 doi:10.20944/preprints202207.0337.v1

Evaluation of the Efficacy of Cryopreserved Human Umbilical Cord Tissue Allograft for the Supplementation of Cartilage Defects Associated to Knee Osteoarthritis: An Observational Data Collection Study

Justine M. Davis[1], Scott M. Martin[1], Mitchell B. Sheinkop[2], Tyler C. Barrett[1]

[1] Regenative Labs, 1700 W Main St., Suite 500, Pensacola, FL 32502
2 Weil Foot & Ankle, 1660 Feehanville Dr., Suite 450, Mount Prospect, IL 60056

Abstract

The primary objective of this study is to report the initial efficacy data observed with the use of cryopreserved human umbilical tissue allograft for the supplementation of cartilage defects in patients with symptomatic knee osteoarthritis. Our primary endpoints were pain, stiffness, and functional recovery scores. In this ongoing study, 55 participants (age 56-93 years) received a single Wharton's jelly tissue allograft application. The study dose consisted of 150mg of Wharton's jelly allograft suspended in approximately 2mL of sterile Sodium Chloride 0.9% solution (normal saline). Each study knee application was performed under ultrasound guidance in a physician's office. The research methodology consisted of NPRS scores and WOMAC subsection scores including pain, stiffness, and physical function. Study enrollment consisted of 55 patients followed for a post-application duration of 90 days. No adverse events or adverse reactions were reported. The results demonstrated statistically significant improvements of NPRS and WOMAC in initial versus 90-day examination. The data represents Wharton's jelly tissue allograft applications are a safe, non-surgical, and efficacious for patients with symptomatic articular cartilage defects associated with osteoarthritis of the knee.

Keywords: Wharton's Jelly, Regenerative Medicine, Osteoarthritis, Cartilage Defects

Introduction

Osteoarthritis (OA) is the most common joint disease amongst adults in the world [1]. Currently, there are 33 million adults in the United States living with osteoarthritis, and that number is expected to rise to 67 million by 2032 [2, 3]. While OA disease primarily affects the elderly, with over half of the cases of those age 65 years old, it is estimated that working-aged adults (45-64 years old) will represent one-third of new cases in the coming years [4]. Osteoarthritis results in inflammation with degeneration of surrounding joint cartilage and the underlying bone. Related symptoms of this degeneration include decreased range of motion, instability, chronic pain, and severe disability [5]. Knee osteoarthritis has been ranked the 11th highest contributor to global disability [6].

In the knee, articular cartilage encases the lower end of the femur, the upper end of the tibia, and the undersurface of the patella. The primary function of articular cartilage is to provide a smooth, lubricated surface for joint movement [7]. Defects in articular cartilage are especially detrimental given that articular cartilage has extremely poor self-renewal capabilities [2]. Adult articular cartilage is not innervated or vascularized and has a tightly condensed extracellular matrix [2, 7, 8]. The unique structure of articular cartilage significantly

 doi:10.20944/preprints202207.0337.v1

complicates the treatment of these defects for the surgeon, the physical therapists, and the patient [7]. The preservation of articular cartilage is highly dependent on conserving the architecture of the cartilage [7]. Untreated defects significantly limit the knee's active range of motion. These defects often progress into osteoarthritis and may eventually require surgical interventions from arthroscopy to a total knee replacement [2, 9]. Early diagnosis and treatment of articular cartilage defects have recently taken precedence due to national and international cost burdens associated with peri and post-surgical care.

Current trends in cartilage replacement therapies are both invasive and require lengthy, painful recovery times. Moreover, procedures such as drilling, microfracture, and osteochondral autographs have low success rates [8]. Physical therapy, activity modification, and Non-Steroidal Anti-Inflammatory drugs (NSAIDs) have demonstrated only temporary benefits and are bereft with operator-dependent limitations and medication side effects. The most common pharmacological agent prescribed to osteoarthritis patients in addition to NSAIDs is acetaminophen [10]. Both pharmacological agents have a long list of side effects, including gastritis, nephritis, dysfunction of the liver, increased mean arterial blood pressure, increase in bleeding time, and cardiovascular events [10].

The most common non-surgical procedures for knee osteoarthritis include cortisone injections, platelet-rich plasma (PRP) therapy, and Hyaluronic Acid (HA) injections. Corticosteroid injections are often used as an initial salvo; however, they rarely prove to be a long-term solution. Repeated injections can result in joint swelling, tendon rupture, stiffness, hyperglycemia, and arthralgia [10].

PRP therapy utilizes patient harvested blood typically by antecubital approach, which is spun by semi-sterile manner in a centrifuge. A concentrated platelet-rich solution is injected into the patient's knee, ideally at the site of the damaged articular cartilage identified by ultrasound guidance. While some benefit has been shown when treating articular cartilage degeneration in younger patients, older patients do not respond as favorably. After the age of 50, the blood platelet count decreases by 20,000 platelets per μL, continuing to steadily decrease with more advanced age [11]. As patients receiving such therapies are typically 60 years and older, the declining platelet count in these patients may limit the effectiveness of PRP treatment. A 2021 randomized, placebo-controlled trial examined PRP effectiveness, and the study demonstrated no statistical improvement in symptomatic knee pain after PRP treatment and recommended pain relief interventions other than PRP [12]. While PRP may be efficacious for a younger patient demographic, there is a growing need for interventions readily accessible patients 60 years and older.

Hyaluronic acid injections are utilized as visco-supplement, ideally replacing the depleted HA in patients with knee osteoarthritis. Injections of additional HA provide lubrication to the joint and temporarily reduce joint pain. While effective in affording temporary pain relief, visco-supplementation therapies lack durability. Repeated injections on a monthly basis or in a series of shots are required for more lasting benefit. Additionally, visco-supplementation has an increased risk of adverse effects [13, 14]. Some may conclude the lack of statistically relevant literature and paucity of clinically significant benefits outweigh the potential adverse events associated with a series of HA injections.

