Recent clinical trials have demonstrated that injecting MSC into the articular joint cavity for the treatment of degenerative knee osteoarthritis can extensively increase the volume of healthy cartilage tissues and healthy meniscus tissues, while reducing patient index-pain, resulting in dramatic improvement in the quality of life of patients. Purpose: The purpose of this study is to understand the frequency of response and duration of effect of a single, two step intra-articular injection of Wharton’s Jelly allograft combined with Amniotic Membrane allograft scaffolding in patients with osteoarthritic knee pain. Study Design: Retrospective Case Series Methods: 47 patients with radiographic evidence of knee osteoarthritis and knee pain greater than 3 months duration were administered a single two step intra-articular injection of Wharton’s Jelly allograft and Amniotic Membrane allograft between 3/1/2017 and 1/1/2019. The effects on pain and function were measured by the 11-point Numeric Rating Scale (NRS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) at pre-treatment, 3 months post-treatment, and 6 months post-treatment. Results: 87% (41/47) of patients were responsive to treatment. Of the responders, NRS pain scores reduced an average of 52-54% (4.0 - 3.9 points) at the 3- and 6- month post-treatment evaluations, respectively, and WOMAC scores reduced (20-22 points) an average of 35 - 39% at the 3- and 6-month post-treatment evaluations, respectively. Pain relief and functional improvements were maximal or sustained at least 6-months in the majority of responders (28/41 per NRS and 25/41 per WOMAC). No adverse reactions to treatment were reported. Conclusion: A single, two step intra-articular injection of this combination treatment was safe and efficacious in reducing pain and/or functional deficits in osteoarthritic patients, with symptom reductions apparent beyond 6 months post treatment. Key Words: Mesenchymal stem cell; Wharton’s jelly; Amniotic membrane; Knee pain; Osteoarthritis.

1. Introduction

Osteoarthritis (OA) is a chronic, progressively degenerative joint disease of the musculoskeletal system resulting in pain and biomechanical dysfunction. OA can originate from a variety of factors including aging, obesity, fatigue, injury, trauma, congenital joint abnormalities, and joint deformity[1]. Due to increases in human life expectancy, OA will become the fourth most disabling disease globally by 2020 [2].

OA most often results in pathological joint
tissue changes such as articular cartilage destruction, subchondral osteosclerosis and synovial hyperplasia [3]. Hyaline cartilage tissues that encapsulate articular joint surfaces have poor regenerative and self-repair capabilities due to the innate lack of neural and vascular innervations. These biological limitations leave traditional treatment methods for OA, including non-drug therapies, pharmacology, and surgical treatments, limited in their capability to relieve pain, repair damaged tissue, and restore function to the pathological joint(s)[3].

In recent years, new stem cell-based therapies for the treatment of pain and symptoms associated with OA have gained increased attention internationally. Mesenchymal stem cells (MSC) are defined by the International Society for Cellular Therapy as stromal cells which have established adherence to plastic, specific surface antigen expression, and multipotent differentiation potential [4].

MSC are optimal for regenerative therapy due to their immunoregulatory properties, paracrine and autocrine functions that generate growth factors (GF), and their ability to differentiate into various cell lineages including osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (smooth muscle cells), adipocytes (fat cells), fibroblasts and hematopoietic stroma according to the requirement of specific biomedical applications[5,6]. In vivo, MSC actively migrate to cartilage ischemia and damaged tissue sites within the articular microenvironment, express enzymes and secrete numerous nutritional factors including GF, cytokines and chemokines which repair cartilage tissue and suppress the secretion of inflammatory factors[7]. These properties collectively make MSC the ideal seed cells for therapeutic OA treatment.

To date, multiple clinical trials around the world have demonstrated that injecting MSC into the articular joint cavity for the treatment of degenerative knee OA can extensively increase the volume of healthy meniscus and cartilage tissues, while reducing patient index-pain and dramatically improving patient quality of life [8-13]. One group conducted a randomized controlled trial (RCT) involving 55 patients with knee OA and concluded that there was evidence of meniscus regeneration and improvement in knee pain following treatment with allogenic human MSC[13]. Another pilot study on the safety and efficacy of using allogenic adipose derived MSC (AD-MSC) for treating chronic lateral epicondylitis demonstrated that the intervention was safe and efficacious in improving pain, performance, and anatomical defects for more than a 52 week follow up period[14].

