag ₂ CO ₃	0	0	0	0
1% Ag ₂ CO ₃	5.5	5.5	4.5	5.5 ± 0.6
2% Ag ₂ CO ₃	6.0	6.0	5.5	5.8 ± 0.3
5% Ag ₂ CO ₃	6.0	6.0	5.5	5.8 ± 0.3

Table 2

Zone of inhibition widths at t=7 days

	Zone of inhibition width (mm)			
	Replicate 1	Replicate 2	Replicate 3	Mean
Blank	0	0	0	0
10µg Gentamicin fresh	11.0	10.5	11.5	11.0 ± 0.5
10µg Gentamicin soaked	3.5	3.5	3.5	3.5 ± 0.0
0% Ag ₂ CO ₃	0	0	0	0
1% Ag ₂ CO ₃	3.5	4.0	3.5	3.7 ± 0.3
2% Ag ₂ CO ₃	3.0	4.0	4.5	3.8 ± 0.8
5% Ag ₂ CO ₃	4.5	5.0	4.5	4.7 ± 0.3

5 **Table 3**

Zone of inhibition at t=14 days

	Zone of inhibition width (mm)			
	Replicate 1	Replicate 2	Replicate 3	Mean
Blank	0	0	0	0
10µg Gentamicin fresh	10.0	10.5	10.5	10.3 ± 0.3
10µg Gentamicin soaked	3.5	3.5	3.0	3.3 ± 0.3
0% Ag ₂ CO ₃	0	0	0	0
1% Ag ₂ CO ₃	3.5	3.0	3.0	3.2 ± 0.3
2% Ag ₂ CO ₃	5.0	5.0	4.5	4.8 ± 0.3
5% Ag ₂ CO ₃	5.0	5.0	4.0	4.7 ± 0.6

Table 4

Zone of inhibition at t=22 days

	Zone of inhibition width (mm)			
	Replicate 1	Replicate 2	Replicate 3	Mean
Blank	0	0	0	0
10µg Gentamicin fresh	10.5	10.5	10.5	10.5 ± 0.0
10µg Gentamicin soaked	3.0	2.5	3.0	2.8 ± 0.3
0% Ag ₂ CO ₃	0	0	0	0
1% Ag ₂ CO ₃	3.0	3.0	3.0	3.0 ± 0.0
2% Ag ₂ CO ₃	3.5	3.5	3.0	3.3 ± 0.3
5% Ag ₂ CO ₃	4.0	2.5	3.0	3.2 ± 0.8

5

Example 4Agar diffusion testing against further organisms

Tables 5-10 detail the performance of tissue-adhesive sheets prepared

10 according to Example 1, against the following organisms:

Escherichia coli ATCC 25922*Pseudomonas aeruginosa* ATCC 9027*Staphylococcus epidermidis* ATCC 4913415 *Staphylococcus aureus* ATCC 6538*Staphylococcus aureus* ATCC 25923

Table 5

Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing 1% silver carbonate (and control discs) against *S. aureus* ATCC 25923 (soaked in artificial wound fluid)

5

	Zone of inhibition width (mm)					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Blank	0	0	0	0	0	0
10 µg Gentamicin	10.5	10.5	10.0	10.5	10.0	10.5
0 % Ag ₂ CO ₃ (t=0)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=0)	4.5	5.0	4.0	5.0	4.5	4.5
0 % Ag ₂ CO ₃ (t=14 days)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=14 days)	1.5 – 2.0	1.5 – 2.0	2.0 – 3.5	2.5 – 3.5	2.5	0.0*

Table 6

Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing 1% silver carbonate (and control discs) against *S. aureus* ATCC 25923 (soaked in DPBS)

5

	Zone of inhibition width (mm)					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Blank	0	0	0	0	0	0
10 µg Gentamicin	10.5	10.5	10.5	10.5	10.5	10.0
0 % Ag ₂ CO ₃ (t=0)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=0)	5.0	4.5	4.0	4.0	4.0	3.5
0 % Ag ₂ CO ₃ (t=14 days)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=14 days)	2.5 – 3.0	3.0	2.5 – 3.0	3.0	3.5 – 4.0	2.0 – 2.5

Table 7

Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing 1% silver carbonate (and control discs) against *S. aureus* ATCC 6538 (soaked in DPBS)

5

	Zone of inhibition width (mm)					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Blank	0	0	0	0	0	0
10 µg Gentamicin	10.0	10.0	10.0	10.0	9.5	9.5
0 % Ag_2CO_3 (t=0)	0	0	0	0	0	0
1 % Ag_2CO_3 (t=0)	3.0	3.0	3.0	3.0	3.0	3.0
0 % Ag_2CO_3 (t=14 days)	0	0	0	0	0	0
1 % Ag_2CO_3 (t=14 days)	1.5	1.0 – 1.5	1.0 – 1.5	2.0	1.5 – 2.0	1.5 – 2.5