Overall, the treatment of articular cartilage defects in patients with symptomatic osteoarthritis of the knee remains a clinical challenge. Most clinicians agree that non-surgical

 doi:10.20944/preprints202207.0337.v1

intervention is the standard of care, yet current treatments lack reproducible and lasting benefits for patients. In that knowledge, we present study results for the use of Wharton's jelly allograft applications as a non-surgical alternative, with statistically significant patient outcomes in the treatment of patients with symptomatic, structural tissue defects of the knee joint.

Wharton's jelly, first discovered by Thomas Wharton in 1656, is a gelatinous-like tissue that encompasses two arteries and one vein of the umbilical cord [15]. Wharton's jelly (WJ) functions to protect the vessels of the umbilical cord from external forces and simultaneously allow for umbilical arterial and venous blood flow [8, 15]. WJ has been reported to contain robust amounts of growth factors, HA, and extracellular vesicles that could potentially reduce inflammation and promote a regenerative microenvironment to aid in healing musculoskeletal injuries [3, 10, 16]. Clinical applications of WJ as a tissue supplement are increasing in popularity due to recent successful outcomes [17]. Supplementation with WJ centers on repairing the structural tissue defects in articular cartilage scaffolding. When the scaffold of articular cartilage is combined with WJ, significant amelioration of defects was observed [18].

WJ is primarily comprised of collagen and glycosaminoglycans, mirroring articular cartilage composition [19]. WJ is similar to articular cartilage in scaffold architecture and bio function, making WJ an ideal homologous allograft to supplement articular cartilage defects in patients with symptomatic knee osteoarthritis. Umbilical cord allografts have shown improvements in WOMAC scores for up to one year in patients with diffuse knee pain due to osteoarthritis [4]. When compared to HA injections, umbilical cord allografts had significantly higher WOMAC improvements [4].

In this study, we examined the effectiveness of umbilical cord tissue allografts in supplementing structural tissue defects in articular cartilage. We examined this through improvement in patient-reported outcomes of both NPRS and WOMAC values**.** We hypothesize that patients receiving umbilical cord allograft supplementation will show an improvement in the Numeric Pain Rating Scale (NPRS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) over a 90-day period when compared to the initial administration date.

Materials and Methods

All methods were completed in compliance with the FDA and American Association of Tissue Banks (AATB) standards.

Human umbilical cords were obtained from consenting mothers following full-term Caesarian section deliveries. Prior to delivery, birth mothers underwent comprehensive medical, social, and blood testing. An independent certified laboratory tested all of the donations for infectious disease in accordance with Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 CFR part 493, and FDA regulations. Each birth mother was tested for Hepatitis B Core Antibody (HBcAb), Hepatitis B Surface Antigen (HBsAg), Hepatitis C Antibody (HCV), Human Immunodeficiency Virus Antibody (HIV-1/HIV-2 Plus O), Human T-Lymphotropic Virus Antibody (HLTV-I/11), Syphilis (RPR), HIV-1/HCV/HBV, NAT, and West Nile Virus (WNV). Each test was performed with an FDA-Approved testing kit (See Appendix A). All test results were required to be negative or non-reactive before processing the umbilical cord tissue.

 doi:10.20944/preprints202207.0337.v1

Wharton's jelly was aseptically dissociated from the rinsed umbilical cord. After dissociation,150mg of Wharton's jelly was suspended in approximately 2mL of sterile Sodium Chloride 0.9% solution (normal saline). Dimethyl sulfoxide (DMSO) was added to the suspension as a cryoprotectant. The volume of DMSO was calculated as 5% of the total suspension volume. The cryoprotectant functions to preserve the integrity of the umbilical cord allograft while being stored in -40^{oC} freezers. . The sample was not combined with cells, tissues, or articles other than the exceptions outlined in 21 CFR Part 1271.10(a)(3) (Human Cells, Tissues, and Cellular and Tissue-Based Product Regulation).

This observational data collection study analyzed 55 adult patients who met predetermined inclusion and exclusion criteria at 12 clinics throughout the United States. Eligible patients included adults older than 18 years of age with a body mass index (BMI) less than 40 kg/m^2 and an initial NPRS pain score of at least 4 on a scale of 1-10. Patients who had not already received at least six weeks of conventional treatment (i.e. PRP, HA supplementation, or corticosteroids) were excluded from this study. Subjects who had completed six weeks of initial treatment and reported it to be unsuccessful then became eligible for umbilical cord allograft supplementation in this study. Subjects were not limited to allograft administration in one knee if both knees were determined to have structural tissue defects.

Umbilical cord tissue allografts were obtained by 12 clinics. The allograft application was administered as an intra-articular knee application by anteromedial approach under ultrasound by a qualified health professional in a private medical setting. Patients were asked to fill out an initial questionnaire consisting of NPRS and WOMAC. Patients answered this same questionnaire at 30 days and 90 days after the initial allograft application.

NPRS is a numerical pain scale employed as a subjective measurement of 0-10 for patients to rate their pain. A measurement of 0 indicates no pain, and 10 indicates the worst pain possible. WOMAC is a combination of three questionnaires measuring the pain, stiffness, and function of the knee and/or hip affected by osteoarthritis. The WOMAC questionnaire consists of 23 questions where patients rate their ability to perform the function from 0 (performs with ease) to 4 (extreme difficulty performing the function). The scores of these three categories were analyzed individually to allow for greater examination of physical mobility of the affected joint.