Although MSC are obtainable from numerous tissues in the human body, a majority of preclinical and clinical research has been initially focused on autologous AD-MSC and bone marrow derived MSC (BM-MSC). However, neonatal tissues such as placenta (P), amniotic fluid (AF), amniotic membrane (AM), umbilical cord blood (UCB-MSC), umbilical cord (UC), and Wharton’s jelly (WJ) are rich sources of perinatal stem cell populations that include MSC, extracellular matrix proteins, tissue reparative GF, cytokines, chemokines, and collagen [15-17]. Though isolated from different tissue sources, the International Society for Cellular Therapy defines all of these types as MSC implying similar standards of regenerative capability in vivo by establishing phenotypical and functional equivalency as a minimum criterion[4]. Thus, BM-MSC and P-MSC are expected to have similar regenerative abilities in vivo. That said, MSC harvested from P-MSC, AF-MSC, AM-MSC, UC-MSC, and WJ-MSC have rapidly escalated in allograft use to enhance healing of wounds because they exhibit superior cell biological qualities such as improved proliferation, life span, and differential potential as compared with BM-MSC[18,19].

UC tissue is the easiest obtainable biological source of MSC[20] and the use of UC-MSC and MSC from surrounding tissues has been proven to be safe and effective[11, 21-23]. One group of researchers conducted a randomized controlled trial involving 20 patients with knee OA who were injected with P-MSC and concluded that a single intra-articular allogenic P-MSC injection for treating knee OA is safe and can result in clinical improvements for at least 24 weeks based upon follow up [24]. Another group of researchers conducted a study in which 128 knee OA patients
were injected with UC blood derived MSC (UCB-MSC) and concluded that implantation of UCB-MSC is effective for treating knee OA based on a 2-year follow-up[22].

In literature, the use of UC-MSC has shown a favorable in vitro potential as they can differentiate toward both the chondrogenic and the osteogenic lineage similar to BM-MSC. Moreover, UC-MSC improve chondrogenic commitment in reaction to low oxygen tension conditions and to pulsed electromagnetic fields much like BM-MSC [24]. One group of researchers documented immediate improvement in pain and function in 2 patients with discogenic lower back pain after a transplantation of UC-MSC and even greater improvement throughout the 2 year follow up period[25]. Another group of researchers concluded that clinical outcomes support the use of UC tissue for augmentation of Achilles tendon repair and that the adjunct use of UC-MSC resulted in decreased postoperative recovery time and a faster return to work and preinjury activity compared with patients who were treated by standard of care only [26]. Furthermore, researchers who assessed the safety and efficacy of utilizing UC-MSC intreating patients for nonhealing wounds concluded that locally delivered allogeneic UC-MSC can contribute to chronic wound repair and provide an additional alternative toward new therapeutic strategies[27].

WJ, first discovered by Thomas Wharton in 1656, is a soft, gelatinous tissue that surrounds the two arteries and one vein of the UC [28]. WJ tissue is classified as a mucoid connective tissue and functions as protection, structural support, and cushioning within the UC[29]. WJ-MSC represents an ideal alternative source of stem cells for cartilage tissue engineering [30]. WJ is a rich source of fetal/placental MSC [31] and studies have consistently shown that WJ-MSC are authentic MSC possessing the same regenerative properties as adult stem cells [32].

WJ tissue has several advantages that make it an attractive choice for use in tissue engineering and regenerative medicine. WJ-MSC are a relatively young cell type compared to most other MSC and can be harvested from the donor painlessly and non-invasively unlike BM-MSC or AD-MSC [18]. Moreover, the yield of MSC from WJ tissue is high compared to other MSC tissue sources [18]. Furthermore, WJ-MSC have multiple embryonic features including high cell proliferation and wide cell differentiation potential while maintaining hypo-immunogenic and non-tumorigenic characteristics, making WJ-MSC safe and ideal for both autologous and allogenic HCT/P clinical use [18].

