

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
7 February 2008 (07.02.2008)

PCT

(10) International Publication Number  
**WO 2008/014665 A1**

(51) International Patent Classification:  
A61F 2/44 (2006.01) A61L 27/54 (2006.01)

(21) International Application Number:  
PCT/CN2007/002024

(22) International Filing Date: 28 June 2007 (28.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/820,378 26 July 2006 (26.07.2006) US

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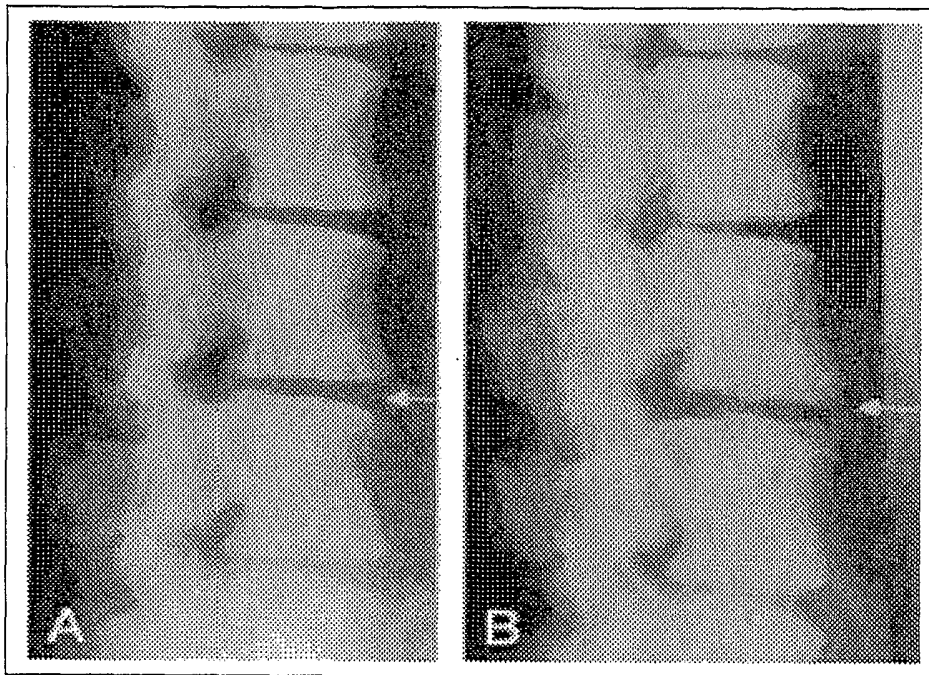
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, 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, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:  
— with international search report

(54) Title: INTERVERTEBRAL DISK TRANSPLANT AND IMPLANTATION METHOD



(57) Abstract: A natural spinal disk including adjacent vertebral structures is removed from a deceased donor, rinsed in normal saline, preserved in a cryopreservative solution, and then frozen by gradually decreasing the temperature using liquid nitrogen or similar method. The implant may be thawed in normal saline solution and then implanted in a patient in need of a vertebral disk replacement.

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## **INTERVERTEBRAL DISK TRANSPLANT AND IMPLANTATION METHOD**

### **CROSS REFERENCE TO RELATED APPLICATION**

This application claims priority based on U.S. Provisional Patent Application No. 60/820,378, filed June 26, 2006, the entire contents of which are incorporated herein by reference.

### **FIELD OF THE INVENTION**

This invention relates to an intervertebral disk transplant, and to methods of preparing, preserving, and implanting such a disk transplant.

### **BACKGROUND OF THE INVENTION**

Intervertebral disks provide mobility and a shock cushion between the vertebrae. Anatomically, a disk includes the nucleus pulposus in the center, which is composed of cells and an insoluble extracellular matrix which is produced by the nucleus itself. The extracellular matrix is composed of collagen, proteoglycans, and water. The nucleus pulposus is surrounded by the annulus fibrosis, which is also composed of cells (fibrocyte and chondrocyte), collagen fibers, and an extracellular matrix. The endplates are attached to the bony tissue, the vertebrae.

To date, there are a few treatments for degenerative disk disease. Spinal fusion has been widely used to obtain mechanical support and stability in various clinical situations,

such as degenerative disk disease or infective and malignant conditions that induce spinal instability. Although results are satisfactory in most cases, long term complications, such as adjacent-segment degeneration, have been reported and remain an issue. The mechanism of this degeneration is still unclear. Cadaver studies demonstrated increasing intradisk pressures in the adjacent unfused intervertebral disks after a simulated spinal fusion. Retrospective clinical analysis suggested that the risk of adjacent-segment failure is higher for patients in whom lumbar fusion with rigid instrumentation is performed. A few studies indicate that the altered mechanical environment after short rigid spinal fusion lead to the accelerated adjacent segment degeneration.

