Cryopreserved Amniotic Membrane Improves Clinical Outcomes Following Microdiscectomy

D. Greg Anderson, MD,† *Victor Popov, MD,*† *Andrew L. Raines, PhD,*‡
and Julie O'Connell, PhD‡

Study Design: Prospective, randomized controlled trial.

Objective: To compare pain, physical/mental functional recovery and recurrent herniation for patients following lumbar microdiscectomy with and without the use of a cryopreserved amniotic tissue graft.

Summary of Background Data: Although microdiscectomy procedures are routinely successful for patients with lumbar radiculopathy due to herniated disc disease, residual low back pain, and recurrent herniation remain unsolved clinical problems.

Methods: Following Investigated Review Board approval, 80 subjects were randomized in a 1:1 ratio to either receive cryopreserved amniotic (cAM) tissue or no tissue following elective lumbar microdiscectomy surgery. cAM grafts were applied to the annular defect at the conclusion of the procedure. Patients provided preoperative and postoperative clinical assessment data out to 24 months using the Oswestry Disability Index (ODI), Short Form-12 (SF-12) Health Survey, and Visual Analog Pain Scale for back and leg pain. Patients with symptomatic recurrent disc herniation were recorded.

Results: In total, 48 males and 32 females with an average age of 47.2 years were included. Mean ODI scores for subjects treated with cAM graft demonstrated statistically greater improvement at 6 weeks (14.49 vs. 21.82; $P = 0.05$) and 24 months (6.62 vs. 14.40; $P = 0.02$) compared with controls. Similarly, SF-12 Physical Component Scores demonstrated statistically greater gains in the cAM group at both the 6 weeks and 24 months. None of the subjects in the cAM graft group sustained a recurrent herniation at the same surgical level, whereas 3 patients in the control group sustained a recurrent herniation at the same surgical level, with 2 requiring fusion to manage persistent pain.

Conclusions: The data demonstrate statistically superior clinical outcomes following lumbar microdiscectomy as measured by ODI and SF-12 (physical composite scale) and a lower rate of recurrent herniation with the use of a cAM tissue graft compared with traditional microdiscectomy.

Key Words: amniotic membrane, microdiscectomy, outcome

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Lumbar radiculopathy secondary to lumbar disc herniation (LDH) is a common health problem that presents a large economic burden to the medical system.[1,2] Nonsurgical treatment options for LDH include activity limitations, pharmacologic therapy, manipulation, physical therapy; and epidural steroid injections.[3] Although nonsurgical treatments are often successful, patients who are nonresponsive to conservative therapies or those with progressive neurological symptoms require surgical intervention.

Surgical treatment is generally successful in relieving or reducing radicular symptoms due to LDH.[4,5] Unfortunately, with longer-term follow-up, residual/recurrent axial back pain and/or recurrent disc herniation with radiculopathy are significant unsolved problems, with studies reporting reoperation rates following surgery for LDH from 18.5%–25%.[4,6] At present, there are no proven treatments available to reduce the incidence of postoperative axial back pain and recurrent disc herniation.

The amniotic membrane (AM) is a placental-derived tissue, sharing the same cellular origin as the developing fetus. A primary function of the AM is to protect the fetus from the maternal immune system and not surprisingly, the AM has been demonstrated to possess potent anti-inflammatory properties,[7] including the ability to reduce proinflammatory and increase anti-inflammatory cytokine levels[8] and induce apoptosis of proinflammatory cells.[8,9] AM tissues have been used clinically in a number of applications in ophthalmology,[10] as well as a dressing for burns, nonhealing skin ulcers, and as an aid to promote wound healing.[11–13] In addition to its anti-inflammatory properties, AM tissues have also been shown to possess antiscarring properties.[14,15] The anti-inflammatory, regenerative and antiscarring activities of AM tissues have recently been shown to be attributable to a unique glycoprotein complex within the extracellular matrix called the HC-HA/PTX3 complex.[7]

One of the hallmarks of disc degeneration is an increase in the expression and levels of various proinflammatory cytokines.[16,17] The anti-inflammatory and antiscarring actions of AM tissues make this tissue a potentially attractive option to reduce postoperative inflammation and promote healing of the annular defect following microdiscectomy.