Researchers have observed that WJ-MSC and BM-MSC respond similarly to inductive cues to differentiate terminally to a DA cell type, and that the neuronal plasticity of human WJ-MSC is comparable with that of BM-MSC[33]. However, while both WJ and BM have made it possible to quickly and easily obtain clinical grade MSC, WJ-MSC has emerged as the more suitable alternative source of MSC due to their primitive nature and easy isolation without stimulating ethical harvesting concerns [34]. Indeed, WJ-MSC have many advantages in isolation time, isolation efficiency, expansion time, passage capacity, expansion capacity when compared with UCB-MSC and BM-MSC (20). Moreover, WJ contains numerous GF, cytokines, hyaluronic acid (HA), and extracellular vesicles which reduce inflammation, reduce pain, and augment healing of musculoskeletal injuries [29]. Furthermore, the amounts of these healing factors contained within WJ are greater compared with other biological tissue sources[29].

Researchers have also shown that WJ-MSC can increase the expression of cartilage-specific genes and can be introduced as a promoting factor for cartilage regeneration[35,36]. WJ-MSC by their inherent nature have high HA, sulfated glycosaminoglycans and collagen expression which to some extent reflect native cartilage tissue[37]. High density WJ-MSC cultures using rotary cell culture systems develop larger, soft, opaque non-scaffold cartilage-like tissues and show higher expression of glycosaminoglycans and collagen II than conventional pellet cultures[37]. Many researchers have noted that WJ-MSC express characteristics of pre-chondrocytes, low immunogenicity and are more easily obtained with higher purity due to the lack of hematopoietic cells contained within WJ.
making WJ-MSC more suitable for constructing tissue-engineered cartilage than other types of MSC[38].

A group of researchers investigating WJ-MSC as a more functional and readily available substitute for BM-MSC in musculoskeletal regeneration determined that while WJ-MSC and BM-MSC osteoblastic activity were similar, WJ-MSC presented a distinct and stronger proinflammatory/chemotactic cytokine profile and significantly enhanced angiogenic activity compared with BM-MSC[39]. WJ-MSC in osteoarthritissynoviocyte significantly reduce the expression of the Prostaglandin E2 gene, Matrix Metalloproteinase-13 gene, and RELA Synoviocyte gene[40,41]. Moreover, WJ-MSC have a higher endothelial differentiation potential than BM-MSC and are therefore more favorable for neovascularization of engineered tissues[42]. Furthermore, the persistent presence of B7 family co-stimulator immune molecule B7-H3 (CD276) in the undifferentiated and chondrogenic differentiated cells of WJ validates its strong immuno-privilege and safety advantage in selecting WJ-MSC over other MSC tissue sources[37].

Leading regenerative medicine clinicians have advanced regenerative cellular medicine treatments by combining MSC with GF, cytokines and scaffolds in order to improve therapeutic efficacy[43]. Commonly used support scaffolds include polymer scaffolds such as HA, fibrin gel, and liquid serum platelet rich plasma suspensions. These support scaffolds are injected directly into the articular joint cavity and serve as a potential tissue source for the treatment and repair of damaged cartilage[43]. Research has demonstrated that WJ-MSC embedded in a three-dimensional hydrogel scaffold are able to adapt to their environment and express specific cartilage-related genes and matrix proteins within 4 weeks[30]. WJ and AM both contain subpopulations of stem cells within a collagenous matrix and are rich in HA and GF[18]. Therefore, these two bioactive tissue matrices are therapeutically promising sources of allografts that can be used in combination to modulate intra-articular cellular microenvironments for optimal healing and repair potential[29,30,44,45].