To avoid altering the biomechanical function of the intervertebral joint, efforts have been made to develop an artificial disk, as reported a few issued patents (U.S. Patent Nos. 6,958,078, 6,989,032, and 6,692,495 (all incorporated by reference herein)). Some of the difficulties with such developments include implant fixation, biocompatibility, and the identification of suitable materials and designs, which duplicate both form and function. Clinically, devices that replace a total disk exhibit certain weaknesses. Total disk replacements rely on attachments of the artificial disk to the vertebral endplates. Various methods of attachment have been described including the use of screws, spikes, and porous ingrowth material. The total disk/vertebral interface can loosen due to non-natural biomaterials. The problems with prosthesis loosening and revision surgery of the same are reported to be similar to that encountered with total knee and hip replacement surgery. As such, the long term outcomes of total replacement by artificial disks are still under review.

The desired treatment for degenerative disk disease therefore lies in treatment which preserves natural disk function. If disk function could be restored with biologic natural disk replacement, the weaknesses of current artificial disk replacement would be minimized, if not eliminated.

### **SUMMARY OF THE INVENTION**

This invention provides a method for treating degenerated, diseased or traumatized human intervertebral disks of the spine. According to the method, a natural allograft disk unit is harvested from a fresh human donor. This donor disk unit preferably includes an intervertebral disk including all of its structures, including the nucleus pulposus, the annulus fibrosis, the extracellular matrix, the endplates, and a portion of the vertebrae bony tissue on either side of the disk.

The donor disk unit can be engineered and processed to alter, stimulate, remove, or insert living cells therein using methods such as temperature changes, saline or other solution washes, and shape osteotomy. The extracellular matrix and original cells may be preserved. Other related cells, for example, nucleus pulposus, annulus fibrosis cells, and stem cells are cultured and transplanted into the natural allograft disk.

The transplantation is surgically performed, and the degenerated or diseased disk is removed from the patient, including the vertebral endplates and a portion of the bony vertebra from less than 1 mm to a whole segment on either side of the disk, thereby creating a void to be filled. The natural allograft disk is positioned within the surgically

created void. The invention further includes the procedures to insert, inject or surgically input one or more tissues, cells, scaffolds or substances to the natural allograft disk.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Further features and advantages of the invention may be understood by reference to the following detailed description of the preferred embodiments, reviewed in conjunction with the following drawings in which:

FIG. 1A is an x-ray of the spine of a patient before implantation of the allograft spinal disk of the present invention; and

FIG. 1B is an x-ray of the spine of the same patient after implantation of the allograft spinal disk of the present invention.

#### **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

This invention relates to a method of treating a degenerated, diseased or traumatized human intervertebral disk of the spine. According to the method, a natural allograft disk is harvested from a fresh human donor. This natural allograft preferably includes an intervertebral disk having all parts of its structure, namely the nucleus pulposus, the annulus fibrosis, the extracellular matrix, the endplates, and a portion of the vertebrae bony tissue from less than 1 mm to the whole bony vertebrae on either side of the disk. The natural allograft disk can further include growth factors, proteins, cytokines, culture media, differentiation factors, hydrogels, artificial scaffolds, antibiotics and/or useful combinations thereof. This natural allograft disk is used to restore function, and to reduce or eliminate pain in patients with degenerative or other disk disease.

Guidelines for tissue procurement including surgical technique of removal, number of hours between death of the donor and tissue procurement, and testing of the donor for infectious disease, are well described in the literature.

Preparation of the Natural Allograft Disk: The spinal column from C1 to S1 of a human donor removed up to a few hours after death is used to produce the natural allograft disk. The donor spine has to test negative for infectious diseases, as described in literature. After removal of the surrounding muscles, posterior elements, and other soft tissues, osteotomy is made at the endplates from less than 1 mm above and below the disk to including the whole vertebra. The disk grafts obtained are washed with saline solution, for example, and immersed in RPMI-1640 cryopreservative solution (10% dimethyl sulfoxide [DMSO] or similar solution) for ten minutes to 2 hours or for any longer time if needed, at 0° C or 4° C up to 200°C. The temperature is then reduced stepwise to 0° and then to -15° C for 1 hour, then to -40° C for 1 hour, or any useful combination thereof, and then -80° C for 1 hour, after which the disk grafts are preserved in liquid nitrogen (-196° C or lower) until surgery. The preservation period can be from one day to two years.

The natural allograft disk can be an autograft, allograft or xenograft disk comprising partially or fully decellularized annulus fibrosus, endplates and nucleus pulposus tissue of a donor vertebra. The annulus fibrosus, endplates and nucleus pulposus tissue can be cross-linked naturally or artificially. The cross-linked annulus fibrosus, endplates, and nucleus pulposus can be combined with natural or artificial scaffolds injected or surgically inserted. Preferably, the donor spinal column section is removed at the

endplates approximately 0 to 60 mm above and below the donor vertebrae or disks. The disk has a predetermined 3-dimensional shape, with a width of about 5-70 mm, a depth of about 5-50 mm, and a height of about 0.5-30 mm.