In the present study, we sought to determine the safety and clinical efficacy of a cryopreserved amniotic (cAM) tissue product as an adjunctive treatment applied to the annular defect following lumbar microdiscectomy surgery. Specifically, we set out to compare in a prospective, randomized manner, the clinical outcomes and symptomatic recurrence rates for patients treated with and without cAM applied to the annular defect following lumbar microdiscectomy surgery.

METHODS

Study Design

A single-center, single-blind, prospective, randomized controlled trial was conducted to determine the safety and effectiveness of cryopreserved AM (cAM; Amniox Medical Inc., Atlanta, GA) after lumbar microdiscectomy. Because of the placement of cAM after microdiscectomy, only the patient could be blinded to treatment assignment. The cAM tissue used in the study is classified by the Food & Drug Administration as a human cell, tissue, and human cellular and tissue product. Tissue collection, processing, and use were followed according to all applicable Food & Drug Administration regulations and guidelines. Following Investigated Review Board approval (Western Investigated Review Board Protocol #20110656), enough patients were screened to be able to enroll 80 patients randomized in a 1:1 ratio (treatment: control) to either receive cAM tissue (treatment) or no tissue (control) following elective lumbar microdiscectomy surgery. The primary exclusion criteria for the study were a history of prior back surgery at the same level as the herniation; inability to walk independently; receipt of corticosteroids, radiation therapy, chemotherapy, or immunosuppressive agents within 1 month of surgery; pregnancy, body mass index > 50, severe renal failure, hepatic insufficiency, cirrhosis; anemia, rheumatoid arthritis; or active local or systemic malignancy. Disc herniations were diagnosed by combining clinical evaluation and magnetic resonance imaging scanning of the lumbar spine. All patients were treated by the same surgeon (D.G.A.). Patients randomized to the treatment arm of the study received placement of cAM in the annular defect after removal of the herniated disc fragments.

Operative Technique

All patients enrolled in the study underwent elective lumbar microdiscectomy for symptoms of severe radiculopathy, unresponsive to nonoperative treatment. Briefly, after the induction of anesthesia, patients were placed prone on a radiolucent spine frame. The incision site was localized fluoroscopically using an 18-G spinal needle. An ∼20-mm incision was made on the symptomatic side and then serial dilation was used to place a tubular retractor (METRx, Medtronic Spine, Memphis, TN) at the affected level. A laminotomy was performed by removing the caudal edge of the proximal lamina and the cranial edge of the distal lamina and intervening ligamentum flavum as required to gain access the disc fragment. The traversing nerve root was protected and retracted as necessary to reach the herniated disc fragment. The membrane over the extruded disc fragment was opened using a Penfield #4 dissector and the herniated disc material was removed using a pituitary rongeur. The annular defect was identified and explored to exclude or remove additional loose disc fragments. After all loose disc fragments had been removed and the nerve root adequately decompressed, cAM was placed into the annular defect in the cohort of patients randomizing to cAM treatment. In the cAM cohort, a 2×2 cm rectangular piece of cAM was placed into the annular defect. Delivery of the cAM to the annular defect was accomplished by placing the tissue on the back side of the nerve root retractor and the pushing the tissue into the annular defect with a nerve hook (Fig. 1). The cAM tissue is a thin membrane that is inserted into the void after removal of the herniated disc material without any physical attachment to retain the graft in position. An important technical pearl is to reduce the intensity of suction during delivery of the cAM tissue to prevent suctioning the tissue up into the suction catheter. After placement of the cAM, the nerve root retractor was removed and the tubular retractor was withdrawn. In all patients, the fascia and skin were closed using 2-0 absorbable suture followed by a subcutaneous injection of 10 mL of 0.25% marcaine (Baxter, Atlanta, GA). Patients randomized to the control arm of the study underwent the same procedure without the placement of cAM.

Postoperatively, patients were encouraged to begin a walking program of at least 30 minutes per day and all patients were referred for postoperative rehabilitation by a physical therapist to improve core muscle strengthening and aerobic conditioning at 2-week postoperative timepoint.

Outcomes Measures

Outcome instruments utilized in this study included the Oswestry Disability Index (ODI), 10 cm Visual Analog Pain Scale (VAS) (separately, for low back and leg pain), and the Short Form-12 (SF-12) Health Survey questionnaire. The data collection timepoints included before surgery (baseline) and at the 2-week, 6-week, 6-month, 12-month, and 24-month postoperative timepoints. At each in-office follow-up visit, outcome questionaires and VAS were filled out by subjects and collected by a research coordinator. At each out-of-office follow-up visit, subjects were contacted by a research coordinator and provided answers to questionaires over the phone.

