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WO 02/48247 A1

(54) Title: BIOCOMPATIBLE, POROUS MATERIAL, METHOD FOR ITS PRODUCTION AND USE OF THE SAME

(57) Abstract: The present invention relates to a method for preparation of a biocompatible, porous material as carrier for cells. The method comprises casting a dispersion containing a polymer phase and a solvent phase, removing the solvent and crosslinking the polymer phase. The invention also relates to use of said material for cultivation of different cell types and use of said material as carrier for cells for production of substances.

BIOCOMPATIBLE, POROUS MATERIAL, METHOD FOR ITS PRODUCTION AND USE OF THE SAME

Field of the Invention

The present invention relates to a method for preparing a biocompatible, porous material as carrier for cells, to said biocompatible, porous material prepared by means of said method and to use of the biocompatible, porous material.

Background Art

Carrier materials can be used for, for instance, chromatographic separation purposes, as ion exchanger in affinity chromatography or in gel filtration and as carrier material in implantation.

The carriers that are currently available for cells are not optimal in every respect.

Macroporous particles which can be used, for instance, in cultivation of cells are described in SE 464,816. The particles in said patent publication are spherical and are mainly used for cultivation of cells on a large scale for preparation of vaccines. The technique for producing the macroporous particles differs from the method according to the invention.

The drawback of previously used macroporous particles for cells is that the particles are not optimal, or even usable, in implantation of the particles in the body of an individual. If any step in the implantation of the macroporous particles should cause serious consequences and it should be desirable to remove the particles, this would be impossible owing to the spherical and minimal structure.

In cultivation of different cells for preparing tissues, entire organs or other three-dimensional structures on different carriers, there is thus a need for a material, the form of which may be varied within wide limits for the specific application. In cultivation of

e.g. skin cells for preparing artificial skin or other three-dimensional forms, it is necessary to have large carriers.

In implantation of the material it is important, for instance, to be able to remove it from the individual's body when required. Important properties of the material are that it is biocompatible and that it is possible to vary the form of the material for the specific purpose. Both the cell types and the different applications determine the form of the carrier.

Summary of the Invention

An object of the present invention therefore is to provide a method for preparing a biocompatible, porous material as carrier for cells, which comprises casting a dispersion containing a polymer phase and a solvent phase, removing the solvent and crosslinking the polymer phase.

Another object of the invention is a biocompatible, porous material as carrier for cells, prepared by casting a dispersion containing a polymer phase and a solvent phase, removing the solvent and crosslinking the polymer phase.

One more object of the invention is use of a biocompatible, porous material, prepared as described above, as carrier for cells.

An additional object of the present invention is a method for implanting a biocompatible, porous material as carrier for cells in an individual for production of substances, comprising injecting said biocompatible, porous material into the individual and then letting the cells on the biocompatible, porous material produce said substances.

The biocompatible, porous material according to the present invention may be used both as carrier for cells in cultivation of cells and as carrier for existing cells for production of a desirable substance before/after implantation in an individual. The cells can either be

the individual's own cells or cells from another source (characteristic of the species or foreign to the species). In some cases, the cells as such can be the desirable product, for instance attached initial stages of adipocytes (preadipocytes) on the carrier which after implantation can propagate so as then to be converted into adipocytes (fat cells). One field of application for this is for instance plastic surgery. Another example is myoblasts (muscle cells) which can be used in treatment of e.g. cardiac infarction. One more example is hepatocytes (liver cells) which can be used to render toxic substances in liver lesions harmless. Also more complex structures, such as the islets of Langerhans, can be attached to and/or in the porous carrier. The islets of Langerhans are composed of a plurality of different cell types and constitute the system that regulates the blood sugar content. These islets are considerably larger and require a pore size of the carrier of 50-200 μm.

The term "substance" used herein relates to the substances that can be produced by various cells or microorganisms, for instance antibiotics, pharmaceutical substances, e.g. dopamine which is a key substance in Parkinson's disease, and different interferons which are active substances in treatment of cancer.

According to an embodiment of the inventive method, the decomposability of the biocompatible, porous material is determined by the degree of crosslinking of the polymer phase.

According to yet another embodiment of the inventive method, an agent is added for enhancing or changing the adhesion of cells to said biocompatible, porous material during casting of the dispersion, or the agent is bound chemically to the polymer or added later. Agents affecting the cell adhesion can be either single molecules or proteins. Examples of the former are positively or negatively charged substances, such as hexamethylene diamine and amino caproic acid. Examples of more complex struc-

tures are peptides containing the amino acid sequence Arginine-glycine-aspargine or derivatives thereof. This sequence promotes the adhesion of cells to the carrier. Examples of proteins are fibronectin and laminin. Also non-defined mixtures of proteins (obtained by extraction of tissues) such as ECM (extracellular matrix) can be used.

