CLINICAL EVALUATION OF A NOVEL ROOM TEMPERATURE STORAGE TECHNOLOGY BONE ALLOGRAFT IN CERVICAL SPINE FUSION

SCOTT GRAHAM, MD,[1] BRUCE MATHERN, MD,[1] BRIAN SAMSELL, BS,[2] ANDREW COTTER, BS,[2] BRANDON KELLY, MD, [1] AND MARK MOORE, PHD[2]
[1]VIRGINIA COMMONWEALTH UNIVERSITY, RICHMOND, VA; [2]LIFENET HEALTH, VIRGINIA BEACH, VA

ABSTRACT

Bone allografts used for interbody spinal fusion are typically preserved using either freeze-drying or low temperature freezing, each having disadvantages involving graft preparation time and material properties. In response, an ambient temperature preservation treatment, trade-named Preservon®, has been developed to maintain biomechanical properties of allografts at ambient temperatures, requiring no thawing or rehydration and only minimal rinse prior to implantation. Here, we report a study whose purpose was to compare the clinical results of Preservon-treated Cloward dowels with freeze-dried Cloward dowels in anterior cervical discectomy and fusion (ACDF) surgeries in a prospective, randomized study. The Preservon and freeze-dried treatment groups had equal rates of intact grafts (95.9% vs. 98.0%) at 3 months follow-up. In an interim analysis, all patients in both treatment groups had evidence of fusion (100%) at 6 months follow-up. Separate biomechanical analysis indicates that the Preservon-treated allografts are less brittle than their freeze-dried counterparts, even after graft rehydration. Taken together, Preservon-treated bone allografts exhibited similar clinical performance results as the freeze-dried grafts, potentially more favorable biomechanical properties, and significantly shorter preparation times, and thus are an attractive graft choice for ACDF procedures.

INTRODUCTION

Traditionally, human tissue allografts are freeze-dried or deep frozen before being sent to a surgeon for implantation. If freeze-dried, the dehydrated allograft must then be reconstituted, often by lengthy means, before it can be implanted. Unfortunately, tissue brittleness and weakness can result if this process is completed too quickly.[4,6] Regarding the frozen tissue option, while deep freezing tissue to -70°C may prevent brittleness and tissue degradation issues, the demands of keeping tissue at such a low temperature during shipment and storage can be challenging especially when freezer space is either limited or remote, necessitating timely graft shipments and inconvenient graft retrieval. The surgical team must also then wait while their chosen graft thaws. In addressing these limitations, a bone allograft processing technology, trade-named Preservon has been developed to eliminate the potential brittleness in freeze-dried allografts as well as the sub-zero temperature requirement of frozen grafts.[3,11,12] Preservon uses a thin, protective coat of glycerol to both preserve the tissue and keep it fully hydrated.[12] Glycerol has long been used to preserve skin grafts[5] and maintains the tissue's biomechanical qualities.[3,11] The end result is a safe, fully hydrated product that can be maintained on a hospital's shelf at ambient temperature. The clinical use of Preservon-treated grafts was assessed in anterior cervical discectomy and fusion (ACDF) surgeries as reported here.

ACDF procedures are used to treat cervical spondylosis which is a common problem that results from degeneration of the cervical intervertebral discs.[16] Most cases can be treated non-surgically but some conditions evolve to cervical radiculopathy or myelopathy and these cases often require surgical intervention. Cervical radiculopathy occurs when pressure is exerted against the nerve root. A common cause of this condition is disc herniation where the nucleus pulposus ruptures the annulus fibrosus and presses against the nerve root.[7] Symptoms include pain, weakness or sensory loss and generally affect the upper extremities.[16] Cervical myelopathy occurs when the spinal cord is compressed.[17] This condition can also be caused by disc herniation or bone spurs or both. The symptoms can affect both upper and lower extremities and do not always include pain. Loss of

September, 2010
68-20-025.00

sensation, weakness, and spasticity are hallmark symptoms.[16] Cervical radiculopathy and myelopathy can be treated through an ACDF surgery using Cloward dowel allografts derived from human bone.[18,19]

The prospective, randomized study reported here compares ACDF surgical outcomes of patients implanted with Preservon-treated Cloward dowels to patients implanted with traditional freeze-dried Cloward dowels.