Over the 24-month follow-up period, patients were encouraged to report any symptoms of new, severe back or leg pain. For patients with recurrent
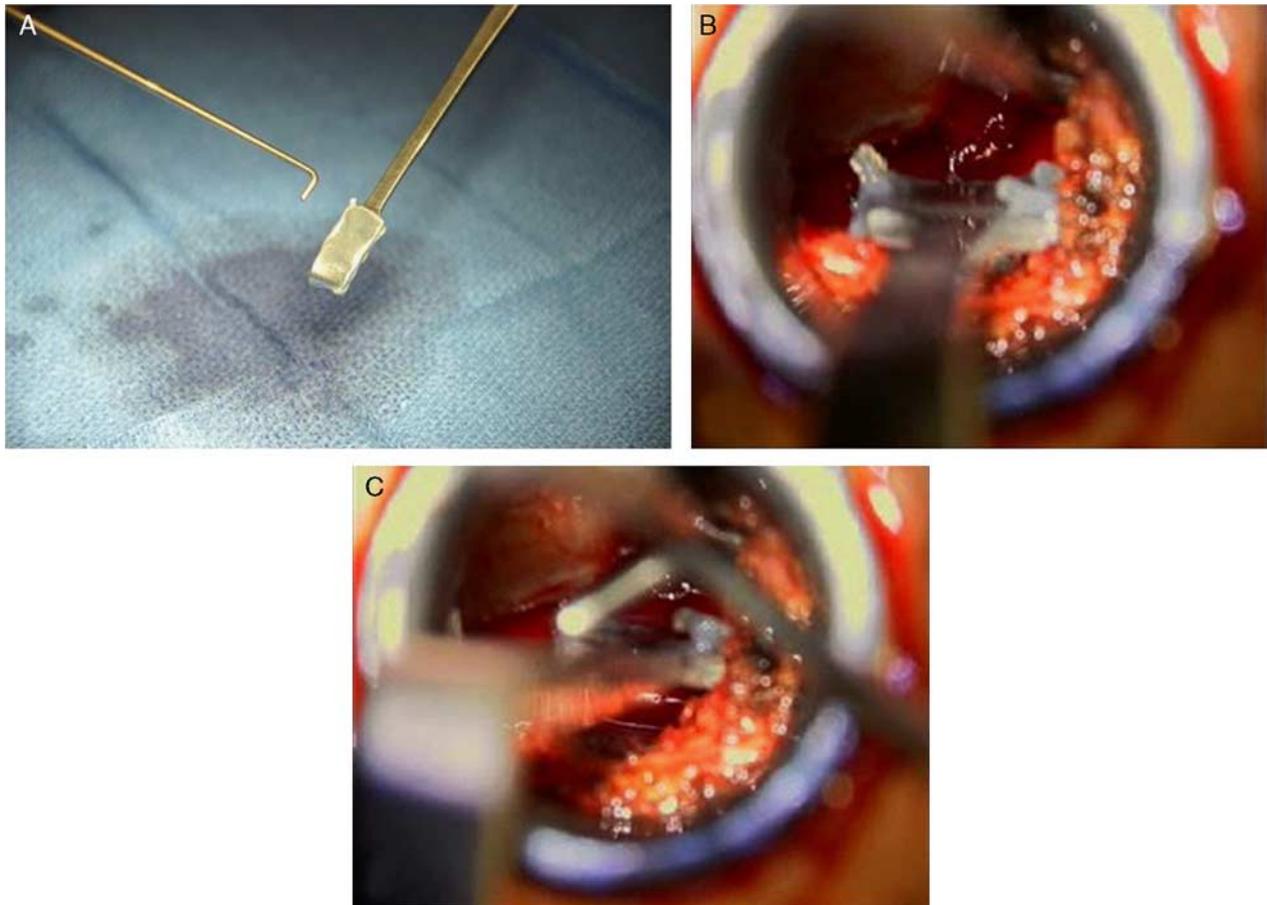
FIGURE 1. AM tissue placement. A 2×2 cm piece of cryopreserved AM tissue (A). Following removal of the herniated disc, cryopreserved AM tissue was inserted through the cannula (B) into the nucleus pulposus of the affected disc (C). AM indicates amniotic membrane.

symptoms following microdiscectomy surgery, gadolidium enhance magnetic resonance imaging scanning was utilized to diagnose recurrent LDH. Patients with imaging evidence of disc herniation that correlated clinically to the patient's symptoms were identified.