Patient-reported outcomes were statistically analyzed for significance in improvement. When comparing the difference between baseline and 90-day data, the average and standard deviation of each patient-reported outcome subsection was calculated. These values were then utilized to run a two-sample Z-test. The p-value of each patient-reported outcome subsection was calculated from the resulting z-score. P-values were determined at a 95% confidence level. A p-value less than or equal to 0.05 was considered to be statistically significant. Percent of change analysis was also conducted between the baseline, 30-day, and 90-day examinations as another statistical endpoint for comparison. The minimal clinically importance difference (MCID) was determined for NPRS and all three WOMAC subsections. MCID was calculated for baseline, 30-day, and 90-day questionnaires. Sample standard deviation and the number of subjects (n=55) was used to calculate each MCID. MCID determined the minimum

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2022 doi:10.20944/preprints202207.0337.v1

improvement in each subsection for the improvement to be considered clinically important. MCID was used as a secondary endpoint to determine the significance of improvement in patient-reported outcomes.

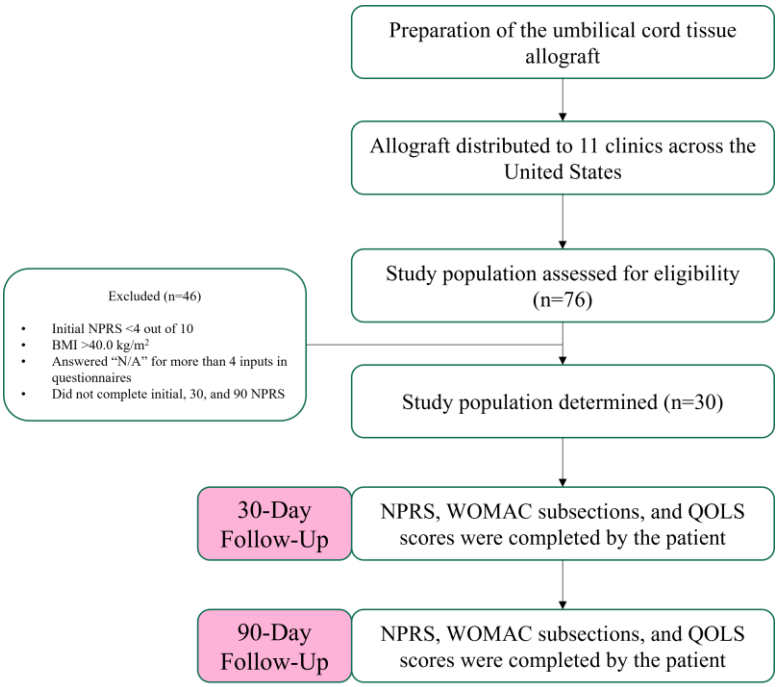

Figure 1. Flow diagram used to depict the flow of procedures in the study. NPRS, Numerical Pain Rating Scale; WOMAC, Western Ontario and McMaster Universities Arthritis Index; BMI, Body Mass Index.

Results

This study was conducted under an Institute of Regenerative and Cellular Medicine IRB-approved protocol (RL-UCT-001) and we obtained informed consent from all patients.

The study population consisted of 55 patients (37 females and 18 males) with a mean age of 73 ± 2.2 years and a mean BMI of 29.6 ± 2.1 kg/m^2. In total, 20 patients received supplementation in the left knee and 35 patients in the right knee. NPRS and WOMAC values were collected at initial application, 30 days, and 90 days for each patient. Changes from baseline (initial application date) for all scores at 30 and 90 days were reported in most patients. The average of NPRS and WOMAC subsection sums are reported in **Table 1** and illustrated in **Figure 2**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2022 doi:10.20944/preprints202207.0337.v1

Table 1. Patient-reported outcomes at initial application, 30-day, and 90-day visit.

		WOMAC		
	NPRS	**Pain**	**Stiffness**	**Physical Function**
Initial Application	6.236	11.127	5.291	37.109
30-Day Follow-Up	4.273	8.527	4.182	28.709
90-Day Follow-Up	4.182	8.782	4.164	29.600
Statistics	$p < 0.00001$	$p < 0.00001$	$p < 0.00001$	$p < 0.00001$

The average change in NPRS, WOMAC subsections, and QOLS reported from initial allograft application. A z-test was performed to determine significance and p-values were reported.