CLINICAL MATERIALS AND METHODS

Design and Objectives

This study is a prospective, randomized, blinded clinical evaluation comparing Preservon-treated and freeze-dried Cloward dowels in ACDF surgery for cervical radiculopathy or myelopathy. The study design, methods and informed consent were reviewed and approved by an institutional review board though Virginia Commonwealth University. The study and surgical technique is largely modeled from Tye et al.[19] who evaluated subsidence rates for ACDF surgery using freeze-dried Cloward dowel allografts. The design includes a total of 60 patients each with Preservon or freeze-dried grafts, respectively, stratified based on expected number of cervical discs to be fused. The objectives included qualitative assessment of subsidence at 3 months and graft fusion at 6 months. The study hypothesis was that the experimental group (Preservon- treated allografts) would not have significantly inferior assessments than the control group (freeze-dried allografts).

Surgical Assessment Methods

Subsidence and evidence of fusion was qualitatively assessed radiographically at 3 months and 6 months, respectively. At three months follow-up, the examining surgeon determined whether the graft was intact or if it had subsided, moved, loosened, dislodged, or fractured. At 6 months follow-up, the examining surgeon determined if the graft showed signs of fusion. The examining surgeon was blind to the treatment type of the patient during the visual and physical assessments of the patients and radiographs.

Patient Population

This interim analysis reports the first 104 of 120 patients to have reached at least 3 months post-implantation. These patients were randomly assigned to receive Preservon treated grafts or freeze-dried grafts. 52 patients were assigned to the Preservon group and 52 patients were assigned to the freeze-dried group. For each treatment group, there were 13 1-level fusions, 22 2-level fusions, 13 3-level fusions, and 4 4-level fusions. Given that the same graft treatment was used in all treated levels in a surgery for a given patient, for each group, there were 13 grafts used for 1-level fusions, 44 used for 2-level fusions, 39 grafts used for 3-level fusions, and 16 grafts used in 4-level fusions. Thus, each treatment group represented a total of 112 levels of surgery.

In addition to providing voluntary informed consent, patients must have met all inclusion criteria as well as not have any exclusion criteria in order to have been included in the study. Inclusion criteria included but were not limited to the patient being between 18 and 70 years of age, the patient having clinically confirmed radiculopathy or myelopathy secondary to neural compression between C3/4 to C6/7, and the patient agreeing to follow all study protocols such as treatment randomization and attending scheduled follow-up appointments. Exclusion criteria included but were not limited to the patient having previous cervical spine surgery, the patient having multi-level fixed/ankylosed cervical spine, and the patient having another condition that would interfere with an accurate pain/function assessment such as rheumatoid arthritis or a progressive neuromuscular disease.

Surgical Procedure

The Cloward dowel surgical procedure[18] used by Tye et al.[19] was followed in this study. All ACDF surgeries were performed with human bone allograft Cloward dowels (LifeNet Health, Virginia Beach, VA). The cervical spine was accessed through an anterior approach through the neck. Degenerated discs were removed and replaced with either Preservon-treated or freeze-dried allograft Cloward dowels depending upon the treatment group. Rigid internal segmental fixation was then performed via

implantation of an Atlantis anterior cervical plate (Medtronic Sofamor Danek, Memphis, TN) with "fixed angle" screws. Size of the plate and the length of the screw fixation were left to the discretion of the operating surgeon.

RESULTS

At three months follow-up (see Table 1), 47 patients in the Preservon group and 48 patients in the freeze-dried group had intact grafts according to radiographic assessment by a blinded evaluating surgeon. Two patients in the Preservon group and 1 patient in the freeze-dried group had subsidence. No patients in either group had evidence that the graft had moved, loosened, dislodged, fractured, or had another problem not specified. Two patients in each group were lost to follow-up.

