



(51) International Patent Classification:

B01J 20/26 (2006.01) **B01D 15/20** (2006.01)
B01J 20/32 (2006.01) **C12N 7/02** (2006.01)

(21) International Application Number:

PCT/GB2010/050294

(22) International Filing Date:

19 February 2010 (19.02.2010)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

0902876.2 20 February 2009 (20.02.2009) GB

(71) Applicant (for all designated States except US): **UNIVERSITY OF SHEFFIELD** [GB/GB]; Firth Court, Western Bank, Sheffield, South Yorkshire S10 2TN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **RIMMER, Stephen** [GB/GB]; 53 Pontefract Road, Ackworth, Yorkshire WF7 7LR (GB). **MACNEIL, Sheila** [GB/GB]; 38 Meadowbank Avenue, Sheffield, South Yorkshire S7 1PB (GB). **SWANSON, Linda** [GB/GB]; 7 Northwood, Sheffield, South Yorkshire S6 7RZ (GB). **DOUGLAS, Charles,**

William, Ian [GB/GB]; 136 Baslow Road, Totley, Sheffield, South Yorkshire S17 4DR (GB).

(74) Agent: **HARRISON GODDARD FOOTE**; Belgrave Hall, Belgrave Street, Leeds, Yorkshire LS2 8DD (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: NOVEL USE OF POLYMERS

(57) Abstract: The invention provides a method for the removal of bacteria from media, the method comprising the steps of: (a) providing a stimulus responsive polymer adapted to be capable of binding bacteria; (b) applying said functionalised polymer to the medium so as to facilitate binding of the polymer to bacteria; and (c) removing the polymer and polymer-bound bacteria from the medium. In preferred embodiments the method provides for the use of thermoresponsive polymers functionalised by the incorporation of bacteria-binding ligands in the removal of bacteria from wounds. The invention also envisages the use of the disclosed polymers for the removal of bacteria from media.

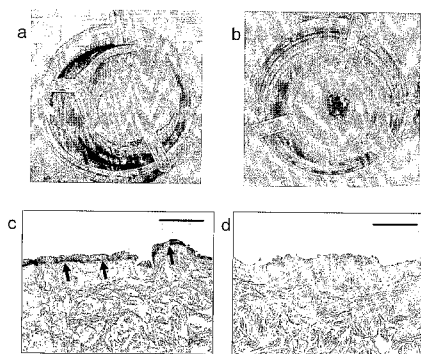


FIG. 3



Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

NOVEL USE OF POLYMERS**Field of the Invention**

5 **[0001]** This invention relates to a novel use of polymers for the binding of bacteria. Specifically, it is concerned with the use of modified stimulus responsive polymers for the binding of bacteria which are found in wounds.

Background to the Invention

10 **[0002]** Medical science constantly seeks new developments for the treatment of wounds particularly with a view to reducing the hazards associated with the possible infection of wounds. To this end, there is a constant requirement for the development of new techniques which can facilitate the efficient removal of bacteria from wounds, and it would clearly be
15 desirable if a technique could be developed wherein a wound could be treated with a material which was capable of efficiently binding to the bacteria and thereby facilitating their removal from the wound.

20 **[0003]** In WO 2008/059274, the present inventors disclosed various polymers adapted to provide a measurable response associated with a change in the properties of a system. The polymers were typically crosslinked polymers including hydrogels and hyperbranched polymers and their use in the detection of certain biomolecules, such as proteins and bacteria, was proposed. It was suggested that such polymers may thereby find application in biological assays, wherein a measurable change, such as an optical colour change, results from the detection of, for example, bacteria in infected wounds. However, the document did not
25 consider the possible use of polymers for the treatment of wounds or the removal of bacteria from wounds.

30 **[0004]** Subsequently, in GB Patent Application No. 0809404.7, the same inventors considered the use of polymers for the separation of cells from samples, and identified polymers which would be suitable for this purpose. Polymers having a temperature-dependent solubility are known as thermoresponsive polymers. For example, polymers such as poly(N-isopropylacrylamide) (PNIPAM or PIPAAm) dissolve in aqueous media below a critical temperature, referred to as the lower critical solution temperature (LCST). As the temperature is raised above the LCST, the polymer forms primary particles which aggregate and then undergo sedimentation in the reaction vessel to form a solid mass.

35 **[0005]** PNIPAM and other thermoresponsive polymers had previously been found to adhere to

cells above the LCST and, consequently, sheets of thermoresponsive polymer had been used as extracellular matrices on which cells or tissue could be cultured. The polymers were then cooled below the LCST, causing the polymers to dissolve and the cells to be released. However, since cell adhesion above the LCST is highly sensitive to temperature fluctuations, the use of this technology was limited to environments in which temperature could be controlled within strict limits. In order to address this problem, thermoresponsive polymers had been functionalised with peptides having cell binding properties, these peptides including those having the sequence Arg-Gly-Asp (RGD), which promotes cell adhesion in mammalian tissues through binding to integrins present on cell surfaces.

[0006] Rimmer *et al* (Soft Matter, 2007, 3, 971-973) had described the formation of a hyperbranched (highly branched) PNIPAM polymer having chain ends comprising an RGD peptide sequence. It was found that when a solution of this hyperbranched PNIPAM was heated above the LCST, a colloidal dispersion of sub-micron particles, rather than a solid mass of polymer, was formed in the solvent.