Those with symptomatic disc herniation were divided into disc herniation at the same level (characterized as recurrent herniation) and herniation at another level (new herniation).

Statistical Analysis

The mean $\pm$ SE for the ODI, VAS, and SF-12 scores were calculated and plotted for both the cAM and control group at all timepoints (Microsoft Excel 2011). Differences between groups were determined using an unpaired t test (SPSS Statistics, IBM, New York) with $P \leq 0.05$ considered to be statistically significant.

RESULTS

A total of 80 patients were enrolled in the study and randomized in a 1:1 ratio to either the cAM cohort or the control cohort. A summary of the patient demographics is presented in Table 1. Briefly, there were 48 males and 32 females included in the study population. The average age was 47.2 years (range, 20–73 y). A breakdown of the affected disc level for the entire study population is as follows: 2 L1–L2; 4 L2–L3; 7 L3–L4; 26 L4–L5, 32 L5–S1; 9 L4–L5, L5–S1. Overall, there were no statistically

TABLE 1. Patient Demographics

	Overall	Control	Amniotic Membrane
#Patients (N)	80	40	40
Age (mean) (y)	45.8 ± 11.3	47.2 ± 9.1	44.3 ± 13.1
Range	20–73	30–66	20–73
#Male	48	20	28
#Female	32	20	12
BMI	29.9 ± 6.1	28.2 ± 5.5	31.7 ± 6.3
Tobacco use	21/80	10/40	11/40
Affected level			
L1–L2	2	2	0
L2–L3	4	3	1
L3–L4	7	4	3
L4–L5	26	15	11
L5–S1	32	13	19
L4–L5; L5–S1	9	3	6

BMI indicates body mass index.

significant differences between groups in terms of sex, age, or affected level. Postoperatively, there were no surgical complications encountered including surgical-site infections, nerve root injuries, or dural laceration in either study cohort.

Scores for ODI were collected before surgery (baseline) and at predetermined timepoints out to the 24-month postoperative timepoint. Before surgery, there was no significant difference in ODI scores between the cohorts (cAM: 48.63 ± 2.69; control: 51.95 ± 2.23). At the 2-week postoperative timepoint, nonsignificant differences between the cAM and control group were observed (cAM: 25.44 ± 3.09; control: 31.64 ± 2.80). At the 6-week postoperative timepoint, the mean ODI score for the cAM group was significantly lower than that of the control group (cAM: 14.49 ± 2.63; control: 21.82 ± 2.75; $P = 0.05$). Nonsignificant differences in ODI scores were again observed between the cohorts at the 6-month (cAM: 11.98 ± 2.50; control: 12.08 ± 2.31) and 12-month (cAM: 9.29 ± 1.96; control: 12.83 ± 2.55) timepoints. At the 24-month timepoint, the mean ODI scores were significantly lower for cAM-treated patients compared with control patients (cAM: 6.62 ± 1.30; control: 14.40 ± 3.29; $P = 0.02$). Reviewing the data trends, it can be observed that the mean ODI score for cAM cohort continued to decrease throughout the 24-month follow-up period, whereas the control cohort experienced its lowest mean ODI scores at the 6-month follow-up timepoint and subsequently experienced a trend of worsening ODI scores at the 12- and 24-month timepoints. A plot of the ODI scores versus time is shown in Figure 2.

Mental and physical health was assessed using the SF-12 Health Survey questionnaire. A plot of the physical composite scores and mental composite scores is shown in Figures 3A, B. Similar to the ODI scores, patients receiving cAM tissue demonstrated a significantly improved mean SF-12 physical composite score at the 6 weeks ($P = 0.018$) and 24 months ($P = 0.05$) postoperative timepoints in comparison with the control cohort. Nonsignificant differences were observed at the other timepoints. No significant differences were observed in the SF-12 mental composite scores between the 2 groups except for the 2-week timepoint where the cAM-treated cohort demonstrated a significant improvement compared with the control group ($P = 0.04$) (Fig. 3B).

