Intervertebral disc transplantation: a biological approach to motion preservation

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Received: 13 May 2008 / Revised: 5 July 2008 / Accepted: 5 July 2008 / Published online: 13 November 2008
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Abstract Intervertebral disc transplantation was developed in a bipedal animal model through the stages of autograft, fresh allograft and fresh frozen allograft. Results showed that the allografts were able to survive through a deep freezing protocol and maintain cell viability after transplantation without significant immunoreaction. Although degeneration of the allograft appeared to be inevitable, it was able to maintain stability and mobility of the functional spinal unit. These findings were similarly reproduced in the human clinical trial with excellent mid-term clinical results at 5 years. The process of evolution and findings were summarized in this review.

Keywords Intervertebral disc · Degeneration · Allograft · Transplantation · Motion preservation

Introduction

Intervertebral disc degenerative disease (DDD) is believed to begin at the nucleus pulposus where there is reduction of aggrecan core proteins, glycoaminoglycan, reduction of matrix turnover, reduction in cell numbers eventually resulting in loss of the water imbibing ability. Reduction of disc height puts excessive load on the annulus fibrosus leading to fissuring and cracks. Uneven loading of the facet

joints causes secondary arthritic changes with osteophyte formation and joint instability. Although commonly asymptomatic, DDD can be associated with pain with or without neurological involvement. The degenerated disc can be a source of pain in its own right. Although the nucleus pulposus is insensible, the annulus fibrosus, particularly the peripheral part, is well innervated with pain fibers. Bulging or rupturing of the annulus with herniation of disc material can also cause pain and neurological deficits when the pain-sensitive ligamentous structures or neural tissues are being implicated, either mechanically or chemically. At the cervical spine, compression of the cervical spinal cord or the nerve roots will lead to clinical signs and symptoms of myelopathy or radiculopathy while at the lumbar spine lumbar root compression may produce signs and symptoms of sciatica. Degenerated facet joints are also another potential source of pain, root compression, and together with the loss of disc height may produce instability, subluxation or deformity of the functional spinal unit (FSU).

Conservative modalities are always the first line of treatment. Surgical intervention is indicated for patients who have failed conservative means or who demonstrate significant neurological compromise. The goals of surgery are to relieve any neural compression and to maintain a stable FSU that is free of deformity. Current treatment for severe back pain usually involves excision of the disc and obligatory fusion of the spine. However, there are known long-term effects on the juxta-fusion segments in form of accelerated degeneration. There is still controversy on whether this phenomenon is part and parcel of the natural history of DDD in a genetically predisposed individual or simply a mechanical consequence of the fusion. Many non-fusion strategies have been attempted in the past five decades aiming at preserving the stability, mobility of the

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spinal segment while relieving the clinical symptoms of neural compression.

There are two general approaches to motion preservation in DDD: regeneration and replacement.

Regeneration or repair of the disc using growth factors, gene therapy and cell therapy are being actively researched [1]. Most of the attention is being focused on the nucleus pulposus on how to rejuvenate the disc through increasing cell numbers thus restoring matrix production. Although this is an exciting area it is still in the experimental stage. The major limitation of this approach is that the nucleus pulposus is not alone in the degenerative process. The problems in the annulus and the bony endplates are equally if not more important. By the time a disc is degenerated to the extent of being clinically symptomatic that needs treatment, the annulus fibrosus is also structurally and mechanically incompetent. The channels in the endplate through which nutrients are delivered to the disc are also jeopardized. The environmental niche in these advanced disc degeneration may be too hostile for sustained cell viability not to mention the expectation of these cells to restore matrix production and repair the structurally damaged annulus fibrosus. Preliminary data from our research team also suggests that cellular therapy is at best only able to maintain the matrix content when the degeneration is mild but is unable to restore it when the degeneration is severe [4, 7].