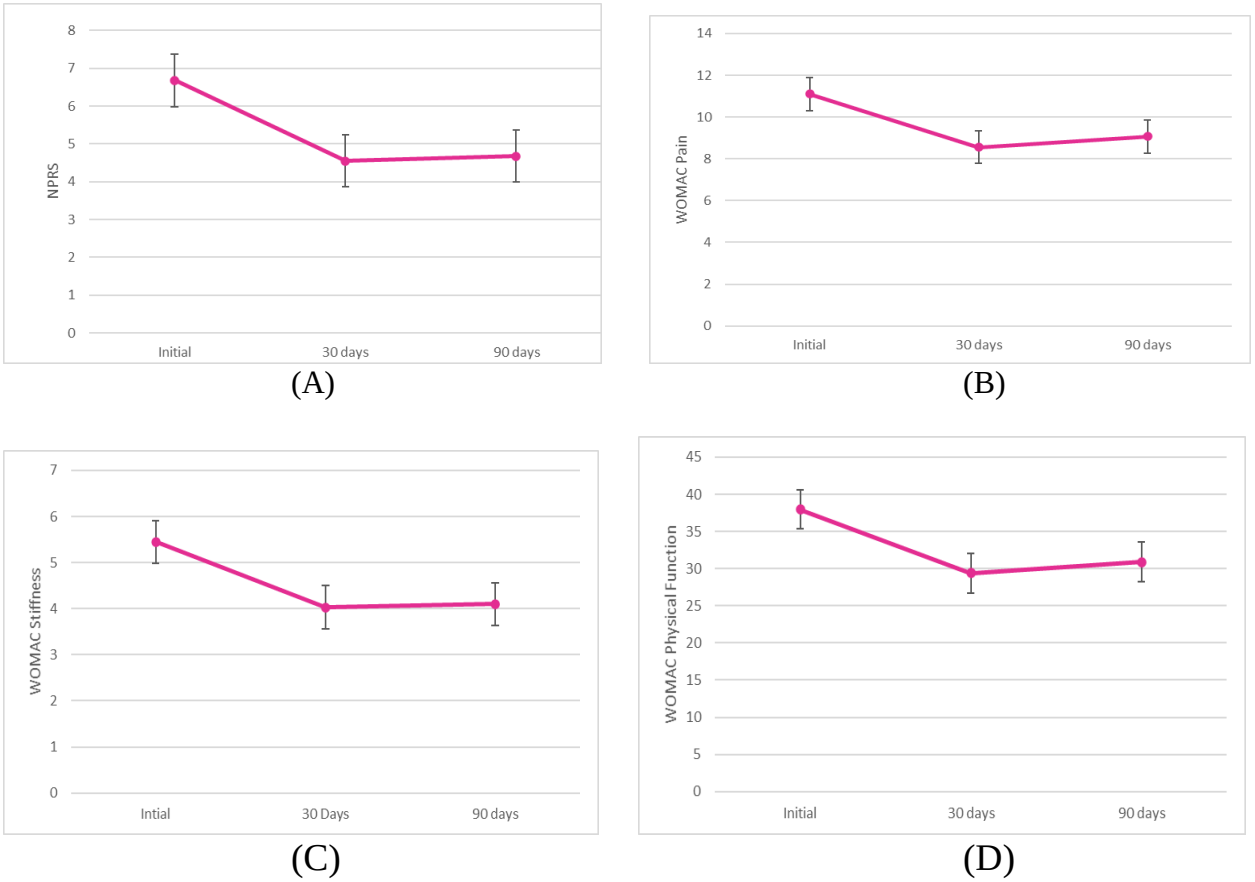

Figure 2. National Pain Rating Scale (NPRS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores reported at initial application, 30-day visit, and 90-day visit.

 doi:10.20944/preprints202207.0337.v1

Average sum ± standard deviation reported for (A) NPRS, (B) WOMAC Pain, (C) WOMAC Stiffness, and (D) WOMAC Physical Function.

When examining the NPRS at baseline, patients reported an average pain score of 6.2 ± 0.4. At 30 days, patients showed a significant decrease in NPRS. The average NPRS was reported to be 4.3 ± 0.4. By day 90, the average NPRS remained similar in value at 4.2 ± 0.4. The average change in NPRS between baseline and 90-day collection was 2.0 points ($p < 0.00001$). The two-point improvement illustrates significantly greater pain relief only 30 days after the initial application of the umbilical cord tissue allograft. There were no significant differences in average NPRS when comparing 30-day and 90-day pain scores. An initial and 30-day x-ray comparison of structural tissue defects in the knee is present in **Figure 3**.

Using WOMAC, we evaluated pain levels of the synovial knee joint impacted by osteoarthritis. At the initial application, the average sum of the WOMAC pain subsection was 11.1 ± 0.9. At the 30-day follow-up, patients reported an average WOMAC pain sum of 8.5 ± 0.9. By day 90, the average sum was 8.8 ± 1.1. The average change in sum of WOMAC scores between the initial application visit and the 90-day visit was 2.3 points ($p < 0.00001$). This average decrease in WOMAC scores indicates greater functionality after the application of the umbilical cord tissue allograft.

Through the stiffness subsection of WOMAC, we examined joint stiffness of the knee impacted by the structural tissue defects associated with osteoarthritis. At the initial application, the average of the WOMAC stiffness sum was 5.2 ± 0.4. 30 days after initial application, the average sum was 4.2 ± 0.5. By day 90, the average sum of the WOMAC stiffness scores was 4.1 ± 0.5. The average change in the sum of WOMAC stiffness scores between the initial application and the 90-day visit was 1.1 points ($p < 0.00001$). The decrease in the average sum of stiffness WOMAC scores demonstrates an improvement in the overall stiffness of the impacted knee.

The last subsection of WOMAC, physical function, was utilized to evaluate the overall functionality of the affected knee before and after application of the allograft. At the initial application, the average sum of the physical function WOMAC scores was 37.9 ± 2.9. At the 30-day follow-up, patients reported an average sum of 28.7 ± 3.3. By day 90, the average WOMAC physical function sum was 29.6 ± 3.6. The average change in the sum of the physical function subset of WOMAC between the initial application and the 90-day visit was 8.3 points ($p < 0.00001$). The decrease in the WOMAC average of physical function indicates greater mobility and function of the affected joint after the application of the allograft.

To ensure both physical and mental improvements in patient health, we employed the use of minimal clinically important difference (MCID) to confirm that improvements in NPRS and WOMAC subsections were significant in patient outcomes. The MCID of NPRS was 0.78, which indicates a significant improvement in pain alleviation after allograft supplementation since the average difference in NPRS sums between the initial and 90-day visit was 2 points. The MCID of the pain subsection of WOMAC was determined to be 1.5. Given that the difference been initial and 90-day average sums was 2.3 points, this again demonstrates a significant improvement in pain alleviation after allograft application. The MCID of the stiffness subsection

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2022 doi:10.20944/preprints202207.0337.v1

of WOMAC was 0.65, again significant when compared to the 1.1-point improvement between initial and 90-day patient visits. The MCID of the physical function subsect of WOMAC was 5.0, proving significant when compared to the 8.3-point improvement between initial and 90-day visits.