Pain was accessed using the 10 cm VAS. Significant improvements in both back and leg pain were observed in both the cAM-treated and control cohorts compared with baseline at all timepoints postoperatively. No significant differences in VAS scores were found between the treatment cohorts (Tables 2, 3).

In the cAM cohort, there were no instances of recurrent herniation at the same level during the 24-month follow-up period. However, in the control cohort, 3 patients presented with a recurrent herniated disc at the same level. All 3 patients failed to respond to a minimum 6-week course of nonsurgical care consisting of activity limitations, analgesic medications, nonsteroidal anti-inflammatory drugs, and epidural steroid injections.
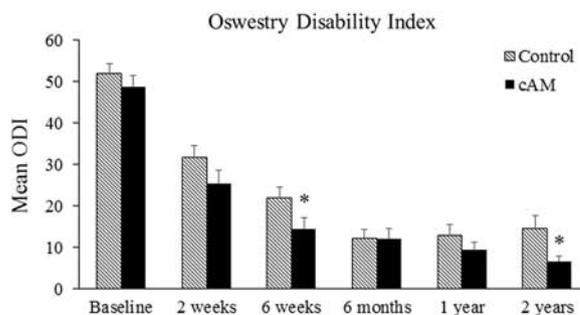
FIGURE 2. ODI. Before surgery and at specified timepoints postoperatively, ODI scores were collected for all patients. Values presented are mean ± SEM. cAM indicates cryopreserved amniotic; ODI, Oswestry Disability Index. *$P < 0.05$ versus control.

These 3 patients were ultimately treated with additional surgery. One of the 3 (2.5% of the control cohort) had predominantly radicular pain and was treated with revision microdiscectomy. The other 2 patients (5% or the control cohort) had a substantial component (> 50% of the overall pain complex) of mechanical low back pain and were treated with lumbar fusion.

No adverse events attributable to the cAM were reported during the study period.
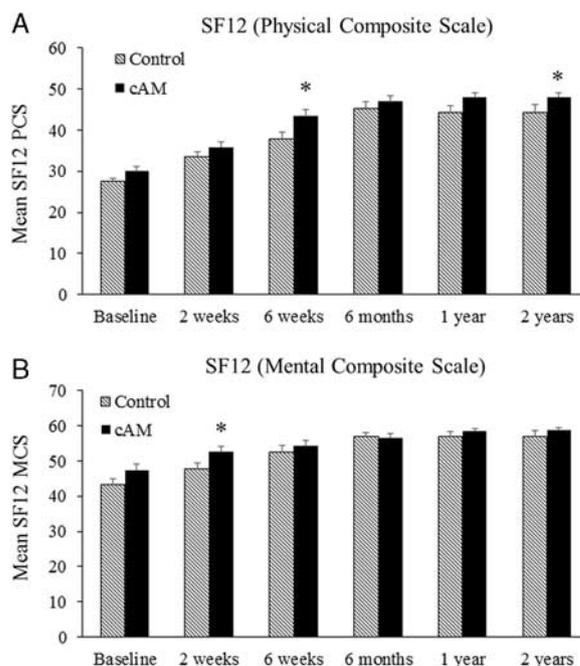
FIGURE 3. SF-12 Health Survey Scores. Before surgery and at specified timepoints postoperatively, patients filled out the SF-12 health survey. Scores for the PCS (A) and MCS (B) were collected for all patients. Values presented are mean ± SEM. cAM indicates cryopreserved amniotic; MCS, mental composite scale; PCS, physical composite scale; SF-12, Short Form-12. *$P < 0.05$ versus control.

TABLE 2. VAS Back Pain Data Summary

| | Amniotic Membrane | | |
	Mean ODI ± SE	% Decrease From Baseline	*P*
Baseline	5.72 ± 0.33	—	—
2 wk	3.31 ± 0.48	42.1	< 0.001
6 wk	2.17 ± 0.41	62.1	< 0.001
6 mo	1.56 ± 0.37	72.7	< 0.001
Control			
Baseline	5.77 ± 0.33	—	< 0.001
2 wk	3.71 ± 0.46	35.7	< 0.001
6 wk	2.97 ± 0.45	48.5	< 0.001
6 mo	1.73 ± 0.47	70.0	< 0.001

ODI indicates Oswestry Disability Index; VAS, Visual Analog Pain Scale.

