Efficacy of human amniotic tissue-derived allograft, NuCel®, in patients undergoing one and two level posteriolateral lumbar fusions for degenerative disc disease

Principal investigator: Joseph A. Shehadi, MD
Sub-investigator: Timothy Meier, MD, Janet Bay, MD

I. INTRODUCTION

Lumbar degenerative disc disease is a common disorder that may cause debilitating symptoms. Although there is discrepancy in the prevalence estimates of the morphological changes associated with degeneration (Battié et al. 2004), lumbar degenerative disc disease is a major cause of chronic disability (Modic and Ross 2007). Risk factors include genetic predisposition, trauma, impaired nutrition of the intervertebral disk, mechanical loading and aging (Battié et al. 2004; Modic and Ross 2007). Patients with lumbar disc disease most commonly present with low back pain and radiculopathy, but many are asymptomatic (Modic and Ross 2007).

When conservative measures such as medication and physical therapy fail, lumbar spinal surgery is used to treat degenerative disc disease. Spinal fusion surgery limits movement of the vertebrae adjacent to the affected intervertebral disc. It typically consists of spinal instrumentation to secure the vertebrae, and bone graft material, to allow the vertebrae to fuse together (Rutherford et al. 2007).

One of the major controversies surrounding lumbar spine fusion is the choice of ideal graft material used to facilitate fusion. Commonly studied properties of graft materials include osteoconduction (scaffolding for bone re-growth), osteoinduction (growth factors that induce bone growth), and osteogenesis (living cells that produce new bone); (Vaccaro 2002). An ideal allograft material is one that is safe, effective in forming bone, forms bone where intended without excessive bone growth, can integrate with the host, and is cost effective.

Autogenous bone graft, obtained from the iliac crest, has traditionally been considered the gold standard. The advantages of an autogenous graft include high fusion rates, good incorporation, predictable results, osteoconduction, osteoinduction, osteogenesis, lack of immunogenicity, circumvents risk of disease transmission from another donor, and is cost-effective. (Arrington et al. 1996; Chau and Mobbs 2009). However, iliac crest harvest is associated with morbidity at the harvesting site. Complications include pain, numbness, injury to nerves or arteries, infection, seromas, hematomas, peritoneal perforation, herniation of abdominal contents, bone fracture, and cosmetic concerns about the scar (Kurz et al. 1989; Arrington et al. 1996; Schwartz et al. 2009). Post-operative pain may lead to a longer hospital stay, and/or possibly lead to difficulty performing basic daily activities (Dusseldorp and Mobbs 2009; Schwartz et al.

2009). Other drawbacks include limited quantity and quality of bone graft, increased blood loss and increased operating time (Chau and Mobbs 2009).

There are several commercially available bone graft substitutes with differing properties. Allograft is bone obtained from cadaveric sources, which has been treated to decrease its immunogenicity and risk of disease transmission. It is osteoconductive, minimally osteoinductive, slower to incorporate and produces more variable results than autogenous bone graft (Vaccaro 2002; Miyazaki and Tsumura 2009). Ceramics have been used in spinal fusions, as they are osteoconductive, available in large quantities and are sterile. However, they are brittle and can withstand little force (Vaccaro 2002; Miyazaki and Tsumura 2009). Demineralized bone matrix (DBM) is both osteoconductive and osteoinductive. It is produced from cortical allograft bone that has undergone acid extraction, removing its mineral component, but retains many proteins, including growth factors (Miyazaki and Tsumura 2009). Osteoinductive growth factors have also been used as bone graft substitutes, and the most studied and used factor is recombinant human bone morphogenetic protein-2 (rhBMP-2). Since its introduction commercially in 2002, rhBMP-2 has shown rapid and robust bone fusion, and gained widespread use, despite its high cost. However, reports of serious complications have emerged, including ectopic bone formation, infection, urogenital events, carcinogenicity and others (Carragee et al. 2011). This lead to the Yale University Open Data access (YODA) Project to re-analyze all the clinical data gathered by the manufacturer of rhBMP-2 (Infuse; Medtronic). The results were published in June 2013, in a special edition of the Annals of Internal Medicine. Two independent groups (Fu et al. 2013; Simmonds et al. 2013) found that rhBMP-2 was not better than autogenous bone graft in terms of fusion, pain or function, but posed an increased risk of adverse events. Other emerging options for bone graft substitutes include autologous platelet concentrate, mesenchymal stem cells and gene therapy (Miyazaki and Tsumura 2009).