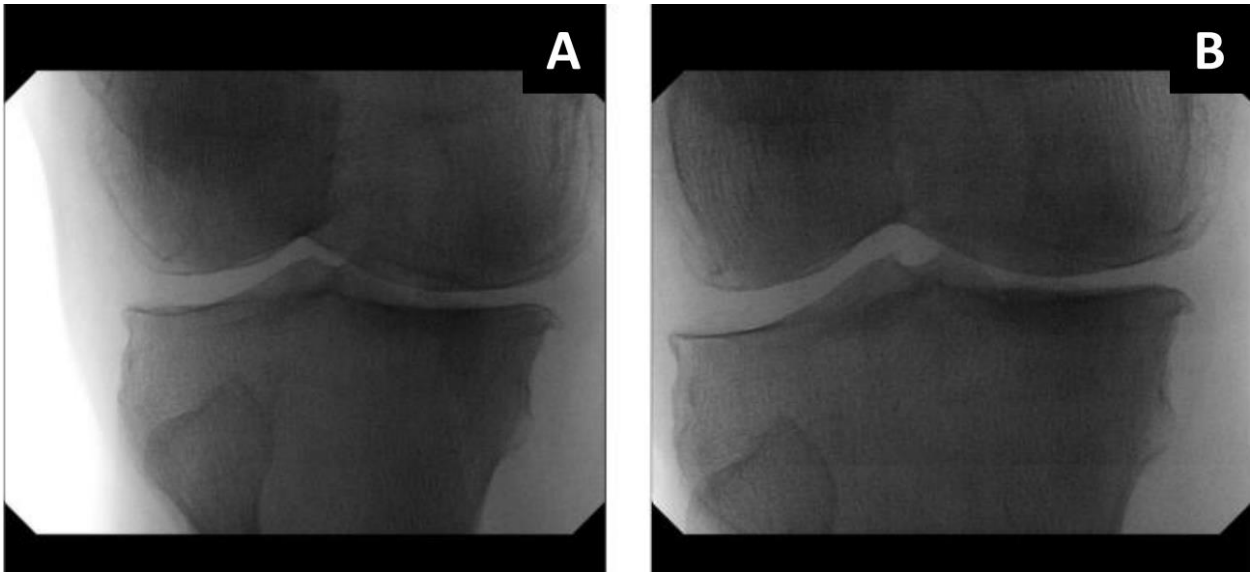

Figure 3. Load-bearing fluoroscopy, anterior-posterior (AP) view of the knee in a 70-year-old female patient. Imaging was performed before allograft application (A) and at the 30-day follow-up visit (B). Patient reported a 4 on the NPRS scale at initial allograft application and 2 on the NPRS scale at the 30-day follow-up. There is significant improvement in both medial and lateral synovial spacing between the joint 30 days after allograft application. X-Ray imaging was provided by Restore Osteo of Colorado.

Table 2. Percent of change analysis.

		WOMAC		
	NPRS	**Pain**	**Stiffness**	**Physical Function**
Baseline to 90-Day	-32.94%	-21.07%	-21.30%	-20.23%
Baseline to 30-Day	-31.48%	-23.37%	-20.96%	-22.64%
30-Day to 90-Day	-2.13%	2.99%	-0.43%	3.10%

Percent of change analysis. The percent of change between NPRS and WOMAC average sums were reported at initial, 30 and 90-day visits.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2022 doi:10.20944/preprints202207.0337.v1

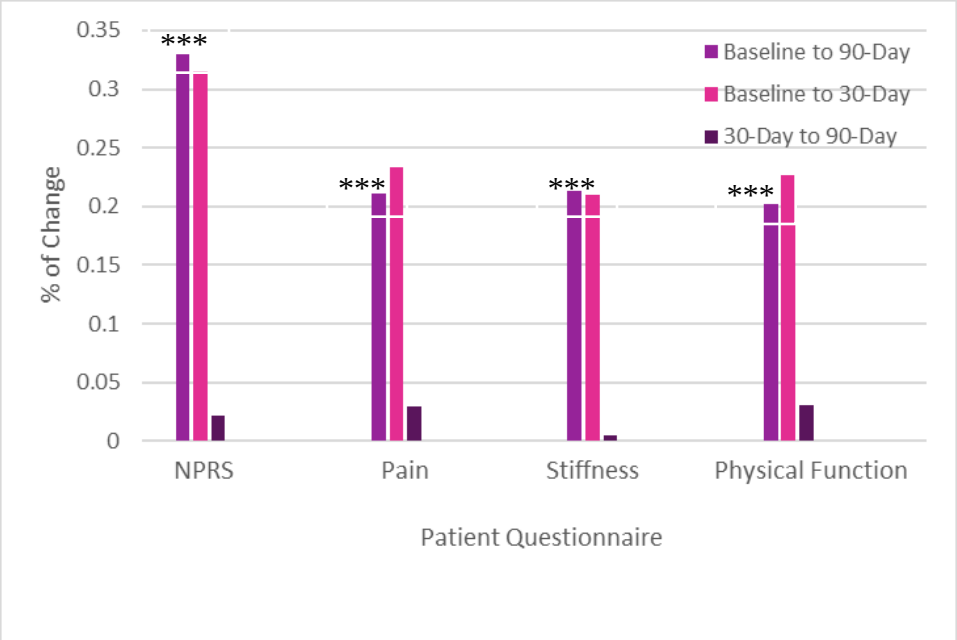

Figure 4. Percent of change analysis. The percent of change between NPRS and WOMAC subsection average sums were reported at initial, 30 and 90-day visits. A z-test was performed to determine significance; *** denotes $p<0.0001$.

Discussion

Institutional Review Board approval (IRCM-2021-297) was obtained before data collection was performed. The product was utilized due to medical necessity.

This observational data collection study found that the results of supplementation with umbilical cord tissue allograft significantly improved pain alleviation, stiffness, and physical mobility of the affected joint. We observed statistically significant differences between baseline and 90-day reported scores in NPRS and WOMAC scores. In addition, all three scales reported improvement by the 30-day follow-up visit as well. Percentage of change between initial, 30-day, and 90-day visit are reported in **Table 2**. A graphical comparison of the percentage of change is presented in **Figure 4**.