The polymer phase is preferably selected among gelatin, polysaccharides and synthetic polymers, gelatin being particularly preferred. The polymer phase is cross-linked preferably chemically or by heating. The polymer phase is preferably aqueous.

To obtain a form that fits the specific application, for instance in cultivation of skin cells on the biocompatible, porous material for production of artificial skin, the dispersion can be cast into membranes, tubes or other convenient forms. Larger forms can be required, for instance, in production of artificial skin or other three-dimensional forms. Preferably, the dispersion is cast according to the present invention into membranes.

In cultivation of cells, the biocompatible, porous material can be used for production of artificial skin, artificial organs, fatty tissue, blood vessels etc.

The biocompatible, porous material is prepared so that the cells are present both inside and on the outside of the biocompatible, porous material. This results in optimal use of the material.

In this description, the expression "carrier for cells" is intended to comprise carriers that can be used in cultivation of various cells, and carriers which can be used for cells to achieve production of desirable substances. The expression "carrier for cells" also includes medical implants for implantation in the human body.

A surprising effect in the preparation of the biocompatible, porous material according to the invention is that the pores obtained in the material are uniformly

distributed through the cross-section of the material. Thus, a more uniform distribution of the cells in the biocompatible, porous material is achieved.

Description of Preferred Embodiments

Example 1

6.7 g gelatin is dissolved in 53 ml deionised water by heating to 50°C. To this solution 11.0 g Tween 80 (polyoxyethylene(20)sorbitanmonooleate) is added. The mixture is agitated. Then the mixture is cooled to 35°C. At this temperature, a mixture of cyclohexane (18 ml) and Tween 80 (0.6 g) is added. The resulting mixture is agitated vigorously. Then this mixture is poured onto a glass slide with a 1-mm-thick packing along the periphery, then another glass slide is placed on top, after which the two glass slides are pressed together by means of clips. The form is chilled with cold water until the temperature is below 20°C. The form is separated and the formed gelatin membrane is washed with acetone until all the water and Tween 80 have been removed. After that the membrane is allowed to air-dry and obtains the appearance of white paper. The membrane formed must be crosslinked so as not be dissolved at temperatures above 25°C.

Crosslinking

A suitable form/size is cut out from the dried membrane by means of a pair of scissors. 0.97 g membrane is rehydrated in 39 ml 0,1 M phosphate buffer, pH 8.0. To this, 97 µl hexamethylene diisocyanate (crosslinking agent) and 0.5 µl triethylamine (catalyst) are added. The crosslinking reaction is allowed to proceed for two hours at 25°C under agitation. The reaction is stopped by adding 0.24 g glycine. Then the mixture is allowed to stand over night. The membrane is washed with deionised water, hot (70°C) deionised water and finally with acetone. The membrane is dried in air. The crosslinked membrane can now be autoclaved at 120°C without being dissolved.

The example above results in a membrane having an ideal pore size of 20-30 μm, which pore size is optimal for cultivation of cells and adhesion of cells.

To obtain a membrane having a pore size in the range 10-20 μm, the example above is applied, except that 7.9 g Tween 80 is added to the gelatin solution. After cooling as described above, a mixture of 6.6 ml cyclohexane and 0.8 g Tween 80 is added.

In the production of the membrane above, larger pores can be obtained by using a higher Tween concentration. An increase of the amount of cyclohexane also results in larger pores and simultaneously a larger total pore volume. An increase of the agitation speed results in smaller pores. There are thus great possibilities of varying the porosity of the membrane within wide limits.

The resistance of the biocompatible, porous material to heat, enzymes etc. is proportional to the crosslinking degree. An increased concentration of crosslinking agent, for instance hexamethylene diisocyanate, results in increased resistance. An increasing crosslinking time also results in increased resistance.

The crosslinking reagents that are used in the preparation can be, for instance, bifunctional, such as diisocyanates and aldehydes.

CLAIMS

1. A method for preparation of a biocompatible, porous material as carrier for cells, which comprises casting a dispersion containing a polymer phase and a solvent phase, removing the solvent and crosslinking the polymer phase.

2. A method as claimed in claim 1, wherein the decomposability of the biocompatible, porous material is determined by the degree of crosslinking of the polymer phase.

3. A method as claimed in claim 1 or 2, wherein an agent for enhancing or changing the adhesion of cells to said biocompatible, porous material is added during casting of the dispersion, or the agent is bound chemically to the polymer or is added later.