Table 1. Summary of patients with intact grafts versus other conditions for both treatments at 3 months follow-up.

Assessment of Subsidence at 3 Months Follow-Up		
	Preservon	Freeze-Dried
All-Intact	47	48
Subsided	2	1
Moved	0	0
Loosened	0	0
Dislodged	0	0
Fractured	0	0
Other	0	0
Patient Did Not Return	2	2
Data Uncollected	1	1
Total	52	52

At six months follow-up (see Table 2), all 44 patients in the Preservon group and all 46 in the freeze-dried group had evidence of graft fusion as reported on the appropriate study case report form. There were no patients in either group that had evidence of nonunion by radiographic evaluation. Patients in each group were lost to follow-up.

Table 2. Evaluation of 6 Month Follow-up Radiographs.

Fusion Data at 6 Months Follow-Up		
Signs of Fusion?	Preservon	Freeze-Dried
Yes	44	46
No	0	0
Patient Did Not Return	5	4
Total	49	50

As shown in Figure 1, representative radiographic images of Preservon-treated and control grafts showed similar signs of fusion at both three and six months follow-up.

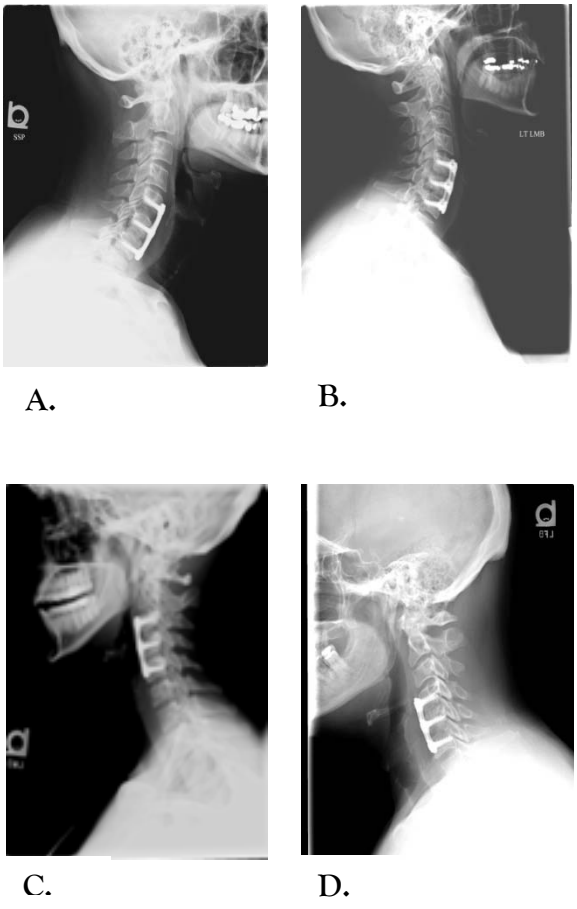

Figure 1. Radiographic images of Control (A, C) and Preservon treated (B, D) grafts at six months follow-up.

In the course of patient follow-up, any Adverse Events were reported and analyzed. As shown in Table 3, there were a total of 97 Adverse Events reported for the 104 patients, with several patients reporting multiple events. Patients were encouraged to report any physical symptoms. Typical events included dysphagia and pain in the neck area or along the arms, shoulders, and back. Many of the reports were not related to the treatment procedure such as torn rotator cuff, foot fracture, panic attacks, etc. Of those still outstanding as possibly graft related, the 3 patients are progressing well and the incidence of adverse events is consistent with this type of surgery.

Table 3. Summary of all Adverse Events recorded in the course of the study.

Adverse Event Reports	Preservon	Freeze-dried
Possibly Graft Related*		
Severe Neck and Right Arm Pain	1	0
Persistent movement on flexion/extension w/no related symptoms	1	0
Possible segmental kyphosis and post-operative infection	0	2 (same patient)
Not Graft Related*		
Dysphagia or Pain in Neck area	18	16
Pain in Arm/Shoulder/Back	14	16
Miscellaneous Other	9	20
Total	**43**	**54**

*As assessed by blinded Data Safety Monitoring Board or examining physician. Note that all (3) patients with AEs deemed possibly graft related exhibit radiographic evidence of fusion.