The second approach of disc replacement can be biological or artificial with the goals of preserving anatomy, motion and stability. Theoretically, one could manufacture a disc scaffold using tissue engineering technology. This can then be populated with the appropriate cells together with the necessary promoter growth factors. However, one should remember that the disc is a complex organ with different tissue components including bone, cartilage, different collagens and cell types that have different mechanical properties serving different functions. It will not be easy, if at all possible, to build such a complex organ that can stand the test of time. Artificial discs made of metals, polymers or a combination of materials have been attempted in the past five decades. The modern designs are certainly more sophisticated than the early ones and are gaining popularity in the recent years. The early results of total disc replacement (TDR) are undoubtedly encouraging and are deemed comparable to spinal fusions. However, there are still many unanswered questions relating to the choice of material, design kinematics, recipient factors, surgical precision, and, in particular, the long-term outcome and possible salvage procedures. It is ironic to note that at least in one long-term study of artificial disc replacement the best results occurred in patients where the disc replacement went into spontaneous fusion, making the TDR a very expensive spacer for fusion [6, 12].

In 1991, Olson et al. [11] described in a quadruped model transplantation of a vertebral body together with the two adjacent discs as a spacer for reconstruction of a spinal column defect. They found that there was incomplete revascularization of the intervening vertebral body and the intervertebral discs were able to retain a relatively normal mobility and stability. Three years later, Frick et al. [3] of the same group reported for the first time a fresh autograft disc transfer in a dog model and examined the biochemical and histological changes. Their conclusion was that the morphology and the metabolic functions of the transplanted discs were not normal, but the structure and function were maintained. However, they also admitted that the major limitation of their study was the transplanted discs were rigidly fixed internally, which could have jeopardized the nutrition of the discs. Subsequently, Katsuura and Matsuzaki [5, 10] have conducted similar studies using cryopreserved allografts also in quadruped models, but again all the grafts were internally fixed rigidly with plates and screws.

Intervertebral disc transplantation—animal research

Our group conceived a similar idea of disc transplantation in 1991, around the same time as Olson's first report. We adopted a different approach of not constraining the transplanted graft and conducting the experiments in an upright primate model, which we believe has closer resemblance to the human biomechanics. A series of experiments were performed firstly to establish the animal model, followed by autografting, fresh allografting and fresh frozen allografting. Finally a small scale proof-of-concept clinical trial was commenced in 2000.

The first autograft experiment was carried out in 1992 at the Tangdu Hospital, Affiliated Hospital of the 4th Military Medical University, Xian, China in collaboration with Dr. DK Ruan. In total 14 male rhesus monkeys were divided into four groups with four animals in each of the 2-, 4-, and 6-month follow-up groups and two in the 12-month follow-up group. The L34 intervertebral disc was mobilized completely by osteotomizing the bony endplate using a sharp osteotome about 1.5 mm above and below the disc, taking care not to fracture the endplate or damage the neural tissue. The composite graft was then repositioned into the slot and anchored with two silk sutures from the outer annulus to the adjacent soft tissue. No rigid internal fixation or external immobilizer was used and the animal was allowed free activity inside the cage. Serial radiographs were taken to monitor the changes in disc height or other degenerative changes. Upon euthanization of the animals, the autografts were subjected to biochemical, histological and biomechanical testing. Healing of the

osteotomies took place uneventfully in the first 2 months. There was a gradual reduction in the disc height which seemed to have stabilized at 2–4 months post-surgery. There was a suggestion that the disc height regained slightly up to the 12-month final follow-up. No definite conclusion on this was drawn because the number of animals studied was small. Biochemically, none of the changes of water, proteoglycan and hydroxyproline contents with time were found to be significant statistically although in the nucleus fibrosus the trend was a continuing drop of water content, an initial drop followed by an increase in the proteoglycan, and a persistently raised hydroxyproline content in the grafts. Histologically, viable cells were seen both at the annulus and the nucleus although the lineage of the cells was not tracked in the experiment. The morphology and structure of the annulus was well preserved. Biomechanical testing showed that there was an initial period of hypermobility of the grafted disc at all ranges of motion at 2–4 months which returned to normal by 6 months. We concluded from this experiment that a bipedal animal model was successfully established for further study of intervertebral disc transplantation. The cells in the composite autograft were able to withstand a transient period of ischaemia and the organ was able to recover its function to a certain extent both biochemically and biomechanically [8].