DISCUSSION

The present investigation was performed to study the clinical efficacy and safety of a cAM tissue product placed in the annular defect for patients undergoing routine lumbar microdiscectomy. This prospective, randomized controlled trial, demonstrated that cAM was able to produce better clinical outcomes at the 6-week and 24-month timepoints as measured by the ODI and SF-12 physical composite scores compared with the current standard of care. In addition, in patients treated with cAM, there were no recurrent herniations at the same level.

Recurrent disc herniation following microdiscectomy remains an unsolved problem. In a systematic review of the microdiscectomy literature between 2009 and 2015, the rate of recurrent herniation varied from as little as 0.2% to 20%,[18] although most of the 57 articles reviewed reported recurrence rates between 2%–10%. In the present study, the rate of recurrent disc herniation in the control group (7.5%) was consistent with prior studies, whereas patients receiving cAM did not experience any recurrent herniation at the same level (0%). This finding is potentially quite significant and deserves more investigation using larger patient cohorts to confirm these early observations.

The AM has been used clinically for over a century[19] in a number of applications in ophthalmology[8] and wound healing.[20–22] The clinical success of cAM as a

TABLE 3. VAS Leg Pain Data Summary

| | Amniotic Membrane | | |
	Mean ODI ± SE	% Decrease From Baseline	*P*
Baseline	8.19 ± 0.24	—	—
2 wk	3.54 ± 0.46	56.8	< 0.001
6 wk	2.68 ± 0.47	67.3	< 0.001
6 mo	1.66 ± 0.40	79.7	< 0.001
Control			
Baseline	8.14 ± 0.18		< 0.001
2 wk	3.82 ± 0.50	53.1	< 0.001
6 wk	3.09 ± 0.56	62.0	< 0.001
6 mo	1.81 ± 0.54	77.8	< 0.001

ODI indicates Oswestry Disability Index; VAS, Visual Analog Pain Scale.

potent anti-inflammatory and antiscarring agent has recently expanded interest into the potential applications of this unique tissue form in reconstructive procedures where inflammation and adhesion formation might be harmful. Clinical protocols have been studied for tendon[23] and nerve repair[24–26] and investigations are ongoing for various spinal applications.[27]

A well-characterized hallmark of disc degeneration is the increased expression of proinflammatory cytokines, including Interleukin-1β, resulted on activation normal T-cell expressed, tumor necrosis factor-α, and substance P.[24,25] These proinflammatory cytokines likely play a role in the clinical pain syndromes that require medical intervention. cAM has been shown to downregulate proinflammatory and upregulate anti-inflammatory cytokine signaling.[26] In addition, cAM has been shown to decrease adhesion and proliferation of proinflammatory cells and induce selective apoptosis of proinflammatory cells.[26,27] cAM also downregulates TGF-β1 signaling, an important component in the pathway of scar formation following injury.[13,28] This tissue acts to limit the differentiation of fibroblasts into myofibroblasts to prevent collagen contraction, the mechanism responsible for inducing scar and adhesion formation.[29,30] AM has been investigated as a material to reduce epidural adhesions after laminectomy in both rat[31] and canine[32] models. These studies documented a reduced volume and density of scar tissue, less inflammatory cell infiltration, and reduced fibroblast proliferation in animals treated with AM.

The immunomodulatory and antiscarring actions of cAM tissue are modulated by a unique component of the extracellular matrix called the HC-HA/PTX3 complex. This complex is formed by tight association between pentraxin 3 (PTX3) and HC-HA, which consists of high molecular weight hyaluronic acid (HA) covalently linked to heavy chain 1 of inter-α-trypsin inhibitor (IαI) through the catalytic action of tumor necrosis factor-stimulated gene-6 (TSG-6).[33,34]

The potential mechanisms of the benefits seen in the current study for patients treated with cAM could theoretically be due to the down-regulation of proinflammatory cytokines, proinflammatory cells, and/or inhibition of the scar formation pathway resulting in an enviroment more conducive to healing of the intervertebral tissue.[26–28] It is compelling to note the ongoing clinical improvement was seen at each timepoint in the mean ODI scores of the cAM treatment cohort compared with the control group. Additional research to better characterize the mechanism of action of cAM within the annulus fibrosus is certainly warranted.