After removal from the donor, the disk is washed with saline and immersed in a preservative solution such as RPMI-1640 cryopreservative solution (10% dimethyl sulfoxide [DMSO] and/or serum) for 1 minute to one month.

The temperature can also be reduced stepwise or continuously in liquid nitrogen or other liquefied gas such as helium in a laboratory freezer, or with dried ice to 0°C, then to -40°C, then to -80°C and then finally to -200°C where the disk is preserved in liquid nitrogen or other suitably cold fluid, or in a cryogenic freezer.

Following disk preservation, the natural allograft disk is used to replace a degenerated, diseased or traumatized human intervertebral space. Surgical techniques to remove the disk and a portion of the vertebrae are well known to spine surgeons. The natural allograft disk is either not affixed or affixed using a fixation method including suturing, tissue glue, or an adhesive bonding material to surrounding tissues of the said disk area to be treated. A resorbable or nonresorbable bone implant material can be used where deemed beneficial to help affix the disk to the adjacent bone. The natural allograft disk is then placed into the surgically created void. The surgical procedure can be as follows, for example:

With the patient under general anesthesia, the diseased intervertebral disk is exposed. After removal of the diseased disk and the adjacent bony endplates, the preserved natural allograft disk of the most compatible size is selected and quickly thawed by immersion in physiologic saline at 37°C. The disk allograft is then positioned into the slot of the excised disk without internal fixation. On recovering from anesthesia, the patient is allowed free mobilization in a few hours to a few days with or without external support.

Growth factors, such as bone morphogenic proteins, living cells and stem cells are added to the natural allograft before and/or during the surgery, and a few hours, or a few weeks, or months following the surgery. The delayed insertion of the living cells may allow revascularization of the donor bony endplates prior to cell insertion. This endplate revascularization is important for diffusion of nutrition to and from the transplanted natural allograft disk cells. Alternatively, the living cells, growth factors, or other related substances can be added to the natural allograft before, during and after surgical transplantation.

Radiological Assessment is carried out before, during and after surgical transplantation as in Fig. 1. Anteroposterior and lateral radiographs of the transplanted spine unit of the patient can be taken before surgery and immediately after the surgery, and at any period of time post surgery. The anterior, middle, and posterior disk heights are measured on the lateral radiographs using a computerized digitizer. Segmental motion can be monitored by dynamic flexion-extension radiographs. The status of hydration or degeneration of the transplanted disk can be monitored with MRI.



References Cited

The following references are incorporated by reference herein:

U.S. Patent No.: 5,893,889

U.S. Patent No.: 5,899,941

U.S. Patent No.: 5,928,284

U.S. Patent No.: 6,080,579

U.S. Patent No.: 6,231,615

U.S. Patent No.: 6,228,118

U.S. Patent No.: 6,245,107

U.S. Patent No.: 6,332,779

U.S. Patent No.: 6,340,369

U.S. Patent No.: 6,352,558

U.S. Patent No.: 6,454,804

U.S. Patent No.: 6,648,919

U.S. Patent No.: 6,682,562

U.S. Patent No.: 6,692,495

U.S. Patent No.: 6,706,068

U.S. Patent No.: 6,716,245

U.S. Patent No.: 6,958,078

U.S. Patent No.: 6,989,032

The invention has been described with reference to the preferred embodiments. Various modifications can be made without departing from the spirit and scope of the invention. It is therefore intended that the invention be defined by the following claims.

**What is claimed is:**

1. A method for preparing and preserving an intervertebral disk for transplantation from one human to another, comprising:
  - obtaining surgical access to an intervertebral disk in a recently deceased or soon to be deceased patient;
  - removing the intervertebral disk including at least a portion of bony vertebrae on each side thereof;
  - rinsing the intervertebral disk with normal saline;
  - immersing the intervertebral in a cryopreservative solution; and
  - freezing the disk by gradually reducing the temperature thereof stepwise to about 0°C, then to about -15°C, then to about -40°C, then to about -80°C; and
  - maintaining the frozen disk at a temperature below about -150°C.
2. A method according to claim 1, wherein the disk is preserved in liquid nitrogen.
3. A method according to claim 2, wherein the disk is thawed after preservation by immersing the disk in saline at room or body temperature.
4. A method of replacing a damaged intervertebral disk in a patient comprising:
  - obtaining surgical access to the damaged intervertebral disk and removing the disk along with a portion of bony vertebrae on both sides of the disk;
  - inserting an intervertebral disk having a portion of bony vertebrae on both sides of thereof harvested from a deceased donor patient, the intervertebral disk being rinsed with normal saline, preserved in a cryopreservative solution and then frozen stepwise to

about 0°C, then to about - 15°C, then to about - 40°C, then to about -80°C, then to about -150°C and then thawed to room or body temperature.