The next question we asked was how a fresh allograft would behave if it were to be transplanted from a live or freshly dead donor, in particular the problem of immunogenicity. The experimental design was to perform surgery on two monkeys at the same time switching between them the L34 discs. We believed that similar to the cornea in the eye and the meniscus in the knee, the intervertebral disc composite, other than the bony endplate, was immuno-privileged because of its avascularity. The fact that massive allogenic bone graft was already being widely used clinically in joint replacement revisions or tumor reconstructions without the need for immunosuppressants, no rhesus or even blood group matching was done for our experimental animals. Two monkeys of similar age and size were operated by two teams of surgeons and anesthesiologists to minimize operating time and blood loss. The experiment was essentially a total failure because of repeated subluxation and dislocation of the grafts due to graft size mismatch. We learned from this second experiment that in order to achieve primary stability of the transplantation without the need for rigid internal fixation, appropriate size matching and press-fit fixation was essential.

To move one step further to making disc transplantation feasible clinically, we moved onto the third experiment using a fresh frozen allograft. Information from this study will help resolve issues of organ donation, preservation,

physical size and immunocompatibility of the grafts, distribution of the grafts to different geographic regions, etc. Seventeen monkeys were used for this experiment. The first two were sacrificed as donors of the discs, and three others were used as controls. The remaining 12 animals were divided into three groups for short-term (2–8 weeks), medium-term (6 months) and long-term follow-up (24 months). Briefly, the whole spine from T10 to L7 were harvested from the donor monkeys and cut up into individual disc segments carrying 1–2 mm of adjacent endplates. After recording the dimensions of the grafts, they were immersed in a DMSO solution and cooled stepwise to -196°C for storage in liquid nitrogen. During the experimental surgery, after removing the target disc, a graft of the appropriate size was thawed and snugly fitted into the recipient site. The postoperative regime was similar to the autograft experiment and no immunosuppressant was used. Similar to the autograft findings, union of the bony endplates took place uneventfully in all cases without graft subluxation or dislocation. There was a slow but progressive reduction of disc height up to the 24 months final follow-up with secondary changes of traction osteophytes similar to that seen in human DDD. Different from the autograft finding, there was a steady decrease of both the water and proteoglycan content from 6 to 24 months. The mechanical stability and mobility of the grafted FSU remained comparable to the controls throughout. The major focus of the histologic examination was the presence of signs of immunoreactivity. Light microscopy examination showed inflammatory cell infiltration with lymphocytes and fibroblastic proliferation limited to the osteotomy site but not beyond. This reaction was significantly reduced at 8 weeks. The number of cells in the annulus and the nucleus was similar between the transplanted allograft and the controls at the early follow-up but at 24 months post-surgery, the NP like cells showed features of irregular nuclear shape, mitochondrial swelling and karyopyknosis compatible with degeneration. We concluded from this study that a cryopreserved allograft was able to retain some cell viability and maintain mechanical properties similar to the autografts [2]. The cryopreservation process could also reduce the immunogenicity of the allograft which when transplanted would induce minimal or no immunoreaction. Since the tissue reaction was only observed at the bony interface, in our subsequent experiments and clinical trial we have emphasized mechanical washout of the cancellous endplates in the allograft before preservation. In this phase of the experiment however, similar to the previous ones, degeneration of the transplanted allograft was apparently still unavoidable. Further work could be done to refine the preservation protocol that might increase the cell viability or reduce early graft degeneration [9].