[0007] In GB Patent Application No. 0809404.7, the inventors went on to disclose the use of a liquid medium comprising a hyperbranched thermoresponsive polymer for the separation of cells from a sample, the polymer forming a particulate dispersion in the medium above the LCST. The liquid medium was contacted with the sample at a temperature above the LCST of the polymer, such that the polymer was present as a particulate dispersion. The polymer particles subsequently became bound to the cells, allowing the liquid medium to be separated from the sample. Cooling of the liquid medium below the LCST then caused the polymer particles to dissolve and thus release the cells.

[0008] Thus, WO 2008/059274 is concerned with the detection of bacteria by the use of polymers adapted to provide of a measurable response, such as an optical colour change, whilst GB Patent Application No. 0809404.7 relates to the separation of cells from a sample by the use of thermoresponsive polymer. However, neither of these documents considers the use of polymers for the binding of bacteria, thereby facilitating the removal of bacteria from various media, especially from wounds, and it is this issue that the present invention seeks to address, by the provision of polymers adapted to bind to bacteria, and of methods for the removal of bacteria from media such as wounds.

Summary of the Invention

[0009] The present invention, therefore, concerns the use of a stimulus responsive polymer, adapted such that it is capable of binding bacteria, in the removal of bacteria from media, and

associated methods for the removal of bacteria. Binding of the bacteria rapidly triggers the collapse of the polymer, such that the collapsed polymer containing bacteria may readily be removed from the media. Typically, the polymer is adapted so that it is capable of binding bacteria by the attachment of suitable ligands capable of binding to bacteria, such as peptides.

5 **[0010]** Thus, according to a first aspect of the present invention, there is provided a method for the removal of bacteria from media, said method comprising the steps of:

- (a) providing a stimulus responsive polymer adapted to be capable of binding bacteria;
- 10 (b) applying said functionalised polymer to the medium so as to facilitate binding of the polymer to bacteria; and
- (c) removing the polymer and polymer-bound bacteria from the medium.

[0011] Said stimulus responsive polymer comprises a polymer in which a change is caused to occur by a change in the surrounding environment. Typically, for polymers in solution, the
15 stimuli to which said polymers respond include, for example, changes in temperature, pH, ionic strength, or concentrations of specific materials, such as salts, in the solution. Particularly suitable stimulus responsive polymers in the context of the present invention are thermoresponsive polymers, examples of which include hyperbranched or highly branched polymers that show LCST behaviour in aqueous media. Examples of suitable polymers include,
20 but are not limited to, poly(N-isopropyl acrylamide) and copolymers thereof, highly branched and hyperbranched poly(methyl vinyl ether) and copolymers thereof, and highly branched and hyperbranched poly(vinyl caprolactam) and copolymers thereof.

[0012] Said stimulus responsive polymer is adapted to be capable of binding bacteria by the attachment of suitable ligands which are selected due to their facility to bind to bacteria.
25 Suitable ligands include various moieties capable of binding to bacteria, and these may include groups derived from peptide antibiotics and their bacteria binding derivatives, non-peptide antibiotics and their bacteria binding derivatives, antibody fragments that bind to bacteria, and oligo- and mono-saccharides that bind to bacteria. Other examples of suitable ligands include any low molecular weight compounds that selectively bind to any cell surface receptor on
30 bacteria. Especially suitable ligands for present purposes are bacteria-binding peptides, which may include antibiotic and non-antibiotic peptides. Particularly preferred examples of such ligands include peptides of vancomycin, polymyxin, beta-lactam and teicoplanin antibiotics, and cationic peptides such as the cecropin and melittin hybrid, CEME and defensins. Attachment of said ligands to the stimulus responsive polymers is most preferably achieved by linkage to
35 binding groups located at the ends of the polymer chains.

[0013] Application of said stimulus responsive polymer to a bacteria-containing medium may be conveniently achieved by the application of the functionalised polymer in a solution or tethered to a surface or gel. In both variants, the polymer is below the LCST (in the open coil state) in the absence of bacteria. The interaction with bacteria drives the polymer into its collapsed state and, in this state, polymer plus bacteria can be removed. If subsequently cooled the polymer changes back to its open coil state and releases the bacteria.

[0014] It is necessary that said polymer is applied to the medium at a temperature below the lower critical solution temperature (LCST) of the functionalised polymer, which is typically above 60°C. Consequently, there is no requirement for precise control of temperature in the handling of this polymer because it will not spontaneously change conformation at room temperature or body temperature, but will only collapse when bacteria bind to it. Removal of the polymer from the medium then allows for removal of the polymer-bound bacteria. The method of the invention finds particular application when the medium is a wound, i.e. in the removal of bacteria from wounds.

[0015] According to a second aspect, the present invention provides for the use of a stimulus responsive polymer adapted to be capable of binding bacteria in a method for the removal of bacteria from media. Preferred media comprise wounds.

Brief Description of the Drawings

[0016] A better understanding of the invention will be facilitated by reference to the accompanying illustration, wherein:

Figure 1 provides a comparison between (A) the prior art application of polymers for the lifting of mammalian cells from cell culture surfaces, and (B) the method of the present invention for the removal of bacteria from wounds.

Figure 2 provides an illustration of how a stimulus responsive polymer is insolubilised on incubation with bacteria according to the method of the invention.

