

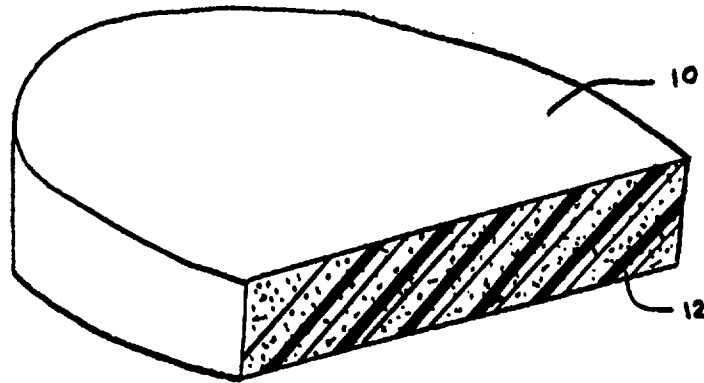


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : A61F 2/44</p>	<p>A1</p>	<p>(11) International Publication Number: <b>WO 98/05274</b> (43) International Publication Date: 12 February 1998 (12.02.98)</p>
--	-----------	---

<p>(21) International Application Number: PCT/US97/13854 (22) International Filing Date: 6 August 1997 (06.08.97) (30) Priority Data: 08/694,191 8 August 1996 (08.08.96) US (71) Applicant: THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; Suite 300, 3700 Market Street, Philadelphia, PA 19104 (US). (72) Inventors: GAN, Jean, Chin, Chin; 222 East Montgomery Avenue #206, Ardmore, PA 19003 (US). DUCHEYNE, Paul; 763 Applegate Lane, Rosemont, PA 19010 (US). VRESILOVIC, Edward; 3207 Baring Street, Philadelphia, PA 19104 (US). SHAPIRO, Irving; 426 South 26th Street, Philadelphia, PA 19146 (US). (74) Agents: ELDERKIN, Dianne, B. et al.; Woodcock Washburn Kurtz Mackiewicz &amp; Norris LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103 (US).</p>	<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i></p>
--	---

(54) Title: COMPOSITIONS AND METHODS FOR INTERVERTEBRAL DISC REFORMATION



(57) Abstract

Methods of reforming degenerated intervertebral discs are provided in accordance with methods of the invention. Hybrid materials useful in methods of the present invention are also provided.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece	<b>ML</b>	Mali	<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>MN</b>	Mongolia	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MR</b>	Mauritania	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MW</b>	Malawi	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MX</b>	Mexico	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>NE</b>	Niger	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NL</b>	Netherlands	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NO</b>	Norway	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NZ</b>	New Zealand	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>PL</b>	Poland		
<b>CM</b>	Cameroon	<b>KR</b>	Republic of Korea	<b>PT</b>	Portugal		
<b>CN</b>	China	<b>KZ</b>	Kazakstan	<b>RO</b>	Romania		
<b>CU</b>	Cuba	<b>LC</b>	Saint Lucia	<b>RU</b>	Russian Federation		
<b>CZ</b>	Czech Republic	<b>LI</b>	Liechtenstein	<b>SD</b>	Sudan		
<b>DE</b>	Germany	<b>LK</b>	Sri Lanka	<b>SE</b>	Sweden		
<b>DK</b>	Denmark	<b>LR</b>	Liberia	<b>SG</b>	Singapore		
<b>EE</b>	Estonia						

## COMPOSITIONS AND METHODS FOR INTERVERTEBRAL DISC REFORMATION

### FIELD OF THE INVENTION

The present invention concerns methods and materials useful for reforming degenerated discs of the spine of a vertebrate and in particular the spine of a human.

### BACKGROUND OF THE INVENTION

Back pain is one of the most frequently reported musculoskeletal problems in the United States. 80% of the adults will miss work at least three times in their career due to back pain. The most common factor causing low back pain is the degeneration of the disc. At the ages between 35 to 37, approximately a third of the US population have suffered from a herniated disc.

The main functions of the spine are to allow motion, transmit load and protect the neural elements. The vertebrae of the spine articulate with each other to allow motion in the frontal, sagittal and transverse planes. As the weight of the upper body increases, the vertebral bodies which are designed to sustain mainly compressive loads, increase in size caudally. The intervertebral disc is a major link between the adjacent vertebrae of the spine. The intervertebral disc, the surrounding ligaments and muscles provide stability to the spine.

- 2 -

The intervertebral discs make up about 20-33% of the lumbar spine length. They are capable of sustaining weight and transferring the load from one vertebral body to the next, as well as maintaining a deformable space to accommodate normal spine movement. Each disc consists of a gelatinous nucleus pulposus surrounded by a laminated, fibrous annulus fibrosus, situated between the end plates of the vertebrae above and below.

The nucleus pulposus contains collagen fibrils and water-binding glycosaminoglycans. At birth, the nucleus pulposus contains 88% water, however, this percentage decreases with age. This water loss decreases its ability to withstand stress. The annulus fibrosus consists of fibrocartilaginous tissue and fibrous protein. The collagen fibers are arranged in between 10 to 20 lamellae which form concentric rings around the nucleus pulposus. The collagen fibers within each lamella are parallel to each other and runs at an angle of approximately 60 degrees from vertical. The direction of the inclination alternates with each lamellae. This crisscross arrangement enables the annulus fibrosus to withstand torsional and bending loads. The end-plates are composed of hyaline cartilage, and are directly connected to the lamellae which form the inner one-third of the annulus.

When under compressive loads, the nucleus pulposus flattens and bulges out radially. The annulus fibrosus stretches, resisting the stress. The end-plates of the vertebral body also resist the ability of the nucleus pulposus to deform. Thus, pressure is applied against the annulus and end-plate, transmitting the compressive loads to the vertebral body. When tensile forces are applied, the disc is raised to a certain height straining the collagen fibers in the annulus. At bending, one side of the disc is in tension while the other side is in compression. The annulus of the compressed side bulges out.