Table 8

Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing 1% silver carbonate (and control discs) against *S. epidermidis* ATCC 49134 (soaked in DPBS)

5

	Zone of inhibition width (mm)					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Blank	0	0	0	0	0	0
10 µg Gentamicin	14.5	14.0	14.5	14.0	14.0	14.5
0 % Ag_2CO_3 (t=0)	0	0	0	0	0	0
1 % Ag_2CO_3 (t=0)	6.0	6.0	6.0	5.5	6.0	5.5
0 % Ag_2CO_3 (t=14 days)	0	0	0	0	0	0
1 % Ag_2CO_3 (t=14 days)	4.5 – 5.0	5.0 – 5.5	4.5 – 5.5	4.0 – 5.5	5.0	3.5 – 4.5

Table 9

Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing 1% silver carbonate (and control discs) against *E. coli* ATCC 25922 (soaked in DPBS)

5

	Zone of inhibition width (mm)					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Blank	0	0	0	0	0	0
10 µg Gentamicin	8.5	8.0	8.0	9.0	8.5	9.0
0 % Ag ₂ CO ₃ (t=0)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=0)	2.5	3.0	3.0	3.0	2.5	3.0
0 % Ag ₂ CO ₃ (t=14 days)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=14 days)	1.5 – 2.0	2.0 – 2.5	2.0	2.0	1.5	2.0

Table 10

Zone of inhibition widths obtained by applying sheets (prepared according to Example 1) containing 1% silver carbonate (and control discs) against *P. aeruginosa* ATCC 9027 (soaked in DPBS)

5

	Zone of inhibition width (mm)					
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6
Blank	0	0	0	0	0	0
10 µg Gentamicin	8.0	7.5	7.5	7.5	7.0	7.5
0 % Ag ₂ CO ₃ (t=0)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=0)	2.5	2.5	2.5	2.0	2.5	3.0
0 % Ag ₂ CO ₃ (t=14 days)	0	0	0	0	0	0
1 % Ag ₂ CO ₃ (t=14 days)	2.5	1.5 – 2.5	1.5	2.0	1.5 – 2.0	1.5 – 2.0

Example 5Preparation of second embodiment of a multi-lamellar sheet

10

A second embodiment of a multi-lamellar tissue-adhesive sheet according to the invention is shown schematically in Figure 5. The sheet 20 comprises six layers:

- a) a first, tissue-contacting layer 21 of poly(VP-AAc-AAc(NHS));
- 15 b) a second layer 22 of PLC that also incorporates silver carbonate;
- c) a third layer 23 of poly(VP-AAc-AAc(NHS));
- d) a fourth layer 24 of PLC;
- e) a fifth layer 25 of poly(VP-AAc-AAc(NHS)); and

f) a sixth (barrier) layer 26 of PLC.

- The second and fourth layers 22,24, which comprise PLC, each have a thickness of approximately $4\mu\text{m}$, and the third and fifth layers 23,25 have a thickness of approximately $3\mu\text{m}$. The tissue-contacting first layer 21 has a thickness of approximately $12\mu\text{m}$, and consists of three sub-layers 21a-c of approximately equal thickness. The barrier layer 26, of PLC, has a thickness of approximately $10\mu\text{m}$.
- 5
- 10 The sheet 20 is prepared by a method analogous to that of the first embodiment (Example 1), but using PLC in place of PLGA and using eight casting operations (successively for the layers 26, 25, 24, 23, 22 and the three sub-layers 21a, 21b and 21c).

Claims

1. A multi-lamellar tissue-adhesive sheet having a first tissue-contacting layer of material containing tissue-reactive functional groups, to which first
5 layer is conjoined a second layer comprising a synthetic polymer, and wherein the second layer additionally comprises one or more therapeutic agents.
2. A sheet as claimed in Claim 1, which comprises alternating layers of material containing tissue-reactive functional groups and layers comprising a
10 synthetic polymer.
3. A sheet as claimed in Claim 2 which comprises n layers of material containing tissue-reactive functional groups and $\underline{n}$ layers of synthetic polymer, wherein $\underline{n}$ is 1, 2, 3, 4 or 5.
15
4. A sheet as claimed in Claim 3, wherein $\underline{n}$ is 2 or 3.
5. A sheet as claimed in any preceding claim, wherein the therapeutic agent is an anti-infective agent
20
6. A sheet as claimed in Claim 5, wherein the anti-infective agent is a metal or a compound containing metal ions.
7. A sheet as claimed in Claim 6, wherein the metal is silver, gold or
25 copper.
8. A sheet as claimed in Claim 7, wherein the metal is silver.
9. A sheet as claimed in Claim 6, wherein the compound containing
30 metal ions is a silver salt.

