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ESTABLISHING A MESENCHYMAL STEM CELL BANK FROM UMBILICAL CORD TISSUE FOR CELL THERAPY AND INITIAL APPLICATION IN THE TREATMENT OF KNEE OSTEOARTHRITIS AT TAM ANH GENERAL HOSPITAL

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ABSTRACT

Osteoarthritis (OA) is a natural aging process of joints that often occurs as age increases. In knee osteoarthritis, the cartilage that cushions the ends of the thigh bone and shinbone within the knee joint deteriorates, leading to a loss of cushioning and flexibility. This can result in pain, swelling, and stiffness when moving the knee joint. In the treatment of knee osteoarthritis, methods often include symptom management with pain-relieving and anti-inflammatory medications, lifestyle modifications to reduce stress on the knee joint; in some cases, surgery may be considered if nonsurgical interventions are ineffective. Stem cell therapy has emerged as a promising approach for treating knee osteoarthritis. This method has been demonstrated to be safe and effective. Still, one of the most important considerations when using stem cell therapy is quality regulation and safety standards regarding cell culture. This has led to the emergence of stem cell banks, where they are manufactured, preserved and stored. In this study, we utilized stem cells derived from allogeneic umbilical cord tissue. The stem cells were expanded and administered to patients via two injections spaced one month apart, with follow-up lasting an additional six months. The initial treatment results using stem cells for patients with knee osteoarthritis have been very promising. Through assessments of mobility, pain levels (measured by the VAS), and improvement in quality of life (measured by the Lequesne index) over 6 months, the research team has demonstrated the effectiveness of using mesenchymal stem cells (MSCs) from umbilical cord tissue for treating knee osteoarthritis.

Keywords: Stem cell bank, umbilical cord-derived mesenchymal stem cells, knee osteoarthritis.

INTRODUCTION

Osteoarthritis is the most common form of joint disorder. It is characterized by the degeneration of joint cartilage and the formation of bone spurs at the joint margins, causing pain and stiffness (Loeser *et al.*, 2012). The pathogenesis of osteoarthritis is multifactorial and involves mechanical and biochemical processes and genetic factors. Current treatments for osteoarthritis are somewhat effective. However, scientific and technological advancements continue with the aim of providing even better therapeutic interventions. Research into the pathophysiology and mechanisms of various therapies will reveal the best ways to develop effective treatments for patients.

Numerous studies worldwide have demonstrated the safety and efficacy of mesenchymal stem cell therapy for treating osteoarthritis. The mechanisms of action of this therapy are gradually being elucidated. The application of stem cell therapy for the treatment of knee joint pathology is a new choice for patients (Makris *et al.*, 2015). It is a non-surgical treatment that can be performed on an outpatient basis. Patients can resume daily activities immediately after using stem cell therapy, eliminating the need to endure postoperative pain and extended recovery times.

In Vietnam, the application of stem cell therapy for treating osteoarthritis has attracted the attention of researchers and clinical physicians. The number of patients seeking treatment is increasing. Stem cell therapy using adipose tissue-derived mesenchymal stem cells has been applied in our country for some time, yielding certain positive outcomes. However, a major obstacle to using adipose tissue-derived mesenchymal stem cells is the difficulty in harvesting fat for stem cell isolation, and the quality of stem cells diminishes with age. Allogeneic mesenchymal stem cell therapy using umbilical cord tissue offers several advantages: noninvasive procedures for harvesting cells, good proliferation and differentiation capabilities, and immune modulation abilities (Makris *et al.*, 2015). The application of stem cell therapy provides patients with access to modern technology. Furthermore, developing procedures for collecting and processing mesenchymal stem cells from umbilical cord (UC) tissue to obtain standard stem cell therapy is another a subject that requires in-depth research.

MATERIALS AND METHODS

Recruitment of expectant mothers (donors) and UC tissue collection

The study complied with ethical regulations in biomedical research, avoided any violations of the ethical standards issued by the Ministry of Health, and was approved by the Ethics Committee of Tam Anh General Hospital and the Hanoi Department of Science and Technology.

Volunteer UC donors were screened and subjected to tests compliant with FDA CFR21 (Code of Federal Regulations Title 21) regulations. The tests may include HBsAg, CMV, HBcAb, HCVAb, HIVAg/Ab, Rubella, and TPHA (Meissner-Roloff *et al.*, 2018). The tests were conducted at the time of UC collection or within seven days before collection.