- 3 -

When the disc is subjected to torsion, there are shear stresses which vary proportionally to the distance from the axis of rotation, in the horizontal and axial plane. The layer of fibers oriented in the angle of motion is in tension  
5 while the fibers in the preceding or succeeding layer are relaxed. Similarly in sliding, the fibers oriented in the sliding direction are in tension while the fibers in the other layers relax.

Repeated rotational loading initiates circumferential  
10 tears in the annulus fibrosus, which gradually form radial tears into the nucleus pulposus until the nucleus degrades within the disc. In addition to the water loss which occurs with age, more water is also lost due to nucleus rupture, thereby reducing its ability to resist compressive loads. As  
15 such, the annulus bulges. As the severity of the tear increases, much of the contents of the disc is lost leaving a thin space of fibrous tissue. This condition is called disc resorption.

Increasing disc collapse can cause facet subluxation  
20 and stenosis of the intervertebral foramen. Subsequently, the degenerative process involves the facet joints equally. As the annulus bulges out posteriorly into the spinal canal, the nerve root may be compressed causing sciatica. Pain is felt from the lower back to the buttocks and the leg. Following the  
25 rupture of the disc, excessive motions such as excessive extension or flexion can occur, resulting in spine segmental instability. The spine is thus more vulnerable to trauma. Herniation can occur due to disc degeneration or excessive load factors especially compression. Pain may result due to nerve  
30 root compression caused by protrusions.

The unstable phase of the degeneration progress allowing excessive movement may result in degenerative spondylolisthesis, which is a breakdown of posterior joints. The nerve is trapped between the inferior articular facet of  
35 the vertebrae above and the body of that below. Thus, sliding of a vertebral body on another damages the posterior joints due

- 4 -

to fatigue and apply traction on the nerve root causing pain.

Surgical treatments for herniated disc include laminectomy, spinal fusion and disc replacement with protheses.

At this time, 150,000 spinal fusion procedures are  
5 performed per year in the US alone, and the numbers are growing exponentially. However, the results of spinal fusions are very varied. Some of the effects include non-unions, slow rate of fusion even with autografts, and significant frequency of morbidity at the graft donor site. In addition, even if the  
10 fusion is successful, joint motion is totally eliminated. Adverse effects of spinal fusions have also been reported on adjacent unfused segments such as disc degeneration, herniation, instability spondylolysis and facet joint arthritis. A long-term follow-up of lower lumbar fusions in  
15 patients from 21 to 52 years of age found that 44% of patients with spinal fusions were currently still experiencing low-back pain and 57% had back pain within the previous year. 53% of the patients tracked were on medication, 5% had late sequela secondary surgery, 15% had a repeat lumbar surgery, 42% had  
20 symptoms of spinal stenosis, and 45% had instability proximal to their fusion. This clinical data shows that significant long-term limitations are associated with spinal fusion.

An alternative to spinal fusion is the use of an intervertebral disc prosthesis. Ideally, a successful disc  
25 prosthesis will simulate the function of a normal disc. The disc replacement must be capable of sustaining weight and transferring load from one vertebral body to the next. It should be robust enough not to be injured during movement and should maintain a deformable space between the vertebral body  
30 to accommodate movement.

Disc protheses should last for the lifetime of the patient, should be able to be contained in the normal intervertebral disc space, should have sufficient mechanical

- 5 -

properties for normal body function, should be able to be fixed to the vertebrae adjacent to the disc, should be possible to implant, should not cause any damage should the disc fail, and should be biocompatible.

5           There are at least 56 artificial disc designs which have been patented or identified as being investigated, McMillin C.R. and Steffee A.D., *20th Annual Meeting of the Society for Biomaterials* (abstract)(1994), although not all these devices have actually been made or tested. They can be  
10 divided into two main categories. Lee et al., *Spine*, Vol. 16, 253-255(1991). A first class includes devices for nucleus pulposus replacements which includes metal ball bearing, a silicone rubber nucleus, and a silicone fluid filled plastic tube. Devices for total or subtotal replacement of the disc  
15 have also been proposed such as a spring system, low-friction sliding surfaces, a fluid filled chamber, elastic disc prosthesis and elastic disc encased in a rigid column.

          An example of total disc replacement is described by Urbaniak et al., *Bio. J. Med. Mater. Res. Sym.*, Vol. 4, 165-186  
20 (1973) who developed and tested, using chimpanzees, an intervertebral disc device made of a central silicone layer sandwiched between two layers of Dacron embedded in the silicone. The open-mesh Dacron was chosen to allow tissue ingrowth for fixation to the adjacent vertebrae. While spinal  
25 mobility was restored and the device tolerated by the host, due to inexact fit of the device, bone resorption and reactive bone formation were observable. Loose fibrous tissue also indicated possible movement of the device.

          Hou et al., *Chinese Medical Journal*, Vol. 104(5),  
30 381-386 (1991), developed a disc implant made of silicone rubber which restored normal disc function. However, the presence of fibrous tissue surrounding the implant indicated possible movement of the device.

          The SB Charite intervertebral disc endoprosthesis,  
35 White and Panjabi, *Clinical biomechanics of the lumbar spine*, Churchill Livingstone, London (1989), which has been tested

- 6 -

clinically, is fabricated from a biconvex polyethylene core sandwiched between two concave-molded titanium end-plates. However, the endoprosthesis shows insufficient mechanical performance and unlikely long-term bone fixation to the device.

5 Two types of disc prostheses were developed and evaluated by Lee et al., *35th Annual Meeting of the Orthopaedic Research Society, Las Vegas, Nevada, February 6-9 (1989)*; Dacron fiber-reinforced polyurethane elastomer (reinforcement located for the annulus section), and a prosthesis made from  
10 thermoplastic polymer which is increasingly rigid moving from the nucleus out to the end-plates. Yet another design is made of cobalt-chromium-molybdenum (Co-Cr-Mo) alloy by Hedman et al., *Spine*, Vol. 16, 256-60 (1991).