10. A sheet as claimed in Claim 9, wherein the silver salt is silver carbonate.

11. A sheet as claimed in any preceding claim, wherein the therapeutic agent is present in the second layer at a concentration of 0.001% to 10%, preferably 0.01% to 5%, by weight of the tissue-adhesive sheet.

12. A sheet as claimed in any preceding claim, wherein the therapeutic agent is present in the second layer at a concentration of 0.01% to 90%, preferably 0.1 to 50% by weight of the second layer of the tissue-adhesive sheet.

13. A sheet as claimed in any preceding claim, wherein the synthetic polymer in the second layer and, where present, the other layers of synthetic polymer is a polyester.

14. A sheet as claimed in Claim 13, wherein the polyester is selected from the group consisting of polylactic acid, polyglycolic acid, polycaprolactones, polyhydroxyalkanoates, and copolymers and blends of any thereof.

15. A sheet as claimed in Claim 14, wherein the polyester is selected from the group consisting of polylactic acid, polyglycolic acid, and copolymers and blends thereof.

16. A sheet as claimed in Claim 14, wherein the polyester is a polycaprolactone.

17. A sheet as claimed in any preceding claim, wherein the tissue-reactive groups are selected from the group consisting of imido ester, p-nitrophenyl carbonate, NHS ester, epoxide, isocyanate, acrylate, vinyl sulfone, orthopyridyl-disulfide, maleimide, aldehyde, and iodoacetamide.

18. A sheet as claimed in Claim 16, wherein the tissue-reactive functional groups are NHS ester groups.

19. A sheet as claimed in Claim 16, wherein the tissue-contacting first layer
5 comprises a tissue-reactive polymer selected from the group consisting of poly(VP-AAc(NHS)) and poly(VP-AAc-AAc(NHS)) terpolymer.

20. A sheet as claimed in Claim 17, wherein the tissue-contacting first layer
10 comprises poly(VP-AAc-AAc(NHS)).

21. A sheet as claimed in Claim 4, wherein the layers of material
comprising tissue-reactive functional groups comprise a tissue-reactive
polymer selected from the group consisting of poly(VP-AAc(NHS)) and
poly(VP-AAc-AAc(NHS)) terpolymer.

22. A sheet as claimed in Claim 21, wherein the tissue-reactive polymer
15 comprises poly(VP-AAc-AAc(NHS)).

23. A sheet as claimed in any preceding claim, which has an overall
20 thickness of from 0.01 to 1mm.

24. A sheet as claimed in any preceding claim, which has an overall
thickness of from 0.015 to 0.05mm.

25. A sheet as claimed in any preceding claim, wherein the tissue-
25 contacting layer accounts for more than 20% of the overall thickness of the
sheet.

26. A sheet as claimed in any preceding claim, wherein the material
30 containing tissue-reactive functional groups comprises a tissue-reactive
polymer with a weight average molecular weight M_w that is greater than

100,000, more preferably greater than 120,000, 140,000, 160,000, 180,000 or 200,000.

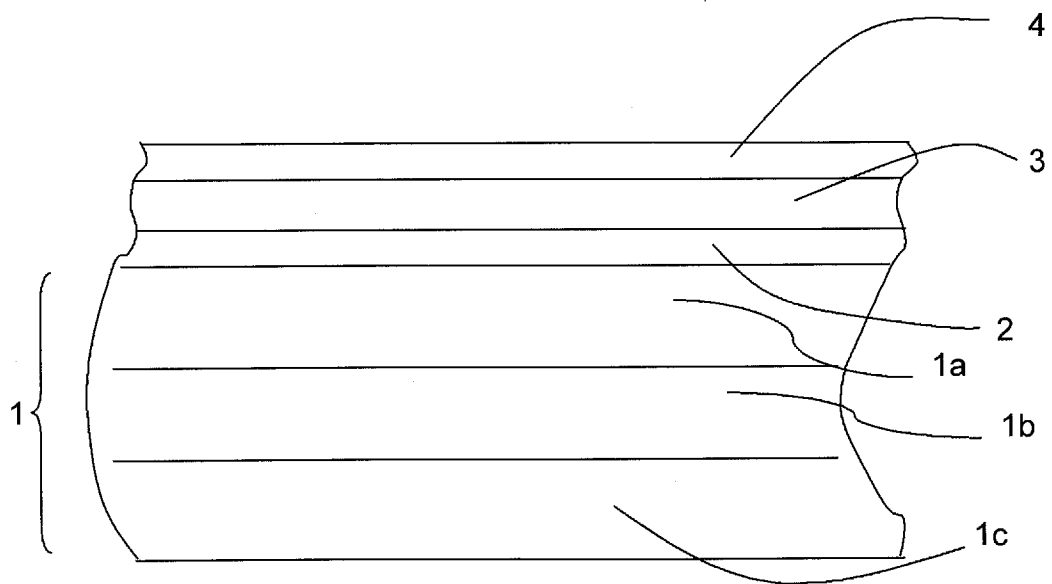
27. A method of manufacturing a sheet as claimed in any preceding claim,
5 which method comprises stepwise formation of the layers, the first, tissue-
contacting layer being formed last.