Therapeutic use of amniotic fluid and the amniotic membrane has existed in medicine for decades in many different capacities (Shimberg 1938). Some applications include healing of the eye, soft-tissue wounds, and bone fractures (Karaçal et al. 2005; Kerimoğlu 2008). Recently, amniotic epithelial cells have been shown to express stem cell markers of pluripotency, including octamer-binding protein 4 (*Oct-4*) and *Nanog* (Miki and Strom 2006; Miki 2011). Cells isolated from the amnion are able to differentiate into all three germ layers: endoderm, mesoderm and ectoderm (Toda et al. 2007).

Amniotic fluid and membrane contain mesenchymal stem cells (MSCs), which may be useful in graft material for bone fusion for several reasons. They have been shown to have osteoinductive and osteogenic properties, and therefore can regenerate bone. They have been shown to selectively adhere to bone substrate and are unlikely to be rejected since they are immune privileged (they do not express human leukocyte antigen (HLA) class II molecules). Also, stem cells from amniotic origin have low risk of tumorigenicity (Siegel et al. 2007). Finally,

stem cells from an amniotic source do not pose ethical issues, as there is no risk to the mother or fetus.

NuCel® is a commercially available (NuTech Medical, Birmingham, AL) source of MSCs derived from human amniotic fluid in combination with morselized amniotic membrane tissue. In addition to stem cells, this tissue contains collagen, proteoglycans, hyaluronic acid, trophic proteins including bone morphogenetic proteins (BMPs), and growth factors.

Therefore, the goal of the proposed research is to use NuCel® in the bone graft material applied to patients undergoing posteriolateral lumbar spine fusion, to assess its ability to promote bone fusion. Its safety and efficacy will be compared to a standard allograft material routinely used for this surgery.

II. METHODS

A. Study Population

Participants will consist of patients presenting with symptomatic, one or two-level lumbar degenerative disc disease, spondylosis or spondylolisthesis. They must have failed traditional conservative treatment measures, such as pain medication, physical therapy, lumbar epidural steroid injections or lumbar bracing support, prior to surgery. They must be candidates for a one or two-level posteriolateral lumbar fusion, with instrumentation, with or without laminectomy. The participants are patients that the surgeon would consider as low risk for non-union, and would have received only demineralized bone matrix (DBM) bone graft material without any other graft material, in particular, no recombinant human bone morphogenetic protein (rhBMP-2). Patients who would be considered higher risk of non-union, and who would have typically received rhBMP-2 will be excluded according to the list below.

Inclusion Criteria

- Between the ages of 18 and 75 years
- Symptomatic, one or two-level degenerative lumbar disc disease, spondylosis or spondylolisthesis
- Failed conservative treatments
- Must be candidates for one or two-level, posteriolateral lumbar spine fusion
- Must be able and willing to give Informed Consent
- English-speaking

Exclusion Criteria

- Smoker (any smoking ≤3 months prior to consent); (Patel et al. 2013)
- Patients with poorly controlled diabetes mellitus (HgbA1c > 7%)
- Documented osteoporosis
- Prior lumbar spinal surgery at the same spinal level, or immediately adjacent spine level, to the level being operated on
- Back pain due to infection, tumour, or metabolic bone disease
- Terminal disease, such as HIV infection, neoplasm
- Autoimmune disease, such as rheumatoid arthritis
- Morbid obesity (body mass index (BMI) of 35 kg/m^2)
- Major psychiatric illness in the last year
- History of alcohol or drug abuse in the last year
- Pregnant women

B. Hypothesis

We hypothesize that the NuCel® group will report, on average, Oswestry Disability Index (ODI) scores 10% lower than those of the DBM group. Assuming a baseline ODI score of 52 (SD=8) for the DBM group, 90% power and an a Type 1 error rate of 5%, a sample size of 42 patients (21 per group) would be sufficient to detect a 10% difference between groups using a repeated measures design with 6 measurement points. To account for drop-out, loss to follow-up, and/or refusal to complete study documents at follow-up, we plan to recruit a total of 60 patients (30 per treatment arm).

C. Study Variables

Clinical outcomes

The following data will be collected at the time of enrollment (pre-operatively), and at 1, 2, 3, 6 and 12 months after surgery: 0-10 numeric pain rating scale for low back and leg pain, Oswestry Disability Index (ODI), and a neurological exam (sensory and motor evaluation). We will also record any adverse events (infection, inflammation, radiculopathy, etc.) that occur intra-operatively through 12 months. Demographic information including gender, age, diagnosis, spinal level for surgery, will be recorded directly into an Excel spreadsheet for each patient.

Radiological outcomes

Radiographs of the lumbar spine will be assessed at the time of surgery (intra-operatively) and at 1 and 2 months (anterior-posterior and lateral) and at 3 months (flexion-extension) after surgery to detect any spinal instrumentation failure. CT scans of the lumbar spine will be taken at 6 and 12 months post surgery to assess fusion. Assessment will be performed by an independent radiologist who will be blinded to the treatment and clinical condition of the participants. The radiologist will score fusion according to the following categories: no fusion, any fusion, or solid fusion.