U.S. Patent No. 4,911,718 (Lee et al.), U.S. Patent  
15 No. 5,002,576 (Fuhrmann et al.), U.S. Patent No. 4,911,718 (Lee et al.) and U.S. Patent No. 5,458,642 (Beer et al.) also teach permanent intervertebral disc endoprosthesis for total disc replacement.

All of foregoing intervertebral disc prostheses,  
20 however, merely replace all or a part of the disc with synthetic materials which must remain in place *ad infinitum*. These prostheses are generally permanent implants which require observation of long term biologic responses throughout the life of the prothesis. Furthermore, discs that are not comprised  
25 of biocompatible material may be rejected by the patient.

Procedures by which the tissues of the intervertebral disc are made to reform or replace the degenerated tissue of the intervertebral disc, would be highly desirable and a significant improvement over the current state of the art which  
30 presently use such permanent implants. Although efforts at tissue-engineering have been reported, no one has, until now, accomplished reformation of intervertebral disc tissue.



- 7 -

Repair of skin tissue has been achieved. For instance, skin deficiencies which arise in severely burnt patients or in decubitus wounds of diabetic patients have been so treated. Sabolinski, *Biomaterials*, Vol. 17, 311-320 (1996).  
5 Cells are seeded onto templates of either resorbable or non-resorbable material. Once tissue begins to form the templates are dressed onto the site in need of treatment. Tissue engineering of the skin, however, is significantly different from tissue engineering of the intervertebral disc because  
10 tissue compositions differ significantly. In addition, the mechanical requirements of engineered skin tissue are significantly different from those of intervertebral disc tissue.

Some intervertebral disc prostheses provide for  
15 regrowth of the intervertebral disc and concurrent resorption of the prosthesis. For example, U.S. Patent Nos. 4,772,287 and 4,904,260 (Ray et al.) teach prosthetic discs having an outer layer of strong, inert fibers intermingled with bioresorbable materials which attract tissue ingrowth. However, this  
20 prosthesis is purely a synthetic material at the time of implantation and does not include any cells or developing tissue. In addition, it provides only partial resorption and the problems associated with permanent implants remain.

U.S. Patent Nos. 5,108,438 and 5,258,043 (Stone)  
25 teach a porous matrix of biocompatible and bioresorbable fibers which may be interspersed with glycosaminoglycan molecules. The matrix serves as a scaffold for regenerating disc tissue and replaces both the annulus fibrosus and nucleus pulposus. However, replacement of this much tissue is a relatively  
30 invasive procedure which requires lengthy recovery time. Furthermore, these matrices do not use any cells to stimulate tissue recovery nor is there any incipient tissue formation in this device at the time of implantation.

Various materials have been seeded with cells in  
35 order to facilitate cell function including proliferation and extracellular matrix synthesis. For instance, El-Ghannam, et al., *Journal of Biomedical Materials Research*, Vol. 29, 359-370

- 8 -

(1974), teaches *in vitro* synthesis of bone-like tissue using bioactive glass templates. Schepers, et al., *J. Oral Rehab.*, Vol. 18, 439-452 (1991), analyzed the use of bioactive glass as fillers for bone lesions. Also, porous polymeric matrices  
5 have been used. The polymers include poly(lactic acid), poly(glycolic acid) and their co-polymers. However, these polymers have not been taught to be appropriate substrates for intervertebral disc cells which until now have not been used to seed implants of any sort.

10           Ideally, intervertebral disc treatment would guide and possibly stimulate the reformation of the tissue of affected intervertebral disc, especially nucleus pulposus and annulus fibrosus tissue. It could also biodegrade while allowing concurrent nucleus pulposus and annulus fibrosus  
15 tissue ingrowth, thereby providing for disc regeneration. Such an intervertebral disc material which is biodegradable while still satisfying the mechanical requirements of an intervertebral disc, has not been available until now.

#### OBJECTS OF THE INVENTION

20           An object of the present invention is to provide a method of inducing and/or guiding intervertebral disc reformation using biodegradable support substrates.

          In yet another object of the present invention is provided biodegradable substrates useful for intervertebral  
25 disc tissue reformation.

          Still another object of the invention is to provide material useful for guiding and/or stimulating intervertebral disc tissue reformation.

          Another object of the invention is to provide methods  
30 of culturing intervertebral disc cells.

- 9 -

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a perspective view of an exemplary hybrid material of the present invention.

**SUMMARY OF THE INVENTION**

5           In accordance with methods of the present invention there are provided methods for repairing damaged or degenerated intervertebral discs. These methods comprise evacuating tissue from the nucleus pulposus portion of a degenerated intervertebral disc space, preparing hybrid material by  
10 combining isolated intervertebral disc cells with a biodegradable substrate, and implanting the hybrid material in the evacuated nucleus pulposus space. In accordance with methods of the invention intervertebral disc cell growth is guided and/or stimulated and intervertebral disc tissue is  
15 reformed.

Methods of culturing intervertebral disc cells are also provided in some aspects of the invention whereby intervertebral disc tissue is digested with collagenase and incubated in medium supplemented with hyaluronidase.

20           In still other aspects of the invention biodegradable substrates are provided comprising polymer foam coated with bioactive materials, which substrates are useful for intervertebral disc tissue reformation.

In yet another aspect of the invention are provided  
25 hybrid materials for reforming degenerate intervertebral disc tissue. The hybrid materials can be made in the form of shaped bodies comprising biodegradable substrate and intervertebral disc cells.

**DETAILED DESCRIPTION OF THE INVENTION**

30           The present invention is directed to methods of inducing intervertebral disc repair by reformation of intervertebral disc tissue. By implanting a hybrid material comprising intervertebral disc cells and a biodegradable

- 10 -

support substrate into the intervertebral disc space, ingrowth of intervertebral disc cells is induced. Thus, the present invention provides methods of inducing self-regeneration of viable tissue and functional joints.