2. Methods and Materials

2.1 Design

This retrospective case series examined the safety and efficacy of an advanced 2 step, human cellular tissue product (HCT/P) procedure on pain and functional outcomes in 47 patients with knee pain associated with OA. A retrospective patient chart review of 906 subjects treated between 3/1/2017 and 1/1/2019 identified the HCT/P patient population for analysis. Patient groups were defined by HCT/P combination (WJ-MSC and AM) as well as by injection site (intra-articular). Independent patient variables such as patient age, ethnicity, and sex have all been eliminated from the data analysis. All patients in this study presented seeking non-surgical intervention for knee pain, had a history of knee pain greater than 3 months duration, and had radiographic evidence of knee OA. Furthermore, no patients had a history of cancer within the 5 years prior to treatment, active infection or cellulitis on extremity to be treated, or prior full or partial joint replacement in the affected knee.

Patients’ 11-point Numeric Rating Scale (NRS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores were recorded at pre-treatment, 3- months post treatment, and 6-months post-treatment. Only subjects with data at all three time points for a given metric were selected for analysis. Data analysis was performed using Microsoft Excel.

Patients were considered responsive if they demonstrated any decrease in NRS or WOMAC scoring, which indicates that they experienced improvement in pain and/or of functional disability. Maximum response was defined as the patient achieving their greatest improvement in NRS and WOMAC outcome measures at either the 3 month or 6-month time frame. The maximum response was considered sustained if the patient maintained that same maximum response throughout the treatment period.

2.2 Materials

The advanced 2 step HCT/P protocol for this
particular study selected AM scaffolding consisting of 1mL of Predictive Biotech’s Amniocyte Plus™, a minimally manipulated human tissue allograft derived from the extracellular matrix of the AM. Amniocyte Plus™ is processed to preserve cytokines, GF and scaffolding proteins in the AM for homologous use. The extracellular matrix of the AM contains a high concentration of cellular scaffolding, cytokines, GF and proteins. The extracellular matrix is a collection of extracellular molecules that provide the structural support needed for surrounding cells comprised of collagens, multi-adhesive glycoproteins, elastin, glycosaminoglycans, GF and cytokines. Amniocyte Plus™ does not contain live cells and, post-processing, cannot be considered a tissue. Amniocyte Plus™ is regulated as a human cell and tissue product (HCT/P) under 21 CFR Part 1271 and Section 361 of the Public Health Service Act [46].

The second step of this advanced protocol utilized 0.5mL of Predictive Biotech’s CoreCyte™, WJ-MSC allograft product. WJ tissue is a gelatinous substance in the UC that provides structural cushioning and support to the umbilical vein and arteries. The structural cushioning and protective elements from WJ consist of a network of proteins, pericytes, WJ-MSC, cytokines, chemokines and GF. Although CoreCyte™ does contain live cells, Predictive Biotech does not claim that CoreCyte™ is dependent on the metabolic activity of living cells for its primary function. The structural cushioning support function of CoreCyte™ is not reliant on the presence nor on the metabolism of the cells in the allograft. CoreCyte™ is processed from donated human tissue from full-term deliveries and is minimally manipulated. CoreCyte™ meets the criteria under 1271.3(d) as an HCT/P, and therefore is regulated under 21 CFR 1271 and Section 361 of the PHS Act. Homologous intra-articular utilization of CoreCyte™ fulfills 21 CFR 1271.10(a) and is regulated under 21 CFR 1271 and Section 361 of the PHS Act. Both Amniocyte Plus™ and CoreCyte™ are manufactured under current good manufacturing practice regulations per CFR 210 and 211 [47,48].

2.3 Intervention

Forty-seven (47) patients were treated in an outpatient clinical setting with an advanced, two step injection procedure that can be performed in under 30 minutes. First, a single intra-articular injection of 1mL AM allograft scaffolding was delivered into the affected joint capsule. Then, with the needle remaining in its intra-articular insertion, HCT/P syringes were exchanged and the second step of injecting 0.5mL WJ-MSC allograft was performed. The target concentration of WJ-MSC for this procedure was 1.05 million MSC/0.5mL WJ [47].