As with any new therapy, the cost of the proposed new treatment versus its benefits should be evaluated. This study was a single-center, early evauation of cAM as an adjunct in lumbar microdiscectomy, and was not designed to have an economic endpoint. However, the lack of recurrent herniation in the cAM group compared with the control group may lead to a cost-savings by reducing office visits, medications, and repeat or additional surgical procedures. The potential ability of cAM to reduce

overall costs associated with lumbar microdiscectomy and recurrent low back pain in particular, should be evaluated in a larger, multicenter clinical study.

Certain limitations of the current study should be acknowledged. First, a relatively small number of patients were enrolled although this deficiency is partially offset by the prospective randomization strategy of the current study design. Second, all operations were performed by a single investigator although the surgical technique was standardized and commonplace for this type of pathology. Third, the 2-year follow-up period represents a medium-term outcome report. For this reason, we have submitted an amended protocol to continue the current research follow-up out to the 5 year timepoint to assess longer-term differences between the cohorts.

CONCLUSIONS

Cryopreserved AM placed in the annular defect following microdiscectomy led to improved clinical outcome as measured by the ODI and SF-12 Physical Component Scale at the 6-week and 24-month timepoints compared with standard microdiscectomy. In this study, there were no reported recurrent disc herniations at the same level in the cohort treated with cAM compared with standard microdiscectomy (control) which saw a 7.5% rate of recurrent disc herniation. Overall, these results are intriguing and further, larger studies are warranted.