5           Methods of the present invention are useful to treat vertebrates suffering from degenerated intervertebral disc conditions, and in particular may be used to treat humans with such conditions.

10           A degenerated intervertebral disc has lost or damaged some or all of its intervertebral disc tissue, primarily including its nucleus pulposus tissue, due to any number of factors discussed herein, including age and stress due to rotational loading. Degenerated discs vary in severity from bulging discs to herniated or ruptured discs. Patients  
15 suffering from a degenerated disc experience a number of symptoms which include pain of the lower back, buttocks and legs and may also include sciatica and degenerative spondylolysis. In accordance with methods of the present invention reformation or regeneration of intervertebral disc  
20 tissue occurs *in situ*, replacing lost or damaged tissue and resulting in amelioration or elimination of the conditions associated with the degenerated disc.

          In accordance with the present invention hybrid materials used to induce and/or guide reformation of  
25 intervertebral disc tissue comprise biodegradable substrates. Biodegradable means that the substrate degrades into natural, biocompatible byproducts over time until the substrate is substantially eliminated from the implantation site and, ultimately, the body. Preferably in accordance with methods of  
30 the present invention, the rate of biodegradation of the substrate is less than or equal to the rate of intervertebral disc tissue formation such that the rate of tissue formation

- 11 -

is sufficient to replace the support material which has biodegraded.

In some aspects of the present invention the biodegradable substrate may be bioactive. Bioactive, as used  
5 herein, is meant to refer to substrates which enhance cell function as compared to cell function of the same cell type in the absence of the substrate. For instance, bioactive glass granules have been shown to enhance cell growth of typical bone cells. Schepers et al., U.S. Patent No. 5,204,106. In  
10 addition, dense bioactive glass discs have been found to enhance osteoprogenitor cell differentiation beyond even those levels of enhanced differentiation elicited by bone morphogenic protein. H. Baldick, et al., Transactions 5th World Biomaterials Conference, Toronto, II-114 (June, 1996).

15 The biodegradable substrate must also have sufficient mechanical strength to act as a load bearing spacer until intervertebral disc tissue is regenerated. In addition, the biodegradable substrate must be biocompatible such that it does not elicit an autoimmune or inflammatory response which might  
20 result in rejection of the implanted hybrid material.

Biodegradable support substrates useful in methods of the present invention include bioactive glass, polymer foam, and polymer foam coated with sol gel bioactive material.

In accordance with some methods of the present  
25 invention bioactive glass is employed as a substrate. Bioactive glass is described in U.S. Patent No. 5,204,104, incorporated by reference herein in its entirety. The bioactive glass contains oxides of silicon, sodium, calcium and phosphorous in the following percentages by weight: about  
30 40 to about 58% SiO<sub>2</sub>, about 10 to about 30% Na<sub>2</sub>O, about 10 to about 30% CaO, and 0 to about 10% P<sub>2</sub>O<sub>5</sub>. In preferred embodiments of the invention the nominal composition of bioactive glass by weight is 45% SiO<sub>2</sub>, 24.5% Na<sub>2</sub>O, 24.5% CaO and 6% P<sub>2</sub>O<sub>5</sub> and is known as 45S5 bioactive glass. Bioactive  
35 glass may be obtained from commercial sources such as Orthovita, Inc. (Malvern, PA).

- 12 -

Granule size of the bioactive glass is selected based upon the degree of vascularity of the affected tissue and generally will be less than about 1000  $\mu\text{m}$  in diameter. In some embodiments of the present invention it is preferred that the  
5 bioactive glass granules be from about 200  $\mu\text{m}$  to about 300 $\mu\text{m}$  in diameter. In still other embodiments of the present invention granule size is from about 50 $\mu\text{m}$  to about 100 $\mu\text{m}$ .

In some embodiments of the present invention bioactive glass has pores. Percent density (100%- percent  
10 porosity) of less than about 80% may be used in some aspects of the invention. Percent density of about 10% to about 68% can be used for other aspects of the invention. In some aspects of the present invention the pore size should be less than about 850  $\mu\text{m}$  in diameter while about 150 $\mu\text{m}$  to about 600  $\mu\text{m}$  pore  
15 diameter is preferred.

One method of preparing porous bioactive glass is by mixing bioactive glass granules of a desired size with sieved sacrificial agent camphor particles of a desired amount and size. The camphor sublimates during sintering leaving pores  
20 in the sintered glass. Thus, the average particle size and weight percent of the camphor particles is chosen to optimize the pore size and percent porosity, respectively, of the glass. In some aspects of the invention particle size may be less than about 850  $\mu\text{m}$  in diameter while about 150 $\mu\text{m}$  to about 600 $\mu\text{m}$  is  
25 preferred. Thereafter the glass may be treated with any aqueous buffer solution containing ions, the identity and concentration of which is found in interstitial fluid. Said treatments result in the formation of a calcium phosphate rich layer at the glass surface. Typical buffers include those  
30 prepared as described by Healy and Ducheyne, *Biomaterials*, Vol. 13, 553-561 (1992), the subject matter of which is incorporated herein by reference in its entirety.

- 13 -

In still other aspects of the present invention the support substrate comprises polymer foam. Polymer foam useful in these aspects of the invention are biocompatible and include polyglycolide (PGA), poly(D,L-lactide) (D,L-PLA), poly(L-lactide) (L-PLA), poly(D,L-lactide-co-glycolide), (D,L-PLGA), poly(L-lactide-co-glycolide) (L-PLGA), polycaprolatone (PCL), polydioxanone, polyesteramides, copolyoxalates, and polycarbonates. D,L-PLGA, which is preferred in some embodiments of the invention, may comprise 10 50% polylactide and 50% polyglycolide. About 75% polylactide and about 25% polyglycolide is still more preferred although it is anticipated that ratios may be varied to optimize particular features of the individual polymers. For instance, the mechanical strength of a polymer may be adjusted by varying 15 the percentage of PLA and the percentage of PGA may be adjusted to optimize cell growth.