Figure 3 shows the visible removal of bacteria from an infected skin wound model when treated with stimulus responsive polymers according to the method of the invention; and

Figure 4 provides a quantitative graphical illustration of the removal of bacteria from an infected skin wound model according to the method of the invention.

Detailed Description of the Invention

[0017] In a preferred embodiment, the invention provides a method for the removal of bacteria from wounds which involves the use of a stimulus responsive polymer to which antibiotic peptides have been attached, such that these polymers are capable of binding bacteria. Application of the polymer to the wound is carried out below the LCST of the polymer, such that the polymer is in open chain form. The subsequent binding of the bacteria to the polymer then rapidly triggers the collapse of the polymer chain, whereupon the collapsed polymer containing bacteria can then be removed.

[0018] The claimed method finds particular application in the treatment of wounds, for reducing the bacterial content of chronic infected wounds. However, the method can also be used for many other applications, including the removal of bacteria from liquids that are passed over the polymer in, for example, microfluidic devices, or wherein the polymer is adhered to a catheter. The key feature of the invention is in the ability of the polymer to physically bind to bacteria, and this is particularly useful in the reduction of bacterial contamination in an infected wound. The method of the invention is also of use in the decontamination of medical devices. For example, natural and synthetic polymer scaffolds for use in tissue engineering can be decontaminated by application of a bacteria binding polymer providing a mild route to disinfection and sterilisation.

[0019] As previously noted, preferred stimulus responsive polymers that show changes in response to changes in the environment in the context of the present invention include polymers of N-isopropyl acrylamide (NIPAM). The macroscopic changes that can occur at critical values of pH, temperature or ionic strength with such polymers come about because the polymer changes from an open (solvated) coil to a collapsed state known as a globule. Any aqueous polymer solution will respond in this way to changes in pH, temperature or ionic strength but, for many systems, the critical points occur at temperatures that are above 100 °C or below 0 °C. However, by the introduction of specific features on either the ends of polymer chains or on crosslinked (hydrogel) chains, the temperature of the transition of a thermoresponsive polymer can be reduced. Thus, if a polymer is observed at a particular temperature that is below the non-bound transition temperature, but above the bound transition temperature, a coil-to-globule transition will be observed on binding.

[0020] In the case of poly(NIPAM) materials, as disclosed in GB Patent Application No. 0809404.7, cell adhesion to the polymer is also enhanced above the transition temperature, whereas below the transition temperature cell adhesion is not supported. The cell adhesive state can be enhanced by the addition of cell adhesive peptides and, thereby, particulate versions of these peptide functional PNIPAM materials can be used to lift cells from cell culture

surfaces.

[0021] In the case of the present invention, it is seen that poly(NIPAM) materials may be functionalised with peptides that bind to bacteria, thereby facilitating the removal of bacteria from media such as wound beds. Preferred bacteria binding peptides are based on antibiotics, but non-antibiotic species that bind bacteria also function effectively.

[0022] Referring now to the illustrations provided in Figure 1, there is initially shown, in section (A) a process for the lifting of mammalian cells from cell culture surfaces, wherein mammalian cells are exposed at a temperature above the transition temperature (10-25°C) to polymer particles functionalised with a cell adhesive peptide. The cells are then lifted from the plate and remain suspended, after which the suspension is transferred and cooled.

[0023] In section (B), on the other hand, there is seen a polymer functionalised with a bacteria-binding-ligand, which is added to a wound below the transition temperature, such that the polymer is in the open chain form. It subsequently binds to the bacteria in the wound and, on so binding, the polymer chain collapses. In this collapsed state, bacteria adhere to the polymer rather than the wound and can thus be physically removed from the wound bed as a bacteria-polymer aggregate.

[0024] Removal of the polymer, with bacteria bound thereto, may be achieved simply by maintaining the polymer at room temperature or above. Thus, removal of bacteria is typically achieved by rinsing the wound with warm sterile saline or swabs soaked in warm saline solution will achieve removal of the polymer with bacteria firmly attached. If the system is then deliberately cooled to a point below the bound transition temperature (for example, a temperature below 10°C), then release of the bacteria from the polymer is observed. This observation is explained by the fact that the method of the invention involves not only the binding of bacteria to the polymer, but also the lowering of the LCST once the polymer becomes so bound. In polymers which are appropriate for use in the disclosed applications, the associated decrease in LCST takes the LCST below the application temperature, such that the polymer open coil chain collapses into the globule on binding. Both chain collapse and binding are required in order for removal of bacteria to occur so that, if the temperature is artificially decreased to below the bound LCST, then the polymer reverts to the open coil state, with the consequence that the bacteria are released.