REFERENCES

1. Tarulli AW, Raynor EM. Lumbosacral radiculopathy. *Neurol Clin*. 2007;25:387–405.
2. Dagenais S, Caro J, Haldeman S. A systematic review of low back pain cost of illness studies in the United States and internationally. *Spine J*. 2008;8:8–20.
3. Sonntag VKH. Treatment of the herniated lumbar disc: persistent problem. *World Neurosurg*. 2010;74:574–575.
4. Aichmair A, Du JY, Shue J, et al. Microdiscectomy for the treatment of lumbar disc herniation: an evaluation of reoperations and long-term outcomes. *Evid Based Spine Care J*. 2014;5:77–86.
5. Shriver MF, Xie JJ, Tye EY, et al. Lumbar microdiscectomy complication rates: a systematic review and meta-analysis. *Neurosurg Focus*. 2015;39:E6.
6. Soliman J, Harvey A, Howes G, et al. Limited microdiscectomy for lumbar disk herniation: a retrospective long-term outcome analysis. *J Spinal Disord Tech*. 2014;27:E8–E13.
7. Tseng SCG. HC-HA/PTX3 purified from amniotic membrane as novel regenerative matrix: insight into relationship between inflammation and regeneration. *Invest Ophthalmol Vis Sci*. 2015;56:1–8.
8. He H, Zhang S, Tighe S, et al. Immobilized heavy chain-hyaluronic acid polarizes lipopolysaccharide-activated macrophages toward M2 phenotype. *J Biol Chem*. 2013;288:25792–25803.
9. He H, Li W, Tseng DY, et al. Biochemical characterization and function of complexes formed by hyaluronan and the heavy chains of inter-alpha-inhibitor (HC-HA) purified from extracts of human amniotic membrane. *J Biol Chem*. 2009;284:20136–20146.
10. Liu J, Sheha H, Fu Y, et al. Update on amniotic membrane transplantation. *Expert Rev Ophthalmol*. 2010;5:645–661.
11. Bose B. Burn wound dressing with human amniotic membrane. *Ann R Coll Surg Engl*. 1979;61:444–447.
12. Gruss JS, Jirsch DW. Human amniotic membrane: a versatile wound dressing. *Can Med Assoc J*. 1978;118:1237–1246.
13. Stern W. The grafting of preserved amniotic membranes to burned and ulcerated surfaces, substituting skin grafts. *JAMA*. 1913;60:973.
14. Li W, He H, Chen Y-T, et al. Reversal of myofibroblasts by amniotic membrane stromal extract. *J Cell Physiol*. 2008;215:657–664.
15. Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. *J Cell Physiol*. 1999;179:325–335.
16. Kepler CK, Markova DZ, Koerner JD, et al. Substance P receptor antagonist suppresses inflammatory cytokine expression in human disc cells. *Spine (Phila Pa 1976)*. 2015;40:1261–1269.
17. Kepler CK, Markova DZ, Hilibrand AS, et al. Substance P stimulates production of inflammatory cytokines in human disc cells. *Spine (Phila Pa 1976)*. 2013;38:E1291–E1299.
18. Anichini G, Landi A, Caporlingua F, et al. Lumbar endoscopic microdiscectomy: where are we now? an updated literature review focused on clinical outcome, complications, and rate of recurrence. *Biomed Res Int*. 2015;2015:417801.
19. Davis JS. Skin grafting at the Johns Hopkins Hospital. *Johns Hopkins Hosp Rep*. 1910;15:542–549.
20. Ellington JK, Ferguson CM. The use of amniotic membrane/umbilical cord in first metatarsophalangeal joint cheilectomy: a comparative bilateral case study. *Surg Technol Int*. 2014;25:63–67.
21. DeMill SL, Granata JD, McAlister JE, et al. Safety analysis of cryopreserved amniotic membrane/umbilical cord tissue in foot and ankle surgery: a consecutive case series of 124 patients. *Surg Technol Int*. 2014;25:257–261.
22. Warner M, Lasyone L. An open-label, single-center, retrospective study of cryopreserved amniotic membrane and umbilical cord tissue as an adjunct for foot and ankle surgery. *Surg Technol Int*. 2014;25:251–255.
23. Demirkan F, Colakoglu N, Herek O, et al. The use of amniotic membrane in flexor tendon repair: an experimental model. *Arch Orthop Trauma Surg*. 2002;122:396–399.
24. Mohammad J, Shenaq J, Rabinovsky E, et al. Modulation of peripheral nerve regeneration: a tissue-engineering approach. The role of amnion tube nerve conduit across a 1-centimeter nerve gap. *Plast Reconstr Surg*. 2000;105:660–666.
25. Kim SS, Sohn SK, Lee KY, et al. Use of human amniotic membrane wrap in reducing perineural adhesions in a rabbit model of ulnar nerve neurorrhaphy. *J Hand Surg Eur Vol*. 2010;35:214–219.
26. Meng H, Li M, You F, et al. Assessment of processed human amniotic membrane as a protective barrier in rat model of sciatic nerve injury. *Neurosci Lett*. 2011;496:48–53.
27. Subach BR, Copay AG. The use of a dehydrated amnion/chorion membrane allograft in patients who subsequently undergo reexploration after posterior lumbar instrumentation. *Adv Orthop*. 2015;2015:501202.
28. Lee SB, Li DQ, Tan DT, et al. Suppression of TGF-beta signaling in both normal conjunctival fibroblasts and pterygial body fibroblasts by amniotic membrane. *Curr Eye Res*. 2000;20:325–334.
29. Abdel MP, Morrey ME, Barlow JD, et al. Myofibroblast cells are preferentially expressed early in a rabbit model of joint contracture. *J Orthop Res*. 2012;30:713–719.
30. Powell DW, Mifflin RC, Valentich JD, et al. Myofibroblasts. I. Paracrine cells important in health and disease. *Am J Physiol*. 1999;277(pt 1):C1–C9.
31. Choi HJ, Kim KB, Kwon Y-M. Effect of amniotic membrane to reduce postlaminectomy epidural adhesion on a rat model. *J Korean Neurosurg Soc*. 2011;49:323–328.
32. Tao H, Fan H. Implantation of amniotic membrane to reduce postlaminectomy epidural adhesions. *Eur Spine J*. 2009;18:1202–1212.
33. Zhang S, He H, Day AJ, et al. Constitutive expression of inter-α-inhibitor (IαI) family proteins and tumor necrosis factor-stimulated gene-6 (TSG-6) by human amniotic membrane epithelial and stromal cells supporting formation of the heavy chain-hyaluronan (HC-HA) complex. *J Biol Chem*. 2012;287:12433–12444.
34. Zhang S, Zhu Y-T, Chen S-Y, et al. Constitutive expression of pentraxin 3 (PTX3) protein by human amniotic membrane cells leads to formation of the heavy chain (HC)-hyaluronan (HA)-PTX3 complex. *J Biol Chem*. 2014;289:13531–13542.