BIOMECHANICAL ANALYSIS

Previously, the successful retention of biomechanical properties following Preservon treatment has been reported.[3,11,12] Here, we specifically consider data related to freeze-dried grafts which are instructed to be adequately rehydrated before use. While freeze-dried allografts have been an attractive option for surgeons who did not want to manage the difficulties of transporting and thawing traditionally processed frozen grafts, problems with this treatment do exist. One issue of concern is reported brittleness or graft fracture that can manifest upon graft insertion.[4,6] Furthermore, sterilized, freeze-dried cortical allografts have demonstrated to be biomechanically weaker than frozen allografts, even after rehydration.[13] As reported in Figure 2, an internally controlled analysis was performed comparing freeze-dried grafts, frozen grafts and Preservon treated grafts. Preservon-treated cortical bone grafts were able to withstand higher flexural stress, or modulus of rupture, prior to breakage in a 3 point bending test than a freeze-dried graft. Freeze-dried grafts broke with considerably less force than the Preservon-treated grafts even after extensive rehydration time (60 min).

The 3 point bending test was also used to determine graft brittleness by measuring the percent strain or the percent the graft is able to bend before breaking. A more brittle graft will have a lower percent strain to time rehydrated for freeze-dried, thawed for frozen, and rinsed for Preservon grafts.[1] As shown in Figure 3, even after rehydrating for 60 minutes, the freeze-dried grafts only exhibited ~0.75% percent strain prior to breakage. In contrast, after a 30 second rinse, the Preservon-treated grafts exhibited over twice the flexural give, at a percent strain of ~1.75%, prior to breakage.

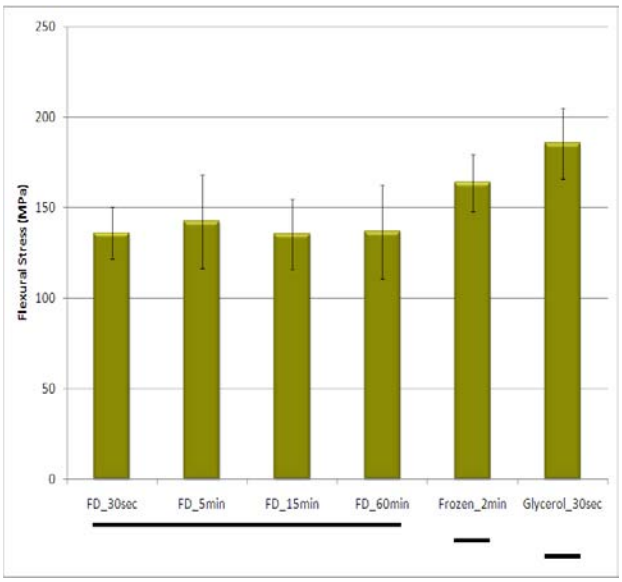

Figure 2. Bar Graph showing the flexural stress, or modulus of rupture, at which freeze-dried (FD), frozen (Frozen), or Preservon (Glycerol)-treated cortical bone can undertake before breaking in a 3 Point Bend Test.[1] Time refers to time rehydrated in room temperature saline solution for freeze-dried, thawed for frozen, and rinsed for Preservon grafts.