[0025] Looking at Figure 2, there can be seen the effect on a stimulus responsive polymer of incubation with bacteria, wherein the polymer becomes insoluble only after such incubation. Thus, in image a) is illustrated a gross view of *S.aureus* bacteria in buffer solution, whilst image b) shows the effect of fluorescence microscopy on fluorescently labelled (fluorescein) *S.aureus* in buffer solution, wherein bacteria are in suspension, and spread evenly throughout. Image c)

is a gross view of a control polymer, which is non-stimulus responsive, in buffer solution alone, and it is seen that this control polymer becomes insoluble even without the presence of bacteria. From the fluorescence microscopy of fluorescently labelled *S.aureus* incubated with control polymer as shown in image d), as with image b) it may be gleaned that the bacteria remain evenly distributed throughout. Image e) presents a gross view of the stimulus responsive polymer BPNIPAM-VAN in buffer solution alone. Unlike the control polymer, the functionalised polymer remains in solution. Image f) is obtained by fluorescence microscopy of BPNIPAM-VAN in buffer solution alone and, as expected, there no fluorescence. Image g) shows a gross view of BPNIPAM-VAN incubated with *S.aureus*, from which it may be seen that binding of bacteria appears to render the polymer insoluble, and causes it to form visible aggregates, whilst the fluorescence microscopy image h) of BPNIPAM-VAN incubated with fluorescently labelled *S.aureus* indicates that the fluorescent bacteria form aggregates as they are bound to the collapsed polymer.

[0026] Figure 3 illustrates the visible removal of bacteria from an infected wound model by the application of a stimulus responsive polymer. Thus, tissue engineered skin constructs (a) are burnt (b) so as to create a burn-wound model and allow bacteria entry to the skin, as occurs *in vivo*. Wounded skin thus obtained was infected with the common pathogenic bacteria *Pseudomonas aeruginosa* for 24 hours, after which time either the polymer, or buffer alone as control, were applied to the surface of the skin. After one hour, the polymer or buffer was removed, and the skin was examined using histology to visualise the level of infection. In infected skin sections incubated with buffer alone, a large number of bacteria were visible within the skin, particularly along the surface, and this is illustrated in image (c) in the depicted layer whereas, in infected skin sections incubated with polymer, the majority of visible bacteria are clearly no longer present within the skin sections as illustrated in image (d). Clearly, therefore, it appears that bacteria become bound to the polymer and are removed upon removal of the polymer.

[0027] A quantitative illustration of the removal of bacteria from an infected wound model by the application of a stimulus responsive polymer is shown in Figure 4, wherein the numbers of viable bacteria remaining in the tissue from engineered skin constructs burnt and infected as in Figure 4 were quantified. Thus from graph a) it is seen that, in skin samples incubated with polymer, the numbers of bacteria remaining in the tissue following removal of polymer are substantially reduced as compared to those incubated with buffer alone whereas, conversely, graph b) shows that the numbers of bacteria found in the polymer solution removed from the wound are substantially increased as compared to those found in buffer alone.

[0028] Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of them mean “including but not limited to”, and they are not intended to

(and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

[0029] Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

[0030] The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

CLAIMS

1. A method for the removal of bacteria from media, said method comprising the steps of:
 - 5 (a) providing a stimulus responsive polymer adapted to be capable of binding bacteria;
 - (b) applying said functionalised polymer to the medium so as to facilitate binding of the polymer to bacteria; and
 - (c) removing the polymer and polymer-bound bacteria from the medium.
- 10 2. A method as claimed in claim 1 wherein said stimulus responsive polymer comprises a polymer in solution in which a change is caused to occur by a change in the temperature, pH or ionic strength of the solution.
- 15 3. A method as claimed in claim 1 or 2 wherein said stimulus responsive polymer comprises a thermoresponsive polymer.
4. A method as claimed in claim 3 wherein said thermoresponsive polymer comprises a hyperbranched or highly branched polymer that shows LCST behaviour in aqueous media.
20
5. A method as claimed in claim 4 wherein said polymer is selected from poly(N-isopropyl acrylamide) (NIPAM) and copolymers thereof, highly branched and hyperbranched poly(methyl vinyl ether) and copolymers thereof, and highly branched and hyperbranched poly(vinyl caprolactam) and copolymers thereof.
25
6. A method as claimed in any one of claims 1 to 5 wherein said stimulus responsive polymer is adapted to be capable of binding bacteria by the attachment to said polymer of ligands possessing the facility to bind to bacteria.
30
7. A method as claimed in claim 6 wherein said ligands comprise bacteria-binding moieties.
8. A method as claimed in claim 7 wherein said bacteria-binding moieties comprise groups derived from peptide antibiotics and their bacteria binding derivatives, non-peptide
35

antibiotics and their bacteria binding derivatives, antibody fragments that bind to bacteria, and oligo- and mono-saccharides that bind to bacteria.

9. A method as claimed in claim 7 wherein said bacteria-binding moieties comprise
5 antibiotic or non-antibiotic peptides.
10. A method as claimed in any one of claims 6 to 9 wherein said ligands are selected
from peptides of vancomycin, polymyxin, beta-lactam and teicoplanin antibiotics, and
10 cationic peptides.
11. A method as claimed in claim 10 wherein said cationic peptides are selected from
the cecropin and melittin hybrid, CEME and defensins.
12. A method as claimed in any one of claims 6 to 11 wherein said ligands are
15 attached to said stimulus responsive polymers by linkage to binding groups located at the
ends of the polymer chains.
13. A method as claimed in any one of claims 1 to 12 wherein application of said
stimulus responsive polymer to a bacteria-containing medium is achieved by the application
20 of the functionalised polymer in a solution or tethered to a surface or gel wherein the
polymer is below the lower critical solution temperature (LCST) of the functionalised
polymer in the absence of bacteria.
14. A method as claimed in any preceding claim wherein the medium is a wound.
25
15. A method as claimed in any one of claims 1 to 13 wherein the medium comprises
a liquid.
16. A method as claimed in claim 15 wherein the liquid is passed over the polymer in a
30 microfluidic device or the polymer is adhered to a catheter.
17. The use of a stimulus responsive polymer adapted to be capable of binding
bacteria in a method for the removal of bacteria from media.
- 35 18. The use as claimed in claim 17 wherein said media comprise wounds.
19. The use as claimed in claim 17 wherein said media comprise liquids.