The table below shows the time line for each data collection activity:

			Post-op months (range in days)				
	Pre-op	**Intra-op**	1 (25-35 days	2 (55-65 days)	3 (85-95 days)	6 (170-190 days)	12 (355-375 days)
Oswestry Disability Index (ODI)[1]	X		X	X	X	X	X
Pain rating scales [1]	X		X	X	X	X	X
Neurological exam[1]	X		X	X	X	X	X
CT scan [1]						X	X
X-ray [2]		X	X	X	X		
Adverse events[1]		X	X	X	X	X	X

[1]Required for research purposes
[2]Standard of care procedure

D. Study Design

The proposed study is a single-blinded randomized controlled trial. Participants will be recruited from patients at Dr. Shehadi's neurosurgery practice, Neurosurgery Associates, L.L.C. (Columbus, OH) or at Neurological Associates, Inc at Riverside Methodist Hospital with Dr. Bay. Patients presenting with symptomatic, lumbar degenerative disc disease, and that meet the inclusion/exclusion criteria will be given information about the study and asked if they would like to participate.

Participants will be allocated to treatment groups using a block randomization scheme to increase the likelihood of balance. Treatment assignments will be stored in sealed, numbered envelopes that will be opened by study personnel in

the pre-operative period. The patient will be blinded to treatment assignment throughout the course of the study.

Participants will receive either standard DBM or experimental NuCel®, and will be blinded to which intervention they receive. All operations will be performed by Dr. Shehadi or Dr. Bay. Instrumentation will consist of titanium pedicle screws and rods. All operations will be done according to standard methods under fluoroscopic guidance and with electrophysiological monitoring.

Allografts

DBM bone graft substitute (DBX; DePuy Synthes, West Chester, PA, USA) will be obtained via the Musculoskeletal Transplant Foundation (MTF), according to standard protocol. DBX putty consists of 93% demineralized bone content in a sodium hyaluronate carrier.

NuCel®, a stem-cell allograft, will be obtained from NuTech Medical, Birmingham, AL, USA. The bioactive amniotic suspension is derived from amniotic fluid and morselized amnion. Donors are pre-screened through questionnaires and laboratory tests. Amniotic tissue is collected at the time of scheduled Cesarian-section, and processed by NuTech under aseptic conditions. The product is approved by the Food and Drug Administration. The frozen contents of the NuCel® vial will be gently thawed, mixed with sterile saline, or the patient's blood, and then mixed with a cancellous bone chip carrier material.

Data will be collected by a member of the study team. Clinical outcome data will be collected in the office through the use of questionnaires (time points described above). Radiological data will be obtained from imaging location (time points described above).

A member of the study team will be responsible for the handling and storage of data. The identity of the participants will be kept confidential at all times. Participants will be given a number identifier for data entry and statistical purposes. Documents containing the identity of the patient will be restricted to those investigators authorized to work on the study. Further, these documents will be kept in a locked cabinet or safe, and stored with the study coordinator or other member of the study team.

Sources of Bias

Efforts were made in designing this study to minimize the chance of study biases. We attempted to minimize the effect of additional medical conditions by having strict exclusion criteria. For example, patients who have any condition, such as diabetes, osteoporosis, or are smokers, are excluded from the study as these factors may have an effect on bone healing. All efforts are made to recruit groups that are as homogenous as reasonably possible. Participant bias will be

mitigated by blinding participants to the intervention they will receive. For clinical outcome measures, evaluator bias is minimized as the data will be collected through objective questionnaires to be filled out by the participants (pain scales, ODI). There is no need for evaluator judgment, which may be subjective, in recording these data. For radiological outcome measures, an independent, blinded radiologist will score the scans, eliminating evaluator bias. The only test that may be subject to evaluator bias is the neurological exam for sensory and motor deficits. Having said that, the neurological exam will be performed by Dr. Shehadi, Dr. Bay, a physician assistant (PA) or a nurse practitioner (NP) who is highly skilled and experienced in evaluating this test.

Since the only experimental intervention is at the time of surgery this study does not depend on patient adherence for the study intervention. The study will, however, depend on patients adhering to follow-up office visits and scans. We expect the "drop-out" rate will be low as most of these visits are required as follow-up to any surgery. Also, the final time point for data collection is 12 months after surgery, therefore, it is unlikely that the patient will seek surgical treatment elsewhere during this time frame.

E. Statistical Analysis

We will first compare the demographic and pre-operative clinical characteristics of the NuCel® and DBM groups to evaluate randomization fidelity. If any characteristics are found to be statistically significant between the groups, this will be accounted for in the analyses of the primary and secondary outcomes. We will describe all categorical variables as percentages and continuous variables with means and standard deviations.