Clinical trial

Although results from the autograft and fresh frozen allograft experiments were not perfect, we believed the allograft transplantation was ready for clinical application because of its ability to maintain cell viability, spinal mobility and stability. In 2000, after obtaining approval from the appropriate institutional-national authority and informed consent of the patients, a carefully executed proof-of-concept clinical trial was started at the Navy General Hospital in Beijing. Between March 2000 and January 2001, disc transplantation was performed in the first five subjects with cervical disc herniation and spinal cord compression. They were followed up carefully for 5 years before this long-term result was published in one of the top medical journals, *The Lancet* [13]. Briefly disc allografts were obtained within 2 h after death from three previously healthy young female trauma victims. Bony abnormality and obvious disc degeneration were excluded using radiographs. They were also screened for transmissible diseases including hepatitis B, C, TB and HIV.

The composite allografts were immersed in 10% dimethyl sulfoxide [DMSO] and 10% calf serum (GibcoBRL/Invitrogen, Carlsbad, CA, USA) in a special container and frozen stepwise to -196°C in liquid nitrogen. At surgery, the offending cervical disc was removed subtotally, including the posterior longitudinal ligament until the dura was completely decompressed and exposed. After measuring the dimensions of the intervertebral slot an allograft of the appropriate size was thawed in a water bath at 37°C and snugly inserted into the recipient site. No internal fixation was used and postoperatively a neck collar was worn full time for 2 weeks and part-time for 4 weeks. One dose of cephalosporin was given preoperatively as prophylaxis and continued for 3 days postoperatively. No immunosuppressant was used. Dynamic flexion–extension radiographs were taken at regular intervals. MRI was done pre- and post-operatively as well as at the final follow-up. The grey scale of the nucleus pulposus in the T2 weighted image was graded with the modified Schneiderman's scale [15]. The neurological function was assessed with the Japanese Orthopedic Association (JOA) scoring system.

The first five patients consisted of one female and four males who all presented with typical pyramidal tract compression. One patient had traumatic cervical disc herniation with acute paraparesis, while the remaining four suffered from chronic cervical spondylotic myelopathy. There was no subluxation or dislocation of the graft but there was one suboptimal positioning of the graft in which it was placed too anteriorly. None of the subjects showed any signs or symptoms of local or systemic immunoreaction. All patients had improvement of the neurology. The incomplete paraplegic patient improved from Frankel B to

D postoperatively. The JOA score of the other four myelopathic subjects increased from a mean of 11–14.8/17. At the 5-year follow-up, none of them had significant neck pain. Radiologically, there was only minimal loss of disc height compared with the preoperative measurements. All except one had between 7 and 11.3 degrees of motion at the grafted disc (Fig. 1). The remaining one had spontaneous fusion of the disc after a revision posterior foraminotomy for residual radiculopathy. There was an increase in the Schneiderman scoring on MRI indicating early degeneration postoperatively. However, there was a trend of recovery of the water content at the final follow-up in which at least two of the allografts showed a T2 signal higher than that of the original defective disc (Fig. 2). For obvious reasons no biopsy of the allograft was available for histological or biochemical study. In the case of the malpositioned graft there was a pleasantly surprising observation of complete remodeling of the graft at the 5th year follow-up. There was relocation of the annulus fibrosus and preservation of the nucleus pulposus indicating that some form of creeping substitution of the allograft must have taken place through biological activity (Fig. 3). This is also inferred that the allograft must have been viable and may even be able to regenerate.

Discussions and possible future works

Degenerated discs are not necessarily symptomatic clinically and can be compatible with normal functions. It is, therefore, unrealistic or unnecessary to prophylactically regenerate all discs that appear degenerated on MRI. It is only when DDD produces secondary sequels of discogenic pain, neurological impingement or instability of the FSU that it requires treatment. Before the etiology and pathomechanism of intervertebral disc degeneration is fully understood and biological rejuvenation or regeneration of the disc becomes clinically applicable, artificial disc replacement is not an unreasonable alternative for motion preservation in DDD. However, to date there is no level one evidence to show that such an artificial disc could produce sustained long-term results superior to that of the time honored spinal fusion operation, or such motion preservation could truly prevent adjacent segment degeneration. The risk of revision surgery and salvage options should an artificial implant fail in the long term are still not completely resolved. There is, thus, major reservation on its application in the young and middle aged population with DDD when disc rejuvenation is preferred.