In some aspects of the invention polymer foam is coated with sol gel bioactive material. Sol gel materials include glasses and ceramics. Such bioactive compounds are prepared by mixing a desired polymer foam with NaCl to create 20 the desired porosity and pore size. Thereafter, the polymer, including the pores and interstices, is coated with sol gel material.

Sol gel glass is prepared by combining a metal 25 alkoxide precursor with water and an acid catalyst to produce a gel. A typical process is described in U.S. Serial No. 08/477,585 (U.S. Patent No. \_\_\_\_\_) which is incorporated by reference herein in its entirety. Once dried the gel consists mostly of metal oxide with a glass consistency. Sol gel 30 bioactive material may be comprised of from about 60 to about 100% silicon dioxide, up to about 40% calcium oxide and up to about 10% diphosphorous pentoxide. A final product of 70% SiO<sub>2</sub>, 25% CaO and 5% P<sub>2</sub>O<sub>5</sub> is preferred in some methods of the present invention although the concentration of each may be adjusted 35 to optimize critical features of the sol gel. Other sol gel materials may be prepared by methods known in the art. For instance, Qui, Q., et al., *Cells and Materials*, Vol. 3, 351-60

- 14 -

(1993), incorporated by reference herein in its entirety, teaches methods of preparing calcium phosphate sol gel bioactive material.

To coat the polymer, the polymer foam is dipped into  
5 the sol during the sol gelation phase. The sol-filled foam is then placed in a syringe filter and the sol is pulled through the foam by creating a vacuum below using the syringe. Thus, the polymer is substantially coated with sol gel, with residual sol gel being evacuated from the polymer matrices. While it  
10 is preferred that most or all of the polymer surfaces, including the surfaces of the pores and interstices, be coated with sol gel bioactive glass, polymers which are only partially coated with sol gel bioactive glass may also be useful in some aspects of the present invention. It is desired in some  
15 embodiments of the invention that greater than about 50% of the polymer surface be coated.

To prepare the hybrid material, intervertebral disc cells are combined with biodegradable substrate material. Intervertebral disc cells may be isolated from tissue extracted  
20 from any accessible intervertebral disc of the spine. For instance, tissue may be extracted from the nucleus pulposus of lumbar discs, sacral discs or cervical discs. Preferably, intervertebral disc cells are primarily nucleus pulposus cells. In some embodiments it is preferred that disc cells are at  
25 least 50% nucleus pulposus cells while 90% nucleus pulposus cells is still more preferred. Cells may be obtained from the patient being treated, or alternatively cells may be extracted from donor tissue.

The present invention provides advantages over prior  
30 art methods in that the entire degenerated disc need not be removed to treat a degenerated disc. Rather, only the nucleus pulposus tissue need be evacuated from the degenerated intervertebral disc. Degenerated nucleus pulposus refers to



- 15 -

a region of the intervertebral disc where the tissue has severely reduced mechanical properties or which has lost some or most of the nucleus pulposus tissue. The present invention thus provides a less invasive procedure than that of the prior art. In addition, the methods and hybrid materials of the present invention prompt biological repair of normal tissue in the disc which will result in better long term results than that obtained with synthetic prostheses.

Evacuation of the degenerated intervertebral disc tissue, and primarily the nucleus pulposus tissue, is performed using known surgical tools with procedures developed to meet the needs of the present invention. Generally an incision or bore is made at the lateral edge in the annulus fibrosus and the intervertebral disc tissue is extracted from the nucleus pulposus via, for example, the guillotine cutting approach. The tissue may be extracted using a scalpel, bore, or curette. Alternatively, tissue may be aspirated. Ideally, the annulus fibrosus, or significant portions thereof, are left intact. It is preferred for instance, that at least 50% of the annulus fibrosus remain intact. It is still more preferred that at least 85% of the annulus fibrosus remain intact. Arthroscopic techniques are most preferred in accordance with methods of the present invention.

Similar surgical techniques are utilized to extract intervertebral disc tissue from other, non-degenerate intervertebral discs of the spine of the patient or donor. For instance, similar techniques may be used to obtain intervertebral tissue from sacral discs. Minor modifications necessary to tailor the procedure to a particular region of the spine would be appreciated by those skilled in the art.

Where there is lag time between tissue evacuation and implantation of the hybrid material, the evacuated space may be temporarily filled with gel foam or other load bearing spacers known in the art.

Intervertebral disc cells are isolated from extracted tissue. Generally, tissue is fragmented and treated with

- 16 -

enzymes such as collagenase to disaggregate the cells into individual cells. Preferably isolated cells are primarily nucleus pulposus cells with 50% nucleus pulposus cells being preferred and 90% nucleus pulposus cells being more preferred.

5 The cells are isolated using centrifugation. Cells may then be combined with a biodegradable substrate and implanted into the evacuated nucleus pulposus. Alternatively, isolated intervertebral disc cells may be cultured alone or seeded onto a biodegradable substrate and cultured together with the

10 biodegradable substrate for later implantation.

In some aspects of the present invention the hybrid material may also include factors to enhance cell growth. For instance, TGF- $\beta$  and EGF may be added to the hybrid material to enhance cell growth. Cells may be incubated alone or seeded on

15 a substrate in a tissue culture medium such as Dulbecco's Modified Eagle Medium (DMEM) (pH 7.0), which may be supplemented with serum such as heat-inactivated fetal bovine serum. Antifungal and antibacterial agents may also be added. In preferred methods of the present invention cells are

20 incubated with about 0.5% to about 1.5% hyaluronidase.

In some aspects of the present invention the end plate may be partially decorticated to enhance vascularization. Thereafter, cells may be implanted or alternatively, after cell attachment, hyaluronidase is removed and incubation is resumed

25 with a medium supplemented with 0.001% ascorbic acid in the absence of hyaluronidase. Medium supplemented with 0.0025% ascorbic acid is used to replenish the cell solutions.