Comparison of the 30-day NPRS to the baseline data showed a significantly greater improvement in pain alleviation (decrease in pain by 32.94%). We noted a slight decrease (-2.13%) change in pain alleviation between the 30- and 90-day NPRS values. There was an overall decrease in pain by 32.94% between the initial allograft application and the 90-day follow-up.. 9 out of the 55 patients reported no change in improvement between initial and 90-day visits and 4 out of the 55 patients reported an increase in pain from the 30-day to 90-day visit.. Since allograft application is performed according to medical necessity, it is likely that a majority of these patients required a second application. However, there is an insufficient amount of literature to presume that patients will benefit from a second allograft application. Future

 doi:10.20944/preprints202207.0337.v1

studies with two-dose allograft applications are required to warrant patient improvement upon a second dose administration.

When examining the pain subsect of the WOMAC scores, there is a significant improvement in pain alleviation between the baseline and 30-day follow-up (decrease in pain by 23.37%). Comparison of the 90-day and 30-day average sums revealed a 2.99% increase in pain. Again, we contribute this to the additional need for supplementation. Regardless of this slight increase, there was an overall 21.07% decrease in the average sum of WOMAC pain scores between initial and 90-day examination. Again, a larger standard deviation (σ= 4.01) was noted in the 90-day average sum which therefore indicates larger variability in patient pain alleviation at 90 days post-application.

The stiffness subsection of the WOMAC scores revealed a 20.96% improvement in the average sum between initial application and 30-day follow-up. We observed a slight decrease in stiffness (0.43% decrease) between the 30-day and 90-day examination. However, the overall 0.43% decrease in stiffness of the knee between the initial and 90-day visit average sums indicates that the allograft application was largely successful in alleviating joint stiffness. Once again, we noted a slightly larger standard deviation (σ= 1.76) in the 90-day average sums.

The final subsection of WOMAC, physical function, demonstrated a 22.64% improvement in joint physical function between the initial allograft application and 30-day follow-up. From the 30-day to the 90-day follow-up, we observed a 3.10% decrease in the overall physical function of the affected knee. Again, we contribute this decrease in mobility to the absence of a second allograft application. From the initial application to the 90-day visit, patients reported an overall 20.23% improvement in the function and mobility of the affected knee. We noted an unusually high standard deviation (σ= 13.36) in the 90-day average sum again, indicating a larger variation in each patient's mobility 90 days after allograft application. However, the notable decrease in all WOMAC subsections between the initial application and 90-day visit indicates a continuous improvement in pain, stiffness, and physical mobility after allograft application.

The results demonstrated a significant minimal clinically important difference (MCID) observed in NPRS and all WOMAC subsection improvements. We believe that the absence of a second application was detrimental to a larger improvement in patient-reported scores. However, changes in the NPRS and WOMAC subsection average sums were greater than the determined MCID and therefore illustrated improvements that were clinically significant in patients after the initial allograft application.

In addition to physical improvement, human umbilical cord tissue allografts are advantageous in that they do not elicit an immune response from the host. Coupled with clinical advantages, WJ also demonstrates structural similarities to articular cartilage. WJ is partially comprised of glycosaminoglycans and collagen, similar to articular cartilage [18]. WJ exhibits similar functions to articular cartilage as well. WJ functions to protect the vessels of the umbilical cord from external forces while also allowing for umbilical arterial and venous blood flow [7, 14]. These primary functions are similar to those of articular cartilage, which supports and distributes external forces in the knee. The structural and functional similarities between WJ and articular cartilage, combined with significant improvement of NPRS and WOMAC indicate

 doi:10.20944/preprints202207.0337.v1

that umbilical cord tissue allografts are an effective supplementation for osteoarthritis cartilage degeneration. These results are consistent with a study in 2022 that found that 30 patients who received umbilical cord tissue (UCT) applications in the knee had decreased pain, decreased medication use (opiates and NSAIDs), and an improvement in physical function lasting over 24 weeks [19].

The main difference in the efficacy of WJ compared to cortisone and HA injections is the duration of effectiveness and pain relief. While HA injections are comparable to corticosteroids in the effectiveness of pain management and reduction related to OA [20] and duration of pain alleviation, neither supplementation provides significant pain alleviation without the need for continual re-injections. HA often requires numerous injections over time, with relief lasting only 8-12 weeks. Commonly reported AE for HA includes pain at the injection site, swelling, lethargy, face rash, and local effusion [21]. Although HA may be relatively safe short-term, it does cause more adverse effects compared to CS [22].

Another study reviewing the efficacy of HA on TKR found a small statistically significant effect on overall function with few adverse events; however, this study did not limit participants to 65 years or older [23]. Due to this limitation, no conclusions can be drawn from the currently available literature. Another study testing a non-animal stabilized hyaluronic acid (NASHA) injection concluded that it was not superior to the placebo for the primary efficacy analysis [24]. Our data suggest statistically significant improvement in NPRS and all three WOMAC subsections after the application of an umbilical cord tissue allograft.

Our observational study has limitations; the design was an analysis of an unblinded data collection. However, the primary outcomes of NPRS and WOMAC were patient-reported, which eliminates the influence of an unblinded investigator. We plan to perform a double-blinded placebo-controlled study in fall of 2022. In addition, we were limited in patient improvement due to the restriction of only one application. In future studies, we plan to analyze NPRS and WOMAC improvements and compare to the MCID in patients with structural tissue defects of the knee after two allograft applications.

Conclusion

In conclusion, this observational study of umbilical cord tissue allografts demonstrated both statistically significant and clinically meaningful improvements overall functionality and joint mobility when the allografts were used as a supplementation in articular cartilage defects in patients with symptomatic knee osteoarthritis. This study provides potential for a more efficacious alternative in comparison to hyaluronic acid supplementation. Additional randomized controlled trials are required to warrant potential allograft applications such as more accessible alternatives to invasive arthroscopy and knee replacement surgeries.