The intervertebral disc is a complex organ composing of bony and cartilaginous endplates, annulus fibrosus and nucleus pulposus which serve different functions. Regenerative strategies must take into consideration in total the

Fig. 1 **a** C5/6 degeneration with reduction of disc height and position of allograft at 6 months after implantation. **b** Flexion–extension lateral radiograph showing excellent range of motion at 15 months postoperatively (modified from InSpine [14])

Fig. 2 Serial MRI T2 images (from left to right) taken pre-, immediate post-, and at 15 months postoperatively showing satisfactory spinal cord decompression and status of hydration of the allograft (modified from InSpine [14])

Fig. 3 **a** Lateral view of the cervical spine showing a malpositioned C5/6 allograft at 3 months post-surgery. **b** Lateral view of the same disc showing complete remodeling at 6 years postoperatively (modified from The Lancet [13])

scaffold, the cells and the environmental niche. It has been shown that the nutritional channels going through the endplates are jeopardized and the annulus fibrosus are structurally and functionally deranged in DDD. Genetic, chemical and cellular manipulation of the nucleus pulposus alone is, therefore, unlikely to be able to reverse all the changes that have occurred in the other two components. Intervertebral disc transplantation theoretically has a number of advantages. The best case scenario would be when the organ is able to completely integrate with the recipient and be substituted by the host over time. Even if it did not and proceeds into degeneration, provided it can serve the mechanical functions of maintaining stability and mobility, it is still an acceptable alternative to a fusion after disc excision. This is very close to what we have demonstrated both experimentally and in our clinical trial. What is not known at this stage is the natural history of the allograft in the long term. Since they are totally denervated, will they ever become painful? Is the degenerative process in an allograft the same as that of a normal disc? From the clinical trial, we are encouraged that the natural remodeling mechanism is able to take care of any technical imperfection which is never possible for artificial disc replacement. Since there is no observed immunoreaction or osteolysis with time, we anticipate that should revision surgery be necessary in the future, it should not be more difficult than revising an artificial implant. The options of revising to another allograft, an artificial implant, or even a fusion are still open.

There are a number of areas for further research. The allograft disc can be considered as a biological scaffold on which cells could be seeded after transplantation. When the nutritional channels through the bony endplate are re-established the young and normal structure is ready for repopulation with the host cells or allogenic stem cells. The latter approach may be preferable if one believes DDD is genetically predisposed, and thus the host cells are also at risk. This can be done in combination with different cytokines to enhance or suppress cell survival or apoptosis. The allograft preservation protocol serves both to maintain cell viability and reduce immunogenicity. This protocol should be improved to increase the shelf life of the allograft after harvesting or to preserve more cells particularly in the deeper part of organ in the nucleus pulposus. Another area of interest is the kinematics of the FSU after transplantation. A malpositioned artificial disc with abnormal kinematics is not only doomed to fail but will also have a deleterious mechanical effect on the adjacent segment. Since the allograft disc is able to be remodeled and the process must have followed biomechanical laws, one would expect the kinematics of the allograft to improve with time and may contribute a protective mechanical effect on the adjacent segment which is one of the goals of any motion preservation strategy. The application of disc transplantation to the lumbar spine remains another challenge. The size of the graft and the load it shall endure are different than the cervical spine. Size matching and preservation protocol may need to be modified accordingly.

In order to take the present pilot clinical trial forward into wider clinical application, the authors believe the following three remaining concerns and controversies have to be further addressed. Firstly, there is always the concern of the risk of transmissible diseases in any live tissue transplantation. The best one could do is to screen for these diseases to the best of our present day medical knowledge. In addition our group is also looking into the feasibility of sterilizing the allograft with radiation and its effect on the biology of the organ. Secondly, unlike conditions like terminal heart, lung or kidney failures, degeneration of the disc is not life-threatening. One may argue whether the benefits of the transplantation could outweigh the risks. Similarly, transplantations for other non-life-threatening conditions of cornea, bone, meniscus, ligaments or even stem cells, will continue to be a subject of ethical controversy. Finally, the issue on the availability of suitable donors depends a lot on ethical, cultural and legal background of the different countries. With the increasing acceptance of major organ donation in developed countries, there is no reason to assume donation of musculoskeletal tissues should be any more problematic in the future. We believe that it is the duty of the clinician-scientist to find the best solution for a pathology based on scientific principles and that it is the responsibility of the community to debate whether it could be accepted.