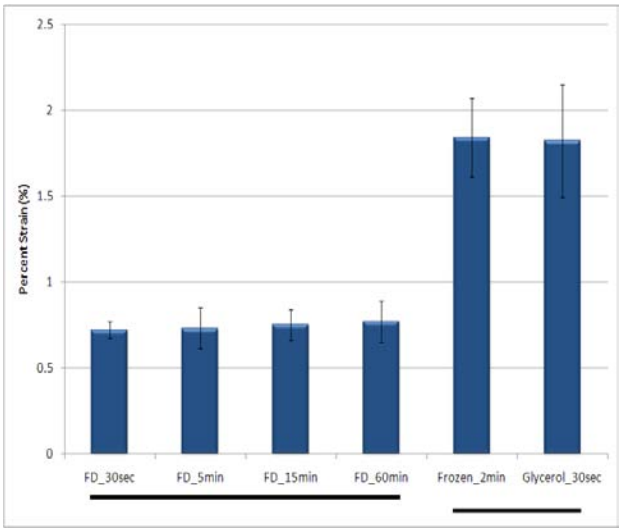

Figure 3. Bar Graph showing the percent strain freeze-dried (FD), frozen (Frozen), and Preservon (Glycerol) treated cortical bone can undertake before breaking in a 3 Point Bend Test.[1] Time refers to time rehydrated in room temperature saline solution for freeze-dried, thawed for frozen, and rinsed for Preservon grafts.

The percent strain exhibited by the Preservon-treated grafts was approximately equivalent to frozen grafts which suggests Preservon maintains the natural properties of bone. In contrast, the freeze-dried grafts remained brittle and even 60 minutes of rehydration was not sufficient to recover the original properties of the graft. In separate studies, it has been shown that the glycerol protectant also maintains compressive properties of bone allografts comparable to traditionally treated bone allografts.[3, 11]

DISCUSSION

Preservon treatment allows allograft tissue to be stored in a fully hydrated state at ambient temperature. After briefly rinsing before implantation (<30 seconds), the graft contains only ~4% glycerol. This is significantly lower than the ~70% glycerol in widely used DBM void filler products such as Optium DBM® and Grafton®. Glycerol has been used for years as a safe and effective preservation treatment for skin allografts in European tissue banks[5,8,14,21] especially in the Euro Skin Bank that developed this method for skin allografts.[15] In support, our initial results indicate no incidence of glycerol related neurologic dysfunction with 52 Preservon graft patients that includes 112 separate levels of surgery.

In addition to the advantages of ambient temperature storage and preservation of biomechanical qualities, glycerolization also has strong antibacterial and virus inactivation properties.[5,8,14,15,20,21] However, this is only useful in highlighting a theoretical benefit of glycerolization since Preservon-treated allograft is processed with the patented Allowash XG® sterilization process.[2] This a process for cleaning disinfecting allograft tissue and can provide sterile tissue that maintains its biomechanical properties. Also, as previously reported,[9,10] the Allowash XG process for bone allograft already ensures a minimum 12.4 log reduction of the HIV viral count.

The ease of use, maintenance of biomechanical properties, antimicrobial, and viral inactivation properties of glycerolization make Preservon an attractive allograft treatment choice. The purpose of this study was to investigate in a clinical setting whether Preservon-treated bone grafts would perform successfully in an ACDF surgery. The results indicate that Preservon-treated Cloward dowels do have equivalent clinical results as freeze-dried Cloward dowels. At 3 months, 47 out of 49 Preservon-treated patients had fully intact grafts with only two patients having signs of subsidence. 48 out of 49 freeze-dried patients had fully intact grafts with only 1 patient having signs of subsidence (Table 1). Similarly all patients seen for both Preservon and freeze-dried groups had signs of fusion at 6 months (43 out of 43 and 46 out of 46 patients respectively; Table 2).

SUMMARY

The Preservon-treated allografts compared favorably with the freeze-dried grafts used in anterior cervical discectomy and fusion surgery. This result, in addition to the maintenance of biomechanical properties and ease of use support the use of Preservon-treated allografts in ACDF procedures.