4. A method as claimed in claim 3, wherein said agent is selected from the group consisting of positively or negatively charged substances and peptides.

5. A method as claimed in claim 4, wherein said agent is selected from the group consisting of hexamethylene diamine, amino caproic acid, peptides containing the amino acid sequence arginine-glycine-aspargine, fibronectin and laminin.

6. A method as claimed in any one of claims 1-5, wherein the polymer phase is selected among gelatin, polysaccharides and synthetic polymers.

7. A method as claimed in any one of claims 1-6, wherein the polymer phase is crosslinked chemically or by heating.

8. A biocompatible, porous material as carrier for cells, prepared according to any one of claims 1-7.

9. Use of a biocompatible, porous material, prepared by means of the method according to any one of claims 1-7 or according to claim 8 as carrier for cells.

10. Use as claimed in claim 9, for production of substances.

11. Use as claimed in claim 9, for cultivation of cells.

12. Use as claimed in claim 11, wherein the biocompatible, porous material is used for cultivation of artificial skin, artificial organs, fatty tissue and blood vessels.

13. Use as claimed in any one of claims 10-12, wherein the cells are present both inside and on the outside of the biocompatible, porous material.

14. A method for implanting a biocompatible, porous material as carrier for cells in an individual for production of substances, comprising injection of said biocompatible, porous material, prepared by means of the method according to any one of claims 1-7 or 8, into the individual and then letting the cells on the biocompatible, porous material produce said substances.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/02778

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C08J 9/28, A61L 27/34, A61L 27/56, A61L 27/60
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)

IPC7: C08J, A61L

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

SE,DK,FI,NO classes as above

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

WPI-DATA, CHEM.ABS-DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4522753 A (IOANNIS V. YANNAS ET AL), 11 June 1985 (11.06.85), column 1, line 30 - line 68; column 2, line 33 - column 3, line 12, abstract, claims --	1-2,4-13
X	US 5856367 A (THOMAS H. BARROWS ET AL), 5 January 1999 (05.01.99), column 2, line 28 - line 34; column 4, line 47 - line 61; column 6, line 66 - column 7, line 3, column 7, line 16 - line 45, example 1, abstract, claims --	1-13

[X] Further documents are listed in the continuation of Box C. [X] 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 application or patent 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	Date of mailing of the international search report
7 May 2002	14-05-2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Monika Bohlin/Els Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/02778

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5686091 A (KAM W. LEONG ET AL), 11 November 1997 (11.11.97), column 2, line 58 - column 3, line 2; column 3, line 36 - column 4, line 13; column 6, line 27 - line 38, examples 1 and 5, claims, abstract --	1-13
Y	EP 0222718 A2 (NILSSON, KJELL G.C.), 20 May 1987 (20.05.87), page 2, line 8 - page 3, line 21, abstract, example 1 --	1-13
Y	US 5939323 A (ROBERT F. VALENTINI ET AL), 17 August 1999 (17.08.99), column 1, line 64 - column 2, line 59; column 6, line 19 - line 38; column 9, line 23 - line 34, abstract, claims --	1-13
Y	US 5723508 A (KEVIN E. HEALY ET AL), 3 March 1998 (03.03.98), column 1, line 66 - column 2, line 43; column 3, line 36 - line 44, abstract, claims --	1-13
Y	US 5290494 A (ALLAN G.A. COOMBES ET AL), 1 March 1994 (01.03.94), abstract, claims --	1-13
X	US 4955893 A (IOANNIS V. YANNAS ET AL), 11 Sept 1990 (11.09.90), column 2, line 10 - line 31; column 5, line 60 - line 61, abstract, claims --	1-2
X	Polymer (Korea), Volume 11, No 3, June 1987, Kim, Kea Yong et al, "Synthesis and Properties of Polymeric Skin Substitute (1) Evaluation of Bilayer Membrane Composed of Crosslinked Gelatin och Polyurethane" page 246 - page 253 --	1-2,4-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/02778

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 0062829 A1 (RUTGERS, THE STATE UNIVERSITY), 26 October 2000 (26.10.00), page 4, line 15 - page 5, line 26, abstract -- --------	1-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE01/02778

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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**
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.

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.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/01/02

International application No.
PCT/SE 01/02778

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		SE 464816 B,C	17/06/91
		SE 8504764 A	16/04/87
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		US 5492697 A	20/02/96
US 4955893 A	11/09/90	WO 8910728 A	16/11/89
WO 0062829 A1	26/10/00	AU 3566399 A	02/11/00
		EP 1173235 A	23/01/02