Hybrids of intervertebral disc cells and biodegradable substrate may then be implanted into the

30 evacuated intervertebral disc space using surgical procedure such as described above.

Hybrid materials are provided by the present invention. Such hybrids can then be shaped for insertion into

- 17 -

the intervertebral disc space of a patient. Exemplary Figure 1 shows a shaped hybrid material comprising biodegradable substrate and intervertebral cells. Intervertebral cells are located on the outer surface 10 and on the surfaces of the pores and interstices 12 of the shaped substrate. It should be noted that Figure 1 depicts one possible embodiment and should not be construed as limiting the invention in any way.

The substrate should generally have a rectangular shape. A cylindrical pad shape is also envisioned.

The following examples are illustrative but are not meant to be limiting of the present invention.

## EXAMPLES

### Example 1

#### Evacuation of Nucleus Pulposus

Mature New Zealand rabbits weighing 4 - 5 kg are used. For each rabbit, L4-L5 or, when possible L4-L5 and L5-L6 disc spaces are accessed as those are the biggest sections. The anesthetics Ketamine, HCl 30 mg/kg, and Xylazine 6 mg/kg, are administered intramuscularly. Using a paraspinal posterolateral splitting approach, the large cephalad-facing transverse process of the lumbar spine is identified and removed with a rongeur. The intervertebral disc can then be seen. An incision is made in the annulus fibrosus. Using a high-power surgical microscope, the nucleus pulposus tissue is scraped out carefully with a curette. The space is then packed with gel foam. The rabbit is closed provisionally.

### Example 2

#### Isolation of Intervertebral Disc Cells

Intervertebral disc tissue is obtained as described in Example 1 or from an amputated tail section. Under aseptic condition, the intervertebral disc tissue is diced with a scalpel and placed in a T25 tissue culture flask with Dulbecco's Modified Eagle Medium (DMEM) adjusted to pH 7.0,

- 18 -

supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin (TCM). The tissue is then treated with 0.25% collagenase for two hours at 37°C. An equal amount of TCM to collagenase is added to stop treatment. The mixture  
5 is centrifuged at 1000r/min for 10 minutes and supernatant is discarded. TCM is added and the mixture is filtered to remove debris. The mixture is again centrifuged and supernatant discarded. Cells are resuspended in TCM supplemented with 1% hyaluronidase (400u/ml).

### 10 Example 3

#### Culture of Intervertebral Disc Cells

Cells are cultured in TCM supplemented with 1% hyaluronidase (400units/ml) at 37°C in 5% CO<sub>2</sub>/95% air. Once cells attach medium is changed to TCM supplemented with 0.001%  
15 ascorbic acid in the absence of hyaluronidase. Cells are resuspended in fresh medium supplemented with 0.0025% ascorbic acid every 3 days.

### Example 4

#### Preparation of Bioactive Glass

20 Bioactive glass granules (45S5) having diameters of 40µm to 71 µm, and 200-300µm, can be obtained from Orthovita, Inc. (Malvern, PA). Prior to implantation or addition to cell culture, the specimens are sterilized in ethylene oxide.

### Example 5

#### 25 Preparation of Sintered Porous Bioactive Glass

Bioactive glass granules having diameters of 40µm to 71 µm can be obtained from Orthovita, Inc. (Malvern, PA). The glass granules are mixed with 20.2 weight % sieved sacrificial agent camphor C<sub>10</sub>H<sub>16</sub>O with grain size of 300µm to 500µm. The

- 19 -

mixture is mechanically mixed overnight, and cold pressed at 350 MPa. The disc obtained is heat treated at 575°C for 45 minutes. The heating rate is 10°C/min. It is then left to cool at room temperature. The disc is immersed in acetone for 30  
5 minutes and dried at 37°C. The disc is cut to the desired dimensions using a diamond-wheel saw. The disc is washed in acetone for 15 minutes. The specimen is then conditioned in tris buffer with electrolytes added (TE) (El-Ghannam, et al.,  
10 *Journal of Biomedical Materials Research*, Vol. 29, 359-370 (1974)), for 2 days to obtain the desired formation of calcium phosphate-rich layer at the glass surface. The specimen is rinsed with methanol and dried at 37°C. The specimen is analyzed using scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) spectroscopy and X-ray diffraction  
15 (XRD). Prior to implantation or introduction to cell culture, the specimen is sterilized in ethylene oxide.

#### Example 6

##### Preparation of Polymer Foam

3g of NaCl with particle sizes 300 $\mu$ m to 500 $\mu$ m, and  
20 2g of D,L-PLGA 75/25 (75% polylactide/25% polyglycolide) polymer foam were mixed. The dispersion is vortexed and cast in a 5cm petri dish. The solvent is allowed to evaporate from the covered petri dish for 48 hrs. To remove residual amounts of chloroform, the petri dish is vacuum-dried at 13 Pa for 24  
25 hrs. The material is then immersed in 250ml distilled deionized water at 37°C for 96 hrs. The water is changed every 12 hrs to leach out the salt. The salt-free membrane is air-dried for 24 hrs, followed by vacuum-drying at 13 Pa for 48 hrs. The material is then cut to the desired geometry with a  
30 razor blade. The membrane is stored in a desiccator under vacuum. The specimens are analyzed using SEM. The specimen will have 60% pore density with pore sizes 300 to 500 $\mu$ m. Prior to implantation or introduction into cell culture, the specimens are sterilized in ethylene oxide.