Acknowledgments

The authors would like to thank Catherine Becker, B.S. for her assistance in data collection. The authors would also like to thank Anthony Olofintuyi, MD, Jennifer Ebert, MD, Carrie Carda, MD, Laura Melsheimer, MD, Holly Nicholson, MD, Jill Harlan, MD, Dean Jones, MD, Joan Ardavanis, MD, Dee Stevens, MD, Ritu Patel, MD and Katrina Babcock, MD for their collaboration in data collection.

Appendix A

Test Kits

1. HBcAb: Catalog number: 06P06, Abbott Laboratories, Abbott Park, IL, USA 223
2. HbsAg: Catalog number: 06P02, Abbott Laboratories, Abbott Park, IL, USA 224
3. HCV: Catalog number: 06P04, Abbott Laboratories, Abbott Park, IL, USA 225
4. HIV1, HIV2, plus O: Catalog number: 06P01, Abbott Laboratories, Abbott Park, IL, 226 USA 227
5. HTLV-I/II: Catalog number: 06P07, Abbott Laboratories, Abbott Park, IL, USA 228
6. RPR: Catalog number: 900025, Arlington Scientific, Springville, UT, USA 229
7. HIV1, HCV, HBV, NAT: Catalog number: 303330, 303331, 303719, 303334, 303344 230
8. WNV: Catalog number: 07001061190, Roche Diagnostics, Indianapolis, IN, USA

References

1. Joern, W. P. M.; Schlüter-Brust, K. U.; and Eysel, P. The Epidemiology, Etiology, Diagnosis, and Treatment of Osteoarthritis of the Knee. *Deutsches Arzteblatt International*. **2010**, *107*, 152-162. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2841860/ (accessed on 10 February 2022).

2. Chen, Y.; Sun, H.; Yao, X.; Yu, Y.; Tian, T.; Xu, W.; Zhou. Y.; and Ouyang. H. Pharmaceutical therapeutics for articular regeneration and restoration: state-of-the-art technology for screening small molecular drugs. *Cellular and Molecular Life Sciences*. **2021**, *78*, 8127-8155. Available online: https://pubmed.ncbi.nlm.nih.gov/34783870/ (accessed on 7 February 2022).

3. Gupta, A.; Maffulli, N.; Rodriguez, H. C.; Carson, E. W.; Bascharon, R. A.; Delfino, K.; Levy, H. J.; and El-Amin, S. F. Safety and efficacy of umbilical cord derived Wharton's jelly compared to hyaluronic acid and saline for knee osteoarthritis: study protocol for a randomized, controlled, single-blind multi-center trial. *Journal of Orthopaedic Surgery and Research.* **2021**, *31*, 352. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8165766/ (accessed 7 February 2022).

4. Hootman, J.M. and Helmick, C.G. Projections of US prevalence of arthritis and associated activity limitations. *Arthritis Rheum.* 2006, *54*, 229-229. Available online: **https://pubmed.ncbi.nlm.nih.gov/16385518/** (accessed 5 July 2022).

5. Ionitescu, M.; Vermesan, D.; Andor, B.; Dumitrascu, C.; Al-Qatawneh, M.; Bloanca, V.; Dumitrascu, A.; and Prejbeanu, R. Potential new treatments for Knee OA: A prospective review of registered trials. *Applied Sciences*. **2021**, *11*, 11049. Available online: https://www.mdpi.com/2076-3417/11/22/11049 (accessed 7 February 2022).

6. Cross, M.; Smith, E.; Hoy, D.; Nolte, S.; Ackerman, I.; Fransen, M.; Bridgett, L.; Williams, S.; Guillemin, F.; Hill, C. L.; Laslett, L. L.; Jones, G.; Cicuttini, F.; Osborne, R.; Vos, T.; Buchbinder, R.; Woolf, A.; and March, L. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. *BMJ Journals.* **2014**, *73*, 1323-1330. Available online: https://ard.bmj.com/content/73/7/1323.long (accessed 9 February 2022).

7. Fox, A. J. S.; Bedi, A. B.; and Rodeo, S. A. The basic science of articular cartilage: structure, composition, and function. *Sports Health*. **2009**, *1*, 461-468. Available online: https://ard.bmj.com/content/73/7/1323.long (accessed 7 February 2022).

8. Zhao, P.; Liu, S.; Lu, S.; Peng, J.; Zhang, L.; Huang, J.; Zhao, B.; Xu, W.; and Guo, Q. hWJECM-Derived Oriented Scaffolds with Autologous Chondrocytes for Rabbit Cartilage Defect Repairing. *Tissue Engineering Part A.* **2018**, *24*, 905-914. Available online: https://www.liebertpub.com/doi/10.1089/ten.TEA.2017.0223?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed (accessed 8 February 2022).

9. Cicuttini, F.; Ding, C.; Wluka, A.; Davis, S.; Ebeling, P. R.; and Jones, G. Association of Cartilage Defects with Loss of Knee Cartilage in Healthy, Middle-Age Adults. *Arthritis and Rheumatism*. **2005**, *52*, 2033-2039. Available online: https://onlinelibrary.wiley.com/doi/epdf/10.1002/art.21148 (accessed 11 January 2022).

10. Gupta, A.; Rodriguez, H. C.; Potty, A. G.; Levy, H. J.; and El-Almin, S. F. Treatment of Knee Osteoarthritis with Intraarticular Umbilical Cord-Derived Wharton's Jelly: A Case Report. *Pharmaceuticals*. **2021**, *14*, 883. Available online: https://www.mdpi.com/1424-8247/14/9/883 (accessed February 7 2022).

11. Jones, C. I. Platelet function and ageing. *Mammalian Genome*. **2016**, *27*, 358-366. Available online: https://link.springer.com/article/10.1007/s00335-016-9629-8 (accessed 10 February 2022).