28. A device suitable for implantation in the human or animal body, which
device carries on at least part of the external surface thereof a coating
10 comprising a synthetic polymer and one or more therapeutic agents, at least
part of said coating being conjoined to a layer of material comprising tissue-
reactive functional groups.

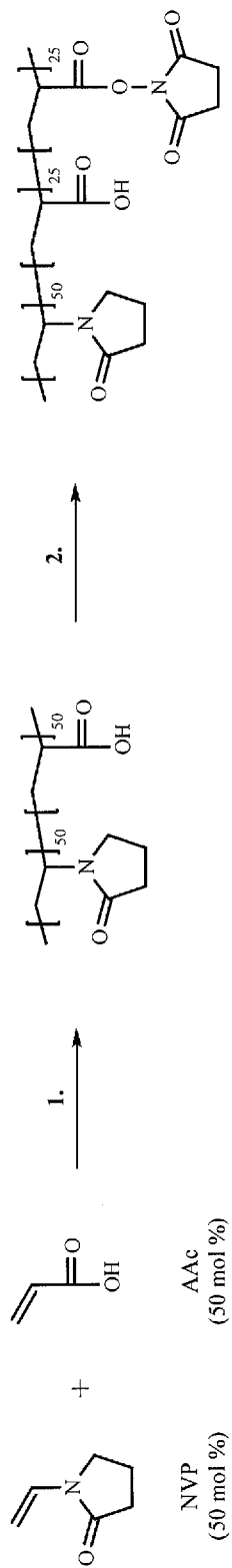
29. A method of joining a tissue surface to another tissue, or of sealing a
15 tissue surface, which method comprises applying to the tissue surface a sheet
as claimed in any one of Claims 1 to 26.

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Figure 1



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Figure 2

1. AIBN (0.01 mol %), DMSO, 80°C, 18 hr

2. NHS (50 mol %), DCC (25 mol %), 20°C, 96 hr

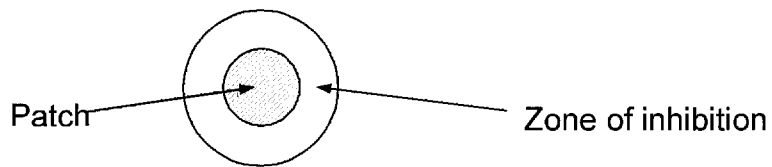
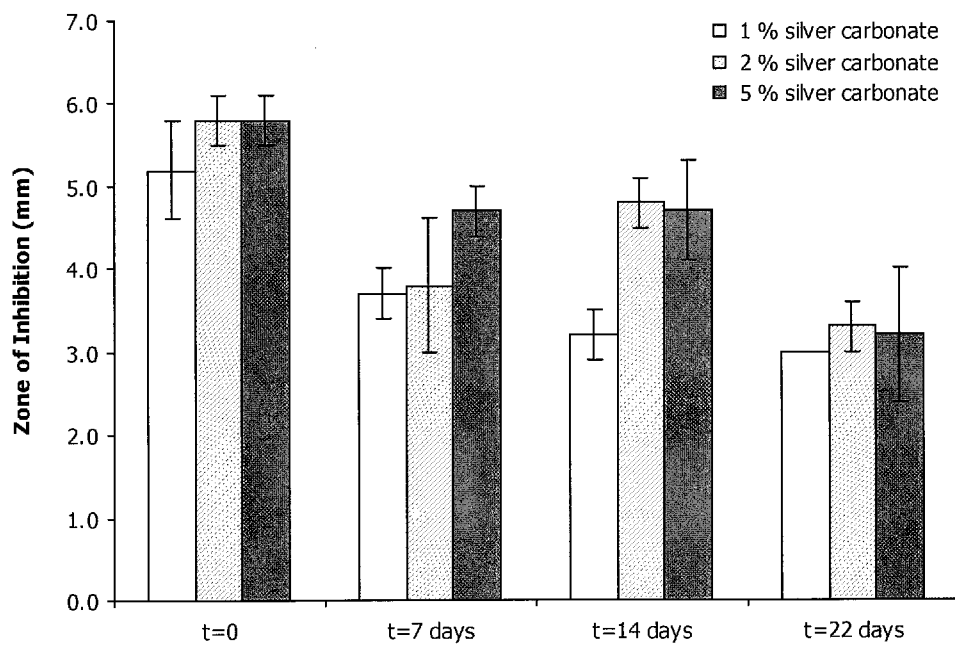
Figure 3**Figure 4**

Figure 5