20. The use as claimed in claim 17 wherein said media comprise medical devices.

5

10

1/4

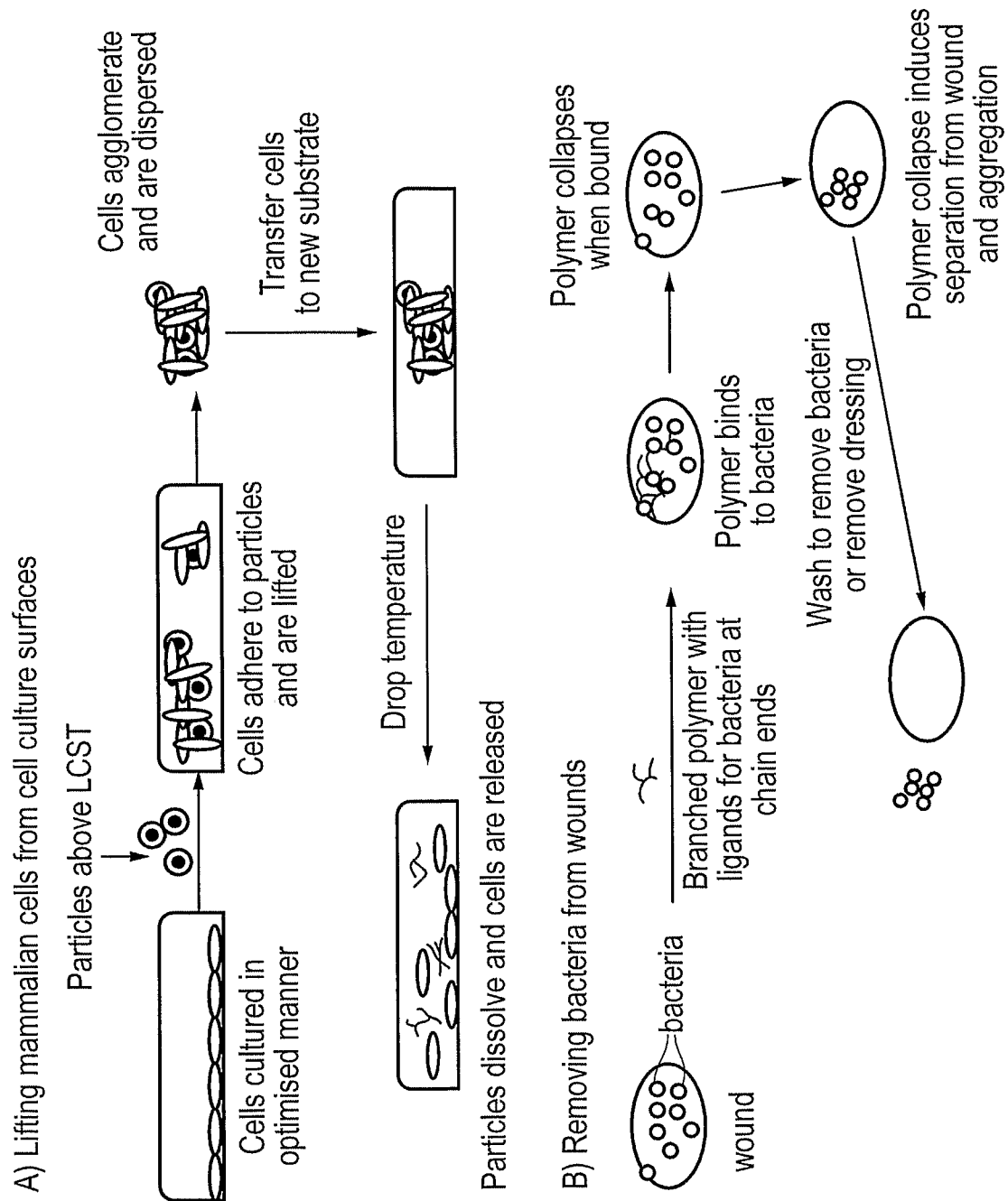


FIG. 1

2/4

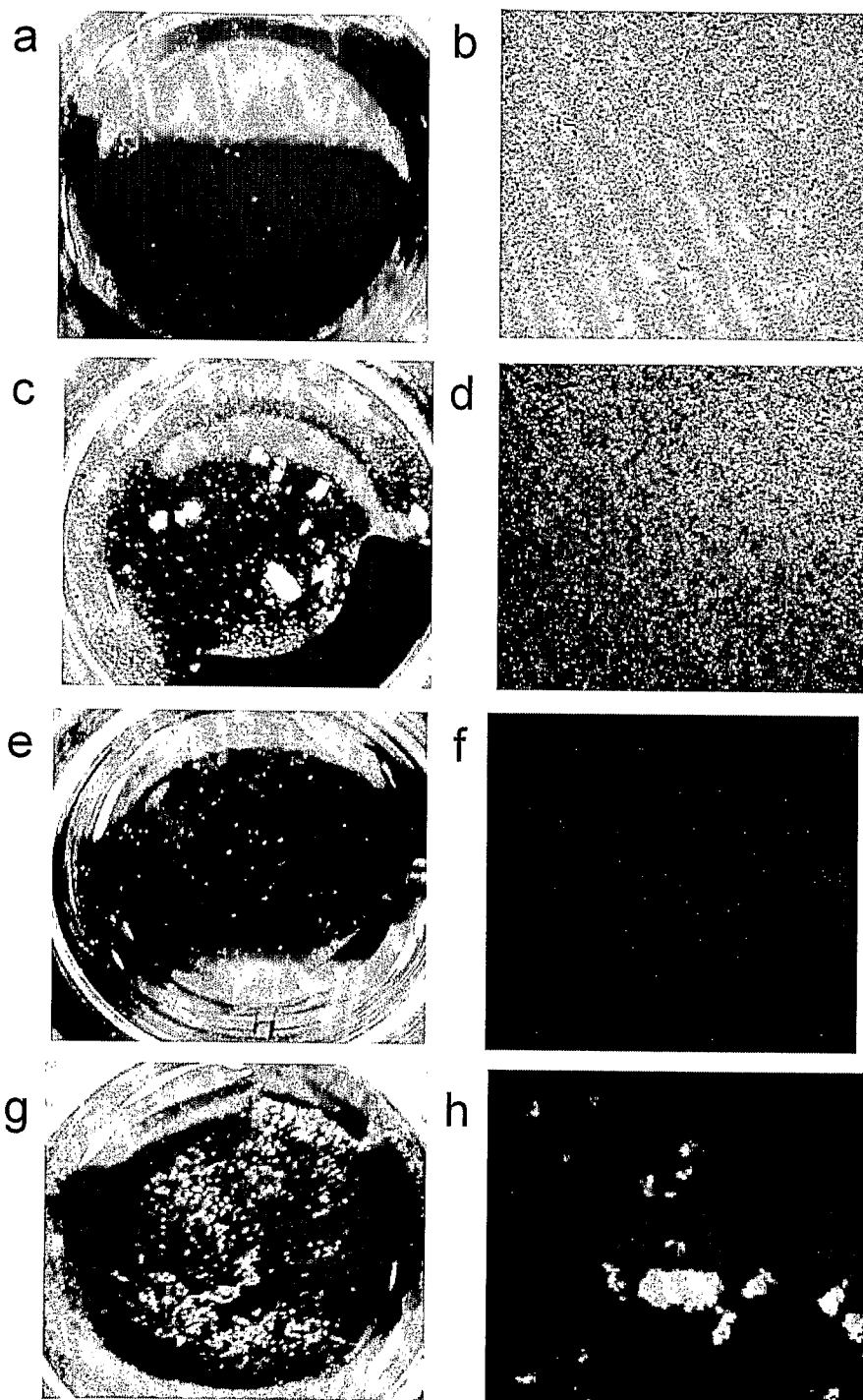


FIG. 2

3/4

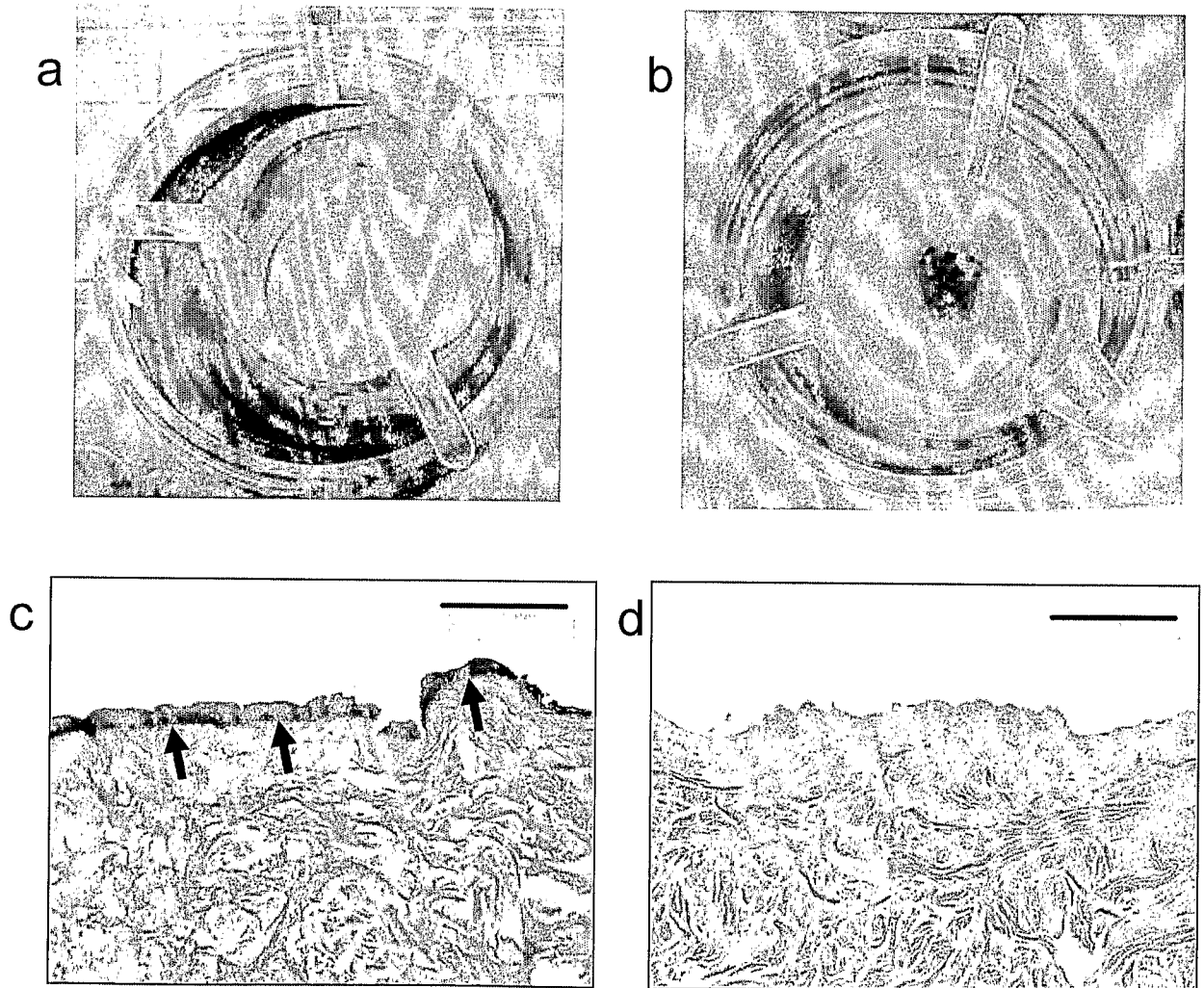


FIG. 3

4/4

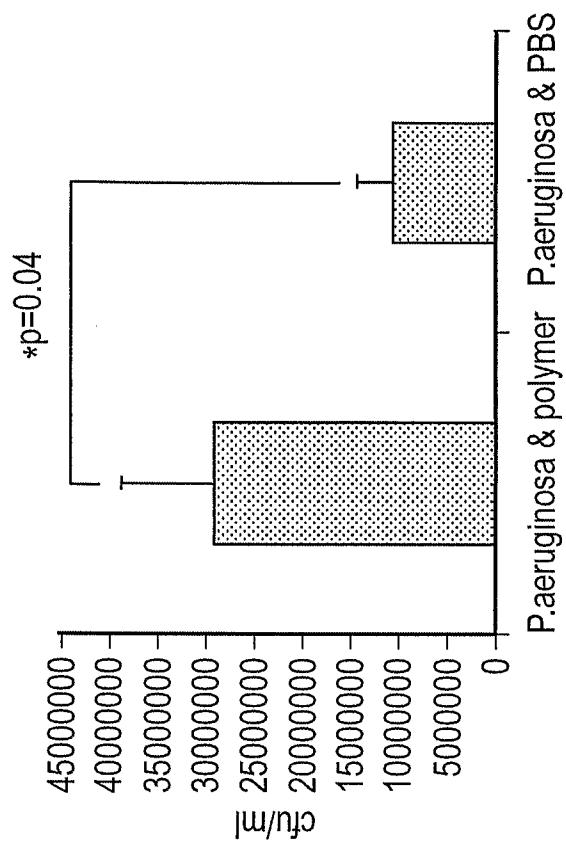


FIG. 4b

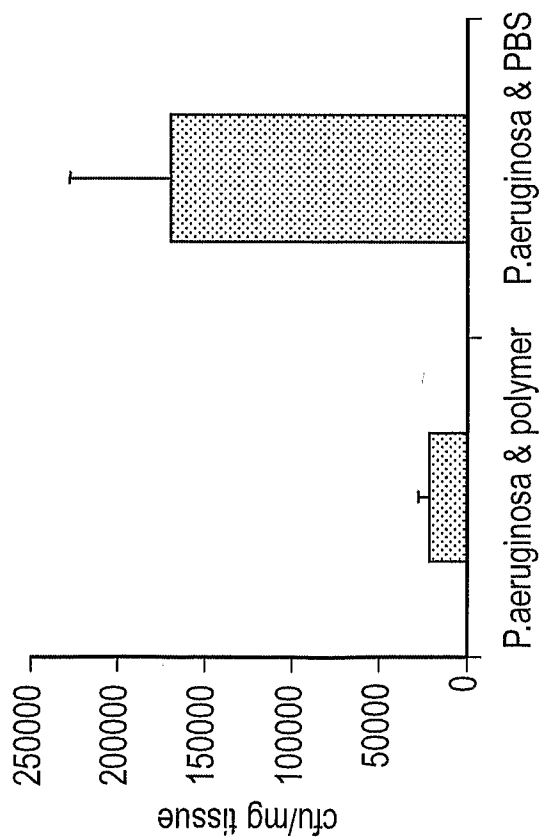


FIG. 4a

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2010/050294

A. CLASSIFICATION OF SUBJECT MATTER

INV. B01J20/26 B01J20/32 B01D15/20 C12N7/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

B01J B01D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAINAIK M B ET AL: "Detachment of affinity-captured bioparticles by elastic deformation of a macroporous hydrogel" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS), NATIONAL ACADEMY OF SCIENCE, US LNKD-DOI:10.1073/PNAS.0508432103, vol. 103, no. 4, 24 January 2006 (2006-01-24), pages 849-854, XP003000336 ISSN: 0027-8424 * abstract Section: "Materials and Methods"; page 853 - page 854 ----- -/-	1-7, 13, 15, 17, 19