12. Bennell, K. L.; Paterson, K. L.; Metcalf, B. R.; Duong, V.; Eyles, J.; Kasza, J., Wang, Y.; Cicuttini, F.; Buchbinder, R.; Forbes, A.; Harris, A.; Yu, S. P.; Connell, D.; Linklater, J.; Wang, B. H.; Oo, W. M.; and Hunter, D. J. Effect of Intra-articular Platelet-Rich Plasma vs Placebo Injection on Pain and Medial Tibial Cartilage Volume in Patients with Knee Osteoarthritis: The RESTORE Randomized Clinical Trial. *JAMA*. **2021**, *326*, 2021-2030. Available online: https://pubmed.ncbi.nlm.nih.gov/34812863/ (accessed on 10 February 2022).

13. Rutjes, A. W. S.; Jüni, P.; Costa, B. R.; Trelle, S.; Nüesch, E.; and Reichenbach, S. Viscosupplementation for Osteoarthritis of the Knee. *Annals of Internal Medicine.* **2012**, *157*, 180-191. Available online: https://www.acpjournals.org/doi/10.7326/0003-4819-157-3-201208070-00473?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed (accessed 8 February 2022).

14. Medina, J. M.; Thomas, A.; and Denegar, C. R. Knee Osteoarthritis: Should your patient opt for hyaluronic injection? *The Journal of Family Practice*. **2006**, *55*, 669-674. Available online: https://pubmed.ncbi.nlm.nih.gov/16882439/ (accessed 10 February 2022).

15. Wampler, A. T.; Pace, L. H.; and Thorne, J. M. Intra-Articular Injections of Wharton's Jelly Allograft Combined with Amniotic Membrane Allograft are Safe and Effective for Relieving Knee Pain Associated with Osteoarthritis; A Retrospective Case Report. *Elite Integrated Medical Journal*. **2021**, *4*, 13. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7585522/ (accessed 8 February 2022).

16. Gupta, A.; El-Amin, S. F.; Levy, H. J.; Sze-Tu, R.; Ibim, S. E.; and Muffulli, N. Umbilical cord-derived Wharton's jelly for regenerative medicine applications. *Journal of Orthopaedic Surgery and Research*. **2020**, *15*, 1-9. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7017504/ (accessed 10 February 2022).

17. Jiang, S.; Tian, G.; Yang, Z.; Gao, X.; Wang, F.; Li, J.; Tian, Z.; Huang, B.; Wei, F.; Sang, X.; Shao, L.; Zhou, J.; Wang, Z.; Liu, S.; Sui, X.; Guo, Q.; Guo, W.; and Li, X. Enhancement of acellular cartilage matrix scaffold by Wharton's jelly mesenchymal stem cell-derived exosomes to promote osteochondral regeneration. *Bioactive Materials.* **2021**, *6*, 2711-2728. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7895679/ (accessed 10 February 2022).

18. Li, Z.; Bi, Y.; Wu, Q.; Chen, C.; Zhou, L.; Qi, J.; Xie, D.; Song, H.; Han. Y.; Qu, P.; Zhang, K.; Wu, Y.; and Yin, Q. A composite scaffold of Wharton's jelly and chondroitin sulphate loaded with human umbilical cord mesenchymal stem cells repairs articular cartilage defects in rat knee. *Journal of Materials Science*. **2021**, *32*. Available online: https://link.springer.com/content/pdf/10.1007/s10856-021-06506-w.pdf (accessed 7 February 2022).

19. Timmons, R. B.; Sugayam K.; and Bane, L. D. Homologous Use of Allogenic Umbilical Cord Tissue to Reduce Knee Pain and Improve Knee Function. *Life*. **2022,** *12*(2), 260. Available online: https://pubmed.ncbi.nlm.nih.gov/35207547/ (accessed 11 March 2022).

20. Askari, A; Gholami, T; NaghiZadeh, M. M.; Farjam, M.; Kouhpaveh, S. A.; and Shahabfard, Z. Hyaluronic acid compared with corticosteroid injections for the treatment of osteoarthritis of the knee: a randomized control trial. *Springerplus.* **2016,** *5*, 442. Accessed online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4828353/ (accessed 6 July 2022).

21. O'Hanlon, C. E.; Newberry, S. J.; Booth, M.; Grant, S.; Motala, A.; Maglione, M. A.; FitzGerald, J. D.; and Shekelle, P. G. Hyaluronic acid injection therapy for osteoarthritis of the knee: concordant efficacy and conflicting serious adverse events in two systematic reviews. *Systematic Reviews.* **2016,** *5*, 186. Accessed online: https://pubmed.ncbi.nlm.nih.gov/27814744/ (accessed 6 July 2022).

22. Zhang, B.; Thayaparan, A.; Horner, N.; Bedi, A.; Alolabi, B.; and Khan, M. Outcomes of hyaluronic acid injections for glenohumeral osteoarthritis: a systematic review and meta-analysis. *Journal of Shoulder and Elbow Surgery*. **2019,** *28*, 596-606. Available online: https://pubmed.ncbi.nlm.nih.gov/30502030/ (accessed 6 July 2022).

23. Main, B. J.; Maffulli, N.; Valk, J. A.; Rodriguez, H. C.; Gupta, M.; El-Almin, S. F.; Gupta, A. Umbilical Cord-Derived Wharton's Jelly for Regenerative Medicine Applications: A Systematic Review. *Pharmaceuticals*. **2021**, *14*. Available online: https://www.mdpi.com/1424-8247/14/11/1090 (accessed 10 February 2022).

24. Newberry, S. J.; Fitzgerald, J. D.; Maglione, M. A.; O'Hanlon, C. E.; Booth, M.; Motala, A.; Timmer, M.; Shanman, R.; and Shekelle, P. G. Systematic review for effectiveness of hyaluronic acid in the treatment of severe degenerative joint disease (DJD) of the Knee. *Technology Assessment Report.* **2015.** Accessed online: https://www.ncbi.nlm.nih.gov/books/NBK343555/ (accessed July 6 2022).