- 20 -

**Example 7****Preparation of Polymer Foam coated with Sol Gel Bioactive Glass**

Tetramethylorthosilane (TMOS), calcium methoxyethoxide and triethyl phosphate are mixed for 5 minutes in an argon atmosphere using a magnetic stirrer. Respective amounts of each are chosen such that the resulting product is 70% SiO<sub>2</sub>-25%CaO-5%P<sub>2</sub>O<sub>5</sub> (upon drying). They are mixed using a magnetic stirrer for 5 min. The PLGA polymer foam prepared according to Example 5 is dipped into the sol approximately halfway to gelation. The foam is dipped 2 to 3 times to make sure that the sol completely fills the polymer foam. The sol-filled foam is then placed in a syringe filter with appropriate filter pore size which only allows the sol to flow through. This syringe filter is attached to a syringe. The sol is pulled through the foam by creating a vacuum below using the syringe. EDAX and SEM are used to analyze pore size, porosity and the thickness/uniformity of the sol gel bioactive glass coating. Prior to implantation or introduction to cell culture, the specimens are sterilized in ethylene oxide.

**Example 8****Cell Phenotype**

Cell phenotype of cells cultured in accordance with the method of Example 3 is examined. Immunofluorescent staining of cells shows positive staining for proteoglycan and collagen type II, markers of intervertebral disc cell phenotype. Substantially negative staining for collagen I, an annulus fibrosus marker, was also observed initially. Subsequently, positive staining for collagen I was also observed.

- 21 -

**Example 9****Cell Reversion**

Intervertebral disc cells cultured as described in Example 3 are tested for reversion. Cells are placed in Eppendorf tube with TCM and spun down to form a pellet. Cell histology is examined after 4, 8 and 12 days by washing the pellet and fixing it with 70% ethanol. The cells are dehydrated, embedded and cut. The sample is stained with hematoxylin-eosin and toluidine blue.

10 The histology of cultured cells is compared to the histology of nucleus pulposus tissue prepared immediately upon retrieval. Histology of the cells evidences a reversion to the original morphology of the cells.

**Example 10****15 Implantation of Biodegradable Substrate**

Cells are prepared in accordance with Examples 2 and 3. Cells are counted. Biodegradable substrates prepared as described in Examples 4-7 each placed in a tissue culture dish and immersed in TCM for 1 hour. The cells are seeded onto each of the sterile biodegradable substrates prepared as described in Examples 4-7 in TCM with hyaluronidase and left to attach for at least one hour before flooding the dish with TCM. Cells are incubated overnight. Attachment is detected using SEM.

25 The rabbit treated as described in Example 1 is reopened per surgical technique described in Example 1, and the intervertebral disc space accessed. The gel foam is retrieved and the cell-biodegradable substrate hybrid material inserted in place. The wound is closed.

**Example 11****30 Effect on Neurological Function**

Regular post-operative neurological functions are evaluated to examine the subject for any spinal injury such as lameness. The effect of the hybrid material on the behavior of

- 22 -

the disc can be observed and generally compared by taking radiographs of the spine immediately pre-operation, post-operation and at 1 month time periods until the animal is sacrificed.

5 **Example 12**  
**Histological analysis**

Histological analysis is performed to determine cell ingrowth, cell types, tissue morphology, and absence of inflammation. To this end, the retrieved disc is fixed in 70%  
10 ethanol and dehydrated. After embedding in methyl methacrylate, sections are cut with a diamond saw, ground, polished with silicon carbide paper and diamond paste, and stained. Histology is done on normal discs and discs retrieved at the  
various time periods. Analysis will show ingrowth of cells  
15 with concurrent degradation of implanted hybrid material with little to no inflammation.



- 23 -

What is claimed is:

1. A method of reforming intervertebral disc tissue of a degenerated nucleus pulposus of a patient comprising the steps of evacuating intervertebral disc tissue from a degenerated nucleus pulposus; preparing a hybrid material by combining intervertebral disc cells with a biodegradable substrate; and implanting the hybrid material in the evacuated nucleus pulposus space.

2. The method of claim 1 wherein the biodegradable substrate is bioactive.

3. The method of claim 1 wherein the biodegradable substrate is bioactive glass, polymer foam, or polymer foam coated with sol gel bioactive material.

4. The method of claim 3 wherein the polymer foam is the copolymer D,L poly(lactide-co-glycolide).

5. The method of claim 4 wherein the copolymer comprises 75% polylactide and 25% glycolide.

6. The method of claim 1 further comprising isolating cells from an intervertebral disc.

7. The method of claim 6 wherein cells are isolated from the nucleus pulposus of an intervertebral disc.

8. The method of claim 6 further comprising culturing intervertebral cells on a biodegradable substrate prior to implantation.

- 24 -

9. The method of claim 6 further comprising culturing intervertebral cells prior to combining cells with a biodegradable substrate.

10. The method of claim 9 wherein intervertebral cells are combined with a biodegradable substrate to form a hybrid material immediately prior to implanting the hybrid in the evacuated nucleus pulposus space.

11. The method of claim 1 wherein the substrate is bioactive glass granules of less than  $1000\mu\text{m}$  in diameter.

12. The method of claim 1 wherein the substrate is porous.

13. The method of claim 12 wherein the substrate contains pores with diameter of less than about  $850\mu\text{m}$ .

14. The method of claim 12 wherein the substrate has a percent density of less than about 80%.

15. A bioactive substrate comprising a porous polymer foam coated with sol gel bioactive material.

16. A bioactive substrate of claim 15 wherein the polymer foam is D,L poly(lactide-co-glycolide).

17. A bioactive substrate of claim 15 wherein the sol gel bioactive material is comprised of from about 60 to about 100%  $\text{SiO}_2$ ; up to about 40%  $\text{CaO}$ ; and up to about 10%  $\text{P}_2\text{O}_5$ .

18. A bioactive substrate of claim 17 wherein the sol gel bioactive material has the nominal composition 70%  $\text{SiO}_2$ , 25%  $\text{CaO}$ , 5%  $\text{P}_2\text{O}_5$ .