Both HCT/P were injected sequentially and directly into the intra-articular space using a 25 gauge 1 ½ inch needle. Patients were positioned in a seated position with the knee bent at 90 degrees and a medial or lateral approach was used to access the joint space based upon radiographic and palpatory findings. The site was prepared using chlorhexidine scrub and Ethyl Chloride. Confirmation of joint space access was achieved through joint fluid aspiration confirmation.

2.4 Outcome Measures

Patient outcomes were measured by the NRS (Numeric Rating Scale) pain scale and the WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) OA disability scale. The NRS is an 11-point numeric rating scale specific to pain, with 0 corresponding to no pain, and 10 defining the worst pain ever possible [49]. The WOMAC metric system consist of a total of 24 items, representing 3 subscales of data collected-pain, stiffness, and physical function. The items are scored by patients on a scale of 0-4, which correspond to: 0=none, 1=mild, 2=moderate, 3=severe, and 4=extreme. Higher scores indicate worse pain, stiffness, and functional limitations. The pain subscale is comprised of 5 items, the stiffness subscale 2 items, and the physical function subscale of 17 items, each given equal weight[50]. Patient NRS and WOMAC scores were recorded at pre-injection, 3 months post injection and 6 months post injection. These outcome assessment scores were collected and the change from baseline was averaged across the cohort.

Medical monitoring for any and all adverse events or reactions possibly resulting from these
regenerative cellular medicine procedures was performed in compliance with FDA Human Cell & Tissue Products (HCT/P) Adverse Reaction Reporting guidelines [51].

3. Results

A total of 47 patients received the AM allograft scaffolding (Amniocyte Plus™) and the WJ-MSC allograft (CoreCyte™) intra-articular injection with assessment of pain and functionality by NRS and/or WOMAC metrics at each baseline, 3-months post-treatment, and 6-months post-treatment. The efficacy of the treatment, as characterized by the percentage of the treated population which self-reported a decrease in NRS and/or WOMAC scores, is presented in Table 1. The mean and average change in NRS pain score at each assessment interval is presented for responders in Table 2, and the duration of effect in NRS scores is presented in Table 3. The mean and average change in WOMAC score at each assessment interval is presented for responders in Table 4, and the duration of effect in WOMAC scores is presented in Table 5.

Of the 47 patients receiving the AM allograft scaffolding and the WJ-MSC allograft intra-articular injection to the affected knee joint capsule, 87% (41 patients) were responsive to treatment, as assessed by a reduction in NRS and WOMAC scores within 6-months post-dose. Among responders, NRS pain scores reduced an average of 52-54% (3.9 – 4.0 points) relative to baseline. While 32% of responders experienced maximal pain relief within 3-months and 29% experienced maximal pain relief within 6-months post-treatment, approximately 29% of respondents exhibited a maximal effect within 3 months post-treatment which was sustained at least through 6-months post-treatment. Moreover, WOMAC disability scores reduced (20-22 points) an average of 35 - 39% relative to baseline. The majority of responders (54%) experienced maximal WOMAC functional improvement within 6-months post-treatment; 7% exhibited maximal effect at 3-months which was sustained through at least 6-months; and 39% of respondents exhibited maximal WOMAC functional improvement at 3-months post-treatment. This data demonstrates that this advanced, two step HCT/P procedure provided patients effective pain relief for knee pain associated with OA while progressively providing functional improvement for 6 months following treatment. Most importantly, no adverse events or reactions to treatment were reported by any of the patients. The results of this study are remarkable and demonstrate the safety, scale and speed of relief, and long-lasting effectiveness of a single injection of this advanced HCT/P procedure.

4. Discussion

Intra-articular injections of AM allograft scaffolding (Amniocyte Plus™) and WJ-MSC allograft (CoreCyte™) show great promise improving pain and functional outcomes in patients with knee OA. Eighty-seven percent of patients demonstrated improvement in pain and functional outcome measures, with an average pain reduction greater than 50% at 3 months. With no adverse effects reported, this treatment offers a safe alternative achieving pain relief and functional improvement.
in patients that have previously been limited to NSAIDs, steroid injections, and topical analgesic options. The delivery of the injection can be performed in an outpatient clinical setting in under 30 minutes, offering a convenient and cost competitive way to deliver effective results.