5. A method according to claim 4, wherein growth factors, living disk cells, or stem cells are added to the disk before, during, or after surgery.
6. A method according to claim 2, wherein the disk is frozen in liquid nitrogen.
7. A method according to claim 4, wherein the implant is thawed by immersion in normal saline solution.
8. A method according to claim 4, wherein growth factors, cytokines, differentiation factors, hydrogels, artificial scaffolds, antibiotics, living cells including stem cells, or living disk cells are added to the disk or the region adjacent the disk before, during or after surgery.

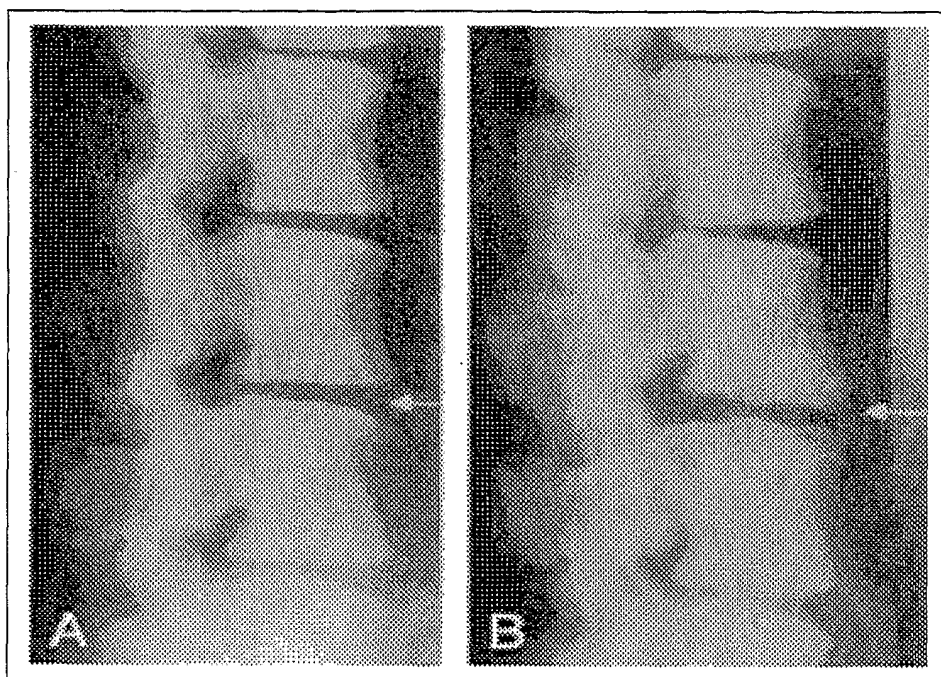


Figure 1.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CN2007/002024

**A. CLASSIFICATION OF SUBJECT MATTER**

see the extra sheet

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)

IPC: A61F A61M A61L A61N

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 practicable, search terms used)

WPI EPODOC CNPAT PAJ CNKI

INTERVERTEBRAL DISK TRANSPLANTAT+ FREEZ+ STOR+ PRESERV+

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN1528469A, (NANJING GEN HOSPITAL NANJING MILITARY RE), 15 Sep. 2004(15.09.2004), page 4 lines 15 to 19	1-3(part of)
Y	YUAN,Dike et al., Frozen Intervertebral Disc Allografting, CHINESE JOURNAL OF ORTHOPAEDICS, January 1999, vol.19, No.1, page 55, ISSN 0253-2352	1-3(part of)

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 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  
25 Sep. 2007(25.09.2007)

Date of mailing of the international search report  
18 Oct. 2007 (18.10.2007)

Name and mailing address of the ISA/CN  
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2007/002024

## 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.: 1-3 (part of); 4-8  
because they relate to subject matter not required to be searched by this Authority, namely:  
for claims 1-3 (part of): when the methods include the step of obtaining surgical access to an intervertebral disk in a soon to be deceased patient, the claims 1-3 (part of) relate to a method carried out on the living human. So the claims 1-3 (part of) belong to the subject-matters under Article 17(2)(a)(i).  
for claims 4-8: the methods relate to the methods for treatment of the human body by surgery, so the claims 4-8 belong to the subject-matters under Article 17(2)(a)(i).
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 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 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.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
PCT/CN2007/002024

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN1528469A	15.09.2004	none	

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/CN2007/002024

**A. CLASSIFICATION OF SUBJECT MATTER;**

A61F2/44(2006.01)i

A61L27/54(2006.01)n