REFERENCES

1. Data On File. LifeNet Health.
2. U.S. Patents 5,556,379; 5,820,581; 5,977,034; 6,024,735.
3. Balsly C, Ruth, K., Moore, M., and Wolfinbarger, L. The Effects of Preservon® treatment on the Biomechanical Strength of Allograft Tissue. *American Association of Tissue Banks Meeting.* Boston, MA; 2007.
4. Bottino M, Jose, M., Thomas, V., Dean, D., and Janowski, G. Freeze-Dried Acellular Dermal Matrix Graft: Effects of Rehydration on Physical, Chemical, and Mechanical Properties. *Dental Materials.* 2009;25:1109-1115.
5. de Backere A. Euro Skin Bank: Large Scale Skin-Banking in Europe Based on Glycerol-Preservation of Donor Skin. *Burns.* 1994;20(1):S4-S9.
6. de Roeck N, and Drabu, K. Impaction Bone Grafting Using Freeze-Dried Allograft in Revision Hip Arthroplasty. *The Journal of Arthroplasty.* 2001;16(2):201-206.
7. Goldberg G, and Hilibrand, A. Anterior Cervical Disectomy and Fusion. *Operative Techniques in Orthopaedics.* 2003;13(3):188-194.
8. Marshall L, Ghosh, M., Boyce, S., MacNeil, S., Freedlander, E., and Kudesia, G. Effect of Glycerol on Intracellular Virus Survival: Implications for the Clinical Use of Glycerol-Preserved Cadaver Skin. *Burns.* 1995;21(5):356-361.
9. Moore M. Inactivation of Enveloped and Non-Enveloped Viruses Seeded on Human Tissues by Validated Low Dose Gamma Irradiation Sterilization Process. *American Association of Tissue Banks Annual Meeting.* Las Vegas, NV; 2009.
10. Moore M. Inactivation of enveloped and non-enveloped viruses on seeded human tissues by validated low dose gamma irradiation process. *Annual Meeting of the Orthopedic Research Society*. New Orleans, LA; 2010.
11. Moore M, Balsly, C., Ruth, K., and Wolfinbarger, L. The effects of an alternate preservation method on the biomechanical strength of allograft tissue. *Annual Meeting of the Orthopedic Research Society*. San Francisco, CA; 2008.
12. Moore M, Crouch, K., O'Neal, A., and Wolfinbarger, L. Properties of human bone allograft bone preserved with glycerol. *BioInterface 2004*. Baltimore, MD; 2004.
13. Nather A, Thambyah, A., and Goh, H. Biomechanical Strength of Deep-Frozen Versus Lyophilized Large Cortical Allografts. *Clinical Biomechanics.* 2004;19:526-533.
14. Pianigiani E, Ierardi, F., Cuciti, C., Brignali, S., Oggioni, M., and Fimiani, M. Processing efficacy in Relation to Microbial Contamination of Skin Allografts From 723 Donors. *Burns.* 2010;36:347-351.
15. Saegeman V, Ectors, N., Lismont, D., Verduyckt, B., and Verhaegen, J. Short- and Long-Term Bacterial Inhibiting Effect of High Concentrations of Glycerol Used in the Preservation of Sking Allografts. *Burns.* 2008;34:205-211.
16. Torrens M. Cervical Spondylosis Part 1: Pathogenesis, Diagnosis, and Management Options. *Current Orthopaedics.* 1994;8:255-264.
17. Torrens M, and Miliaras, G. Cervical Spondylosis. Part II: Surgical Management. *Current Orthopaedics.* 2002;16:300-310.
18. Cloward R. The anterior approach for removal of ruptured cervical discs. J. Neurosurg 1958; 15: 602
19. Tye G, Graham, S., Broaddus, W., and Young, H. Graft Subsidence After Instrument-Assisted Anterior Cervical Fusion. *Journal of Neurosurgery: Spine.* 2002;97:186-192.
20. van Baare J, Buitenwerf, J., Hoekstra, M, and du Pont, J. Virucidal Effect of Glycerol as Used in Donor Skin Pre*servation. Burns. 1994;20(1):S77-S80.*
21. van Baare J, Ligtvoet, E., and Middelkoop, E. Microbiological Evaluation of Glycerolized Cadaveric Skin. *Transplantation.* 1998;65(7):*966-970.*

Acknowledgements: We acknowledge special assistance in this study from Amanda McCluskey (formerly of Medical College of Virginia), Patricia Leach (Medical College of Virginia), and Liisa Eisenlohr and Katrina Ruth (both formerly of LifeNet Health).