Conclusions

Intervertebral disc transplantation has developed from animal experiment to clinical application in the past 16 years. Many issues including graft harvesting, preservation, surgical implantation technique, and immunoreaction have been investigated. It has also opened a wide field for further laboratory and clinical research. The concept has been proven to be viable in a preliminary clinical trial. Despite radiological signs of mild disc degeneration, the motion and stability of the spinal unit was preserved. It represents an attractive possible alternative for preserving motion in the management of degenerative disc disease. Further larger scale clinical trials are needed to verify the benefits and risks.

Conflict of interest statement None of the authors has any potential conflict of interest.

References

1. Alini M, Roughley PJ, Antoniou J, Stoll T, Aebi M (2002) A biological approach to treating disc degeneration: not for today, but maybe for tomorrow. *Eur Spine J Suppl* 2:S215–S220
2. Flynn J, Rudert MJ, Olson E, Baratz M, Hanley E (1990) The effects of freezing or freeze-drying on the biomechanical properties of the canine intervertebral disc. *Spine* 15(6):567–570
3. Frick SL, Hanley EN Jr, Meyer RA Jr, Ramp WK, Chapman TM (1994) Lumbar intervertebral disc transfer. A canine study. *Spine* 19(16):1826–1834
4. Ho G, Leung VY, Cheung KM, Chan D (2008) Effect of severity of intervertebral disc injury on mesenchymal stem cell-based regeneration. *Connect Tissue Res* 49(1):15–21
5. Katsuura A, Hukuda S (1994) Experimental study of intervertebral disc allografting in the dog. *Spine* 19(21):2426–2432
6. Lemaire JP, Carrier H, Soriali el H et al (2005) Clinical and radiological outcomes with the Charite artificial disc: a 10-year minimum follow-up. *J Spinal Discord Tech* 18:353–359
7. Leung VY, Chan D, Cheung KM (2006) Regeneration of intervertebral disc by mesenchymal stem cells: potentials, limitations, and future direction. *Eur Spine J (Suppl 3):S406–S413* [Epub (2006) 15 July Review]
8. Luk KD, Ruan DK, Chow DH et al (1997) Intervertebral disc autografting in a bipedal animal model. *Clin Orthop* 337:13–26
9. Luk KD, Ruan DK, Lu DS et al (2003) Fresh frozen intervertebral disc allografting in a bipedal animal model. *Spine* 28:864–870
10. Matsuzaki H, Wakabayashi K, Ishihara K, Ishikawa H, Ohkawa A (1996) Allografting intervertebral discs in dogs: a possible clinical application. *Spine* 21(2):178–183
11. Olson EJ, Hanley EN Jr, Rudert MJ, Baratz ME (1991) Vertebral column allografts for the treatment of segmental spine defects. An experimental investigation in dogs. *Spine* 16(9):1081–1088
12. Putzier M, Funk JF, Schneider SV et al (2006) Charite total disc replacement—clinical and radiographical results after an average follow-up of 17 years. *Eur Spine J* 15:183–195
13. Ruan DK, He Q, Ding Y, Hou LS, Li JY, Luk KDK (2007) Intervertebral disc transplantation in the treatment of degenerative spine disease: a preliminary study. *Lancet* 369:993–999
14. Ruan DK, Ding Y, He Q, Luk KDK (2008) Allograft disc transplantation—Preserving segment motion and rebuilding stability of the cervical spine. *InSpine* 4(2):20–24
15. Schneiderman G, Flannigan B, Kingston S et al (1987) Magnetic resonance imaging in the diagnosis of disc degeneration: correlation with discography. *Spine* 12:276–281