To evaluate our primary outcome we will use a linear mixed model with ODI score as the dependent variable and treatment group and time as independent variables. Mixed models are often preferred over a two-way ANOVA because they allow for incomplete data from participants and do not require a strictly normal distribution in the dependent variable. This model will also be used to evaluate the effect of treatment group on pain scores over time. Statistical significance will be set at $p<.05$.

We will report the frequency of observed fusion at the 6-month and 12-month follow-up periods via CT scan by treatment group. If our sample size allows, we will compare the NuCel® and DBM groups on fusion rates at each time point using Fisher's Exact tests. Statistical significance will be set at $p<.05$.

We will report the frequency of adverse events experienced by the NuCel® and DBM groups including negative changes in neurological exam (sensory and motor) findings during the follow-up period.

III. REFERENCES

Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA (1996). Complications of iliac crest bone grafting harvesting. *Clinical Orthopaedics and Related Research* 329:300-309.

Battié MC, Videman T, Parent E (2004). Lumbar disc degeneration: epidemiology and genetic influences. *Spine (Phila Pa 1976)* 29:2679-2690.

Carragee EJ, Hurwitz EL, Weiner BK (2011) A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. *The Spine Journal* 11:471-491.

Chau AMT, Mobbs RJ (2009). Bone graft substitutes in anterior cervical discectomy and fusion. *European Spine Journal* 18:449-464.

Dusseldorp JR, Mobbs RJ (2009). Iliac crest reconstruction to reduce donor-site morbidity: technical note. *European Spine Journal* 18:1386-1390.

Fu R, Selph S, McDonagh M, Peterson K, Tiwari A, Chou R (2013). Effectiveness and harms of recombinant human bone morphogenetic protein-2 in spine fusion: a systematic review and meta-analysis. *Annals of Internal Medicine* 158:890-902.

Karaçal N, Koşucu P, Çobanǵĭu Ŭ, Kutlu N (2005) Effect of human amniotic fluid on bone healing. *Journal of Surgical Research* 129:283-287.

Kerimoğlu S, Livaoğlu M, Sönmez B, Yuluğ E, Aynacı O, Topbas M, Yarar S (2008) Effects of human amniotic fluid on fracture healing in rat tibia. *Journal of Surgical Research* 152:281-287.

Kurz LT, Garfin SR, Booth RE (1989). Harvesting autogenous iliac bone grafts. A review of complications and techniques. *Spine (Phila Pa 1976)* 14:1324-1331.

Miki T (2011) Amnion-derived stem cells: in quest of clinical applications. *Stem Cell Research and Therapy* 2:25.

Miki T and Strom SC (2006). Amnion-derived pluripotent/multipotent stem cells. *Stem Cell Reviews* 2:133-142.

Miyazaki M, Tsumura H (2009). An update on bone substitutes for spinal fusion. *European Spine Journal* 18:783-799.

Modic MT and Ross JS (2007). Lumbar Degenerative Disk Disease. *Radiology* 245:43-61.

Patel RA, Wilson RF, Patel PA, Palmer RM (2013). The effect of smoking on bone healing: a systematic review. *Bone and Joint Research* 2:102-111.

Rutherford EE, Tarplett LJ, Davies EM, Harley JM, King LJ (2007). Lumbar spine fusion and stabilization: hardware, techniques, and imaging appearances. *RadioGraphics* 27:1737-1749.

Schwartz CE, Martha JF, Kowalski P, Wang DA, Bode R, Li L, Kim DH (2009). Prospective evaluation of chronic pain associated with posterior autologous iliac crest bone graft harvest and its effect on postoperative outcome. *Health and Quality of Life Outcomes* 7:49.

Shimberg M (1938) The use of amniotic-fluid concentrate in orthopaedic conditions. *The Journal of Bone and Joint Surgery* 20:167-177.

Siegel N, Rosner M, Hanneder M, Valli A, Hengstschläger M (2007) Stem cells in amniotic fluid as new tools to study human genetic diseases. *Stem Cell Reviews* 3:256-264.

Simmonds MC, Brown JVE, Heirs MK, Higgins JPT, Mannion RJ, Rodgers MA, Stewart LA (2013). Safety and effectiveness of recombinant human bone morphogenetic protein-2 for spinal fusion: a meta-analysis of individual-participant data. *Annals of Internal Medicine* 158:877-889.

Toda A, Okabe M, Yoshida T, Nikaido T (2007) The potential of amniotic membrane/amnio-derived cells for regeneration of various tissues. *Journal Pharmacological Sciences* 105:215-228.

Vaccaro AR (2002) The role of the osteoconductive scaffold in synthetic bone graft. *The Ortho Blue Journal* 25:s571-s578.