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "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

Date of the actual completion of the international search

26 July 2010

Date of mailing of the international search report

11/08/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Klemps, Christian

International application No
PCT/GB2010/050294

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PASPARAKIS G; COCKAYNE A; ALEXANDER C: "Control of Bacterial Aggregation by Thermoresponsive Glycopolymers" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 129, no. 36, 16 August 2007 (2007-08-16), pages 11014-11015, XP002593108 ISSN: 0002-7863 DOI: 10.1021/ja074349z Section: "Bacteria adhesion studies"; page S3; figure 3 -----	1-8,13, 15,17,19
X	ISTA L K; VICTOR H P-L; LOPEZ G P: "Surface-Grafted, Environmentally Sensitive Polymers for Biofilm Release" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 65, no. 4, April 1999 (1999-04), pages 1603-1609, XP002593109 ISSN: 0099-2240	1-7,13, 15,17,19
Y	Sections: "Detachment studies"; "Long-term incubation"; page 1604, right-hand column - page 1605, left-hand column Sections: "Detachment studies"; "Long-term incubation"; "Repetitions"; page 1605, right-hand column - page 1607, right-hand column; figures 1-5 -----	16,20
X	CUNLIFFE D; SMART C A; TSIBOUKLIS J; YOUNG S; ALEXANDER C; VULFSON E N: "Bacterial adsorption to thermoresponsive polymer surfaces" BIOTECHNOLOGY LETTERS, vol. 22, no. 2, January 2000 (2000-01), pages 141-145, XP002593110 ISSN: 0141-5492 DOI: 10.1023/A:1005622424821 Section: "Assessment of bacterial attachment to synthetic surfaces"; page 142, right-hand column figure 2 -----	1-7,12, 13,15, 17,19

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARVIDSSON P ET AL: "Chromatography of microbial cells using continuous supermacroporous affinity and ion-exchange columns" JOURNAL OF CHROMATOGRAPHY, ELSEVIER SCIENCE PUBLISHERS B.V, NL LNKD- DOI:10.1016/S0021-9673(02)01114-7, vol. 977, no. 1, 15 November 2002 (2002-11-15), pages 27-38, XP004390892 ISSN: 0021-9673 Section: "2.2.1. Production of continuous supermacroporous IEC-columns"; page 29 Section: "2.2.6. IEC of cells"; page 30, right-hand column Section: "3.4. IEC of cells"; page 35, left-hand column - column 37; figures 9a-c -----	1-7,13, 15,17,19
X	ALARCON C D L H ET AL: "Grafted thermo- and pH responsive co-polymers: Surface-properties and bacterial adsorption" INTERNATIONAL JOURNAL OF PHARMACEUTICS, ELSEVIER BV, NL LNKD- DOI:10.1016/J.IJPHARM.2005.01.037, vol. 295, no. 1-2, 13 May 2005 (2005-05-13), pages 77-91, XP004852843 ISSN: 0378-5173 * abstract Section: "2.4. Surface grafting of acid-functional polymers"; page 79, right-hand column Section: "In vitro adsorption studies"; page 80, left-hand column Section: "3.3. Bacterial adsorption assays"; page 84, right-hand column - page 86, left-hand column ----- -/--	1-7,12, 13,15, 17,19

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2010/050294

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ETIENNE O; PICART C; TADDEI C; HAIKEL Y; DIMARQ J L; SCHAAF P; VOEGEL J C; OGIER J A; EGELS C: "Multilayer Polyelectrolyte Films Functionalized by Insertion of Defensin: a New Approach to Protection of Implants from Bacterial Colonization" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 48, no. 10, October 2004 (2004-10), pages 3662-3669, XP002593111 ISSN: 0066-4804 DOI: 10.1128/AAC.48.10.3662-3669.2004 * abstract Section: "Scanning electron microscopy"; page 3663, right-hand column; figures 6D-C	1-4, 6-11,15, 17,19
Y	GUTOWSKA A; BAE Y H; JACOBS H; MOHAMMAD F; MIX D; FEIJEN J; KIM S W: "Heparin release from thermosensitive polymer coatings: In vivo studies" JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 29, no. 7, July 1995 (1995-07), pages 811-821, XP002187415 ISSN: 0021-9304 DOI: 10.1002/jbm.820290705 Section: "Coating procedure"; page 813, right-hand column - page 814, left-hand column	16,20
A	WO 2006/121396 A1 (PROTISTA BIOTECHNOLOGY AB [SE]; MATTIASSON BO [SE]; GALAEV IGOR YU [SE] 16 November 2006 (2006-11-16) the whole document	1-13, 15-17, 19,20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2010/050294

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☒ Claims Nos.: 14, 18
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
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. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ 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 claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 14, 18

This International Searching Authority considers that the subject-matter of claims 14, 18 of the present international application relates to a method of treatment of the human or animal body by therapy and therefore has decided not to search the subject-matter of said claims. (Article 17(2)(a)(i) and (b) PCT and Rule 39.1(iv) PCT)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2010/050294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006121396 A1	16-11-2006	AU 2006244662 A1	16-11-2006
		CA 2608340 A1	16-11-2006
		EP 1888195 A1	20-02-2008
		JP 2008545379 T	18-12-2008
		US 2009200232 A1	13-08-2009