Results from this present case series parallel many other orthopedic trials utilizing autologous and allogenic MSC from a variety of tissue sources for safe and effective localized orthopedic pain relief and functional support. One triple-blind, RCT confirmed the safety and efficacy of using a single intra-articular injection of autologous BM-MSC in patients with knee OA. Analogous to the results of this study, the BM-MSC treatments provided significant and clinically relevant pain relief for over 6 months versus placebo. Moreover, patients receiving those intra-articular BM-MSC treatments experienced significantly greater improvements in WOMAC total score, WOMAC pain and physical function subscales, and painless walking distance compared with patients who received the placebo. Comparable outcome measures were achieved in this study combining AM and WJ-MSC into a 2 step, single injection therapy.

Another double-blind, RCT exhibited dramatic improvements in OA patient pain and visual analog scale assessments (VAS) following a single superolateral knee injection of allogenic MSC compared with those who received the control [2]. Separately, an additional double-blind, RCT verified the safety and efficacy of utilizing allogenic P-MSC as therapeutic alternatives to treat OA symptoms. That trial demonstrated P-MSC injections providing rapid and substantial pain relief while simultaneously improving functional ranges of motion in OA patients[24]. Patient groups receiving the P-MSC injection therapies experienced decreased OA symptoms and significant improvements in quality of life, activities of daily living, and sports and recreational activities for at least 8 weeks [24].

Additionally, the first study using UC-MSC transplantation for treatment of chronic discogenic low back pain proved to be effective and safe, with rapid and dramatically improved back pain accompanied by a parallel improvement in lumbar function with no side effects [26]. A second clinical study established the safety and efficacy of allogenic AD-MSC in improving elbow pain, performance and structural defects for 52 weeks [14]. This was the first study revealing the therapeutic value of MSC injections for treating chronic tendinopathy [52].

Phase I/II clinical trials for Cartistem, an allogeneic HCT/P consisting of UCB-MSC combined with HA hydrogel, established safety and efficacy over the core trial phases and throughout the extended follow-up[45,53]. Moreover, a parallel study retrospectively recognized that patient mean VAS and WOMAC assessment scores significantly improved compared to preoperative scores in patients who underwent implantation of UCB-MSC combined with HA scaffold [22]. These formentioned studies reinforce the results and conclusions of this present retrospective study and support the combined usage of HA scaffolds with MSC harvested from immuno-privileged, perinatal tissue sources such as UCB-MSC and WJ-MSC as being safe and effective in reducing knee pain and restoring lost function resulting from OA. Finally, it is clinically important to re-emphasize the fact that each of the studies referenced in this report had no adverse reactions nor postoperative complications resulting from the localized and intra-articular orthopedic use of MSC, MSC/HA, and WJ-MSC/AM combination treatments [11,22].

5.Conclusion

This study demonstrates promising effectiveness for patients suffering with knee osteoarthritis. In this study, knee pain, measured by the NRS, was remarkably reduced and functional disability, measured by the WOMAC, was remarkably reduced by administering an advanced 2-step, single intra-articular injection of WJ-MSC allograft and AM allograft scaffolding. The effects were observed within 3-months post-treatment and were sustained for at least 6-months post-treatment. Most importantly, no treatment-related adverse events or reactions were reported. The simplicity and effectiveness of this regenerative cellular procedure opens an alternative treatment to pharmacological and/or surgical treatments.
and has safe, minimally invasive, and cost-effective therapeutic applications in orthopedic medicine, sports medicine, and pain management.

It is acknowledged that this study did not evaluate the relationship between individual age, BMI, normal or adopted changes in activity/exercise regimen, or staging of osteoarthritis and the NRS and WOMAC scores. Additionally, a control group that did not receive any treatment was not assessed for comparison to spontaneous rates of symptom resolution. Future studies should include patient age, sex, BMI, exercise/activity changes, as well as further analysis of the subscale changes present on the WOMAC.

References


