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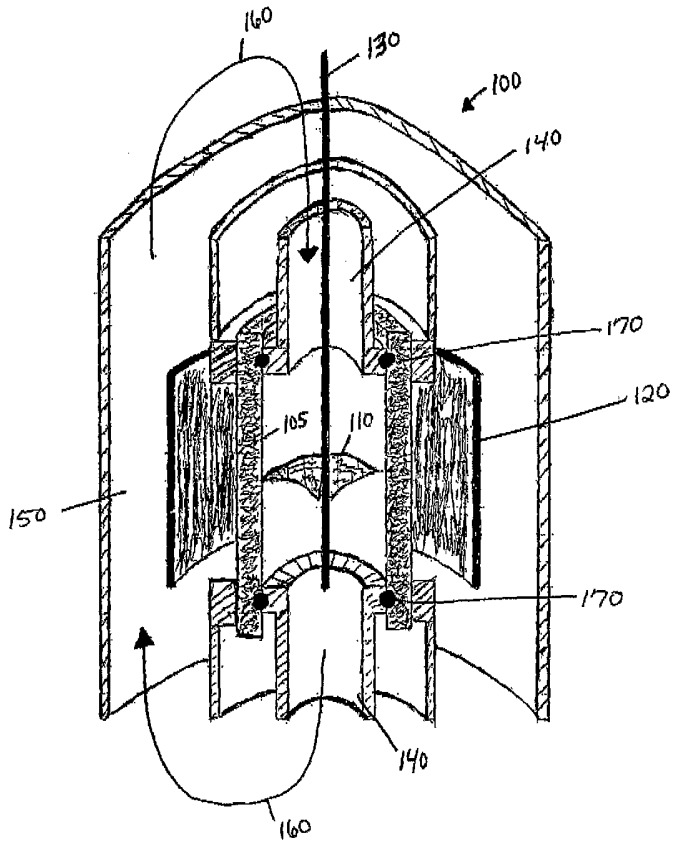
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ,

[Continued on next page]

(54) Title: DECELLURISATION PROCESSES FOR MAKING BIOPROTHESES



(57) Abstract: Methods for decellularizing mammalian tissue for use in transplantation and tissue engineering. The invention includes methods for simultaneous application of an ionic detergent and a nonionic detergent for a long time period, which may exceed five days. One method utilizes SDS as the ionic detergent and Triton-X 100 as the nonionic detergent. A long rinse step follows, which may also exceed five days in length. This long duration, simultaneous extraction with two detergents produced tissue showing stress-strain curves and DSC data similar to that of fresh, unprocessed tissue. The processed tissue is largely devoid of cells, has the underlying structure essentially intact, and also shows a significantly improved inflammatory response relative to fresh tissue, even without glutaraldehyde fixation. Significantly reduced in situ calcification has also been demonstrated relative to glutaraldehyde fixed tissue. Applicants believe the ionic and non-ionic detergents may act synergistically to bind protein to the ionic detergent and may remove an ionic detergent-protein complex from the tissue using the non-ionic detergent. The present methods find one exemplary use in decellularizing porcine heart valve leaflet and wall tissue for use in transplantation.

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OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/018578

A. CLASSIFICATION OF SUBJECT MATTER
A61L27/36

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)
A61L

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, PAJ, COMPENDEX, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 553 974 A (DEWANJEE ET AL) 19 November 1985 (1985-11-19) abstract column 4, line 66 - column 5, line 7; example 1	1-33, 57-72
X	US 4 801 299 A (BRENDEL ET AL) 31 January 1989 (1989-01-31) abstract column 6, line 13 - line 16	1-33, 57-72

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step 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
26 October 2005	10. 02. 2006

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pilling, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KASIMIR M -T ET AL: "Comparison of different decellularization procedures of porcine heart valves." INTERNATIONAL JOURNAL OF ARTIFICIAL ORGANS, vol. 26, no. 5, May 2003 (2003-05), pages 421-427, XP009055893 ISSN: 0391-3988 abstract Materials and Methods	1-33, 57-72
X	JONES M ET AL: "Anticalcification treatments of bioprosthetic heart valves: in vivo studies in sheep." JOURNAL OF CARDIAC SURGERY. MAR 1989, vol. 4, no. 1, March 1989 (1989-03), pages 69-73, XP009055892 ISSN: 0886-0440 abstract figure 1; table 1 page 70, column 2, line 40 - line 43	1-33, 57-72
X	JONES M ET AL: "The effects of anticalcification treatments on bioprosthetic heart valves implanted in sheep." ASAIO TRANSACTIONS / AMERICAN SOCIETY FOR ARTIFICIAL INTERNAL ORGANS. 1988 OCT-DEC, vol. 34, no. 4, October 1988 (1988-10), pages 1027-1030, XP009055891 ISSN: 0889-7190 abstract; figure 1; table 1 page 1028, column 2, line 8 - line 11	1-33, 57-72
X	US 2002/115208 A1 (MITCHELL SHANNON ET AL) 22 August 2002 (2002-08-22) abstract paragraph '0106!	1-33, 57-72
X	WO 95/24873 A (CRYOLIFE, INC) 21 September 1995 (1995-09-21) abstract page 12, line 4 - line 11	1-33, 57-72

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/018578

Box 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.:
because they relate to subject matter not required to be searched by this Authority, namely:

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 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:

see additional sheet

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.:

1-33, 57-72

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-33, 57-72

methods of treating excised cellular tissue or tissue culture constructs to form bioprostheses involving simultaneously contacting the tissue with ionic and neutral detergents; bioprostheses obtainable using these methods

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2. claims: 34-37

methods of sterilizing tissue involving cetylpyridinium chloride or derivatives thereof

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3. claims: 38-56

a tissue product in which proteins/nuclei have been removed

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2005/018578

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