- 25 -

19. A bioactive substrate of claim 15 having a pore size of less than about 850  $\mu\text{m}$ .

20. A bioactive substrate of claim 15 having a percent density less than about 80%.

21. A method of making a bioactive substrate comprising a porous polymer coated with sol gel bioactive material comprising the steps of:

coating a porous polymer with sol gel bioactive material; and

evacuating residual sol gel bioactive material from the pores of the polymer.

22. The method of claim 21 wherein the pores of the polymer are evacuated by creating a suction vacuum.

23. The method of claim 21 wherein the polymer foam is D,L poly(lactide-co-glycolide).

24. The method of claim 21 wherein the sol gel bioactive material is comprised of from about 60 to about 100%  $\text{SiO}_2$ , up to about 40%  $\text{CaO}$ ; and up to about 10%  $\text{P}_2\text{O}_5$ .

25. The method of claim 24 wherein the sol gel bioactive material has the nominal composition 70%  $\text{SiO}_2$ , 25%  $\text{CaO}$ , 5%  $\text{P}_2\text{O}_5$ .

26. The method of claim 21 wherein the polymer foam has a pore size of less than about 850  $\mu\text{m}$ .

27. The method of claim 21 wherein the polymer foam has a percent density of less than about 80%.

28. A method of preparing intervertebral disc cell culture comprising:  
obtaining intervertebral disc tissue;

- 26 -

treating tissue with collagenase;  
washing cells to remove collagenase; and  
incubating cells in a medium comprising hyaluronidase until  
cell attachment.

29. The method of claim 28 further comprising  
seeding cells on a biodegradable substrate.

30. The method of claim 28 further comprising  
replacing hyaluronidase medium with medium supplemented with  
ascorbic acid following cell attachment.

31. The method of claim 28 wherein the cells are  
incubated in 0.25% collagenase for 2 hours.

32. The method of claim 28 wherein the hyaluronidase  
medium comprises 1% hyaluronidase.

33. The method of claim 29 wherein the substrate is  
bioactive.

34. A hybrid material for reforming degenerated  
intervertebral disc tissue of a patient comprising a  
biodegradable substrate and intervertebral disc cells.

35. The hybrid material of claim 34 wherein the  
biodegradable substrate is bioactive.

36. The hybrid material of claim 34 shaped for  
surgical insertion into the intervertebral disc space.

- 27 -

37. The hybrid material of claim 34 wherein the biodegradable substrate is bioactive glass, polymer foam, or polymer foam coated with sol gel bioactive material.

38. The hybrid material of claim 37 wherein the polymer foam is the copolymer D,L poly(lactide-co-glycolide).

39. The hybrid material of claim 38 wherein the copolymer comprises 75% polylactide and 25% glycolide.

40. The hybrid material of claim 34 wherein the substrate is bioactive glass granules of less than 1000 $\mu$ m in diameter.

41. The hybrid material of claim 34 wherein the substrate contains pores with diameter of less than about 850 $\mu$ m.

42. The hybrid material of claim 33 wherein the substrate has a percent density of less than about 80%.

43. The hybrid material of claim 38 wherein the polymer foam is coated with a sol gel bioactive material comprised of from about 60 to about 100% SiO<sub>2</sub>, up to about 40% CaO; and up to about 10% P<sub>2</sub>O<sub>5</sub>.

44. The hybrid material of claim 39 wherein the polymer foam is coated with a sol gel bioactive material having a nominal composition of 70% SiO<sub>2</sub>, 25% CaO, 5% P<sub>2</sub>O<sub>5</sub>.

1/1

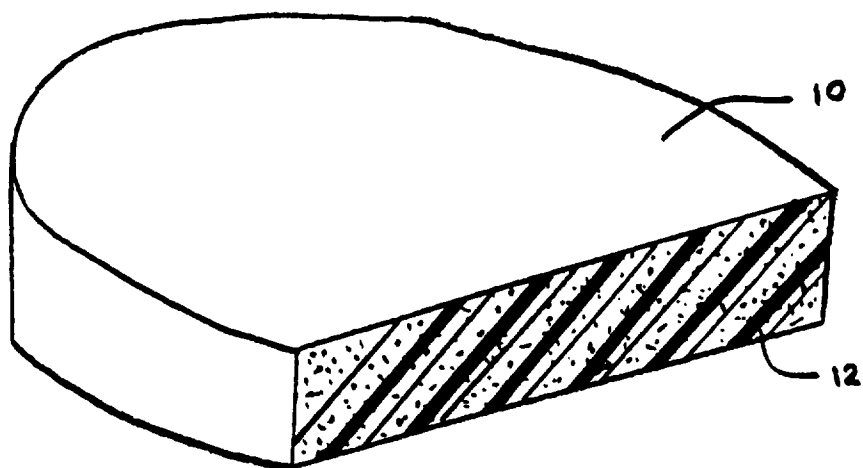


Fig 1

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/13854

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC(6) :A61F 2/44  
 US CL :623/17  
 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)  
 U.S. : 623/16, 17

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)  
 APS  
 Search Terms: sol gel, bioactive, nucleus pulposus

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,084,051 A (TORMALA et al) 28 January 1992, col. 6, lines 22-32; col. 17, lines 6-14; Fig. 2a; and Table 1.	15-17, 19, 20 ----- 18, 21-27, 42
X --- Y	US 5,290,552 A (SIERRA et al) 1 March 1994, col. 5, lines 16-47; and col. 6, lines 36-40.	1-14, 34-40, 43, 44 ----- 42
Y	US 5,074,916 A (HENCH et al) 24 December 1991, Table 1.	18
X	US 5,478,739 A (SLIVKA et al) 26 December 1995, entire document.	28-33

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

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier document published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search: 12 SEPTEMBER 1997  
 Date of mailing of the international search report: 29 OCT 1997

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks  
 Box PCT  
 Washington, D.C. 20231  
 Facsimile No. (703) 305-3230

Authorized official: *Tram Nguyen*  
 TRAM NGUYEN  
 Telephone No. (703) 308-0804