During birth, a technician collected a fresh segment of the umbilical cord approximately 15 cm in length and placed it in a sampling cup containing 50 mL of preservation solution. The collected samples were

promptly transferred to the laboratory. Umbilical cords are selected under the following criteria: the maternity patient does not have accompanying diseases such as genetic disorders or infectious diseases, does not have obstetric complications, the fetus is full-term (natural birth or cesarean), the weight of the newborn is greater than 2600 g, the umbilical cord is intact without damage, and there is no suspicion of infection.

Isolation and primary cultivation

The umbilical cord was cleaned with a saline solution, and then the arteries and veins were removed. After that, the umbilical cord was cut into small pieces. A total of 50-60 pieces were placed into a T-flask (Corning, USA) supplemented with medium. The media used in our research did not contain any animal-derived components provided by a reputable company, ensuring standards suitable for human testing. The flasks containing the cord explants were placed in a CO_2 incubator at 37°C with 5% CO_2 and saturated humidity. The medium was regularly replenished, and cell growth was observed.

Expansion of MSCs

After approximately 12-14 days, once enough cells had developed from the explants, the cells were transferred to the secondary phase. MSCs were seeded at a density of 5000 cells/cm^2 in a T-flask supplemented with medium. The flasks were placed in a CO_2 incubator at 37°C and 5% CO_2. The medium was changed after three days of cultivation until the cells reached more than 80% confluence.

Freezing and revival of UC-MSCs

Upon reaching 80% confluence, the cells were harvested following standard procedures. The cell suspension was transferred to cryogenic vials and cooled down using an automated cryofreezer (Thermo, USA). Then, the cryogenic vial was transferred to a liquid nitrogen tank.

MSCs are only prescribed and infused in the context of a clinical trial. To revive the cell, a cryogenic vial with frozen MSCs was removed from the liquid nitrogen tank and immediately transferred to a beaker containing water at 37°C less than two minutes**.** MSCs were seeded at a density of 5000 cells/cm^2 in a T-flask supplemented with medium. The culture flask was transferred to a CO_2 incubator, and the medium was replaced after three days of cultivation. The cells were harvested when they reached approximately 80% confluence.

Quality Control

Immunophenotyping by flow cytometry

The cells were harvested and suspended in staining buffer (containing 2% FBS) and then stained with monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin-chlorophyll protein (PerCP), and allophycocyanin (APC). Specifically, the MSC-positive cocktail (FITC CD90, PerCP-Cy™5.5 CD105, and APC CD73) leaves the PE channel open for use in combination with the supplied negative MSC cocktail (PE CD45, PE CD34, PE CD11b, PE CD19 and PE HLA-DR). After incubating in the dark for 30 minutes at room temperature, the cells (at a density of approximately 10^7 cells/mL) were washed twice with staining buffer and analyzed on a Navios EX flow cytometry system (Beckman Coulter, USA).

Differentiation assay

Osteoblasts, adipocytes and chrondrocytes differentiation assays were carried out with a differentiation media. The cells were harvested at passage 3 and seeded into 24-well plates. After 24 hours, the cells adhered to the surface, the media was changed to differentiation-specific media, and the cells were cultured for 14-18 days. The media were changed every three days. Differentiated cells were stained with Alizarin Red, Oil Red O, and Alcian Blue (Abcam, United Kingdom) for osteoblasts, adipocytes and chrondrocytes, respectively. Images were observed under a microscope.

Cytogenetics

Karyotyping was performed at the Department of Human Genetics, National Children's Hospital. The cells were cultured and arrested at the metaphase stage using colchicine. Chromosomes were imaged using an automatic imaging system from Zeiss and analyzed using Ikaros software from Metasystem for comparison and mutation checking (if any). The thawed MSCs were karyotyped at passage 5 of culturing.

Microbiology testing

Sterility test: MSC sterility was assessed by bacterial culture (aerobia, anaerobia, and fungi with Bactalert^{R}). First, 10 mL of culture medium was added to each bottle of liquid media—BacT/ALERT® FA Plus and BacT/ALERT® FN Plus (BioMérieux, France) using sterile syringes and incubated at 37°C for 14 days or until contamination was detected.

Endotoxin: An EndoSafe®-PTS Kit (Charles River, USA) was used to test endotoxin. The cells were harvested and then suspended in a saline solution. The cell suspension was diluted with endotoxin-free water and used to be tested according to the kit instructions.

Mycoplasma: MycoAlert™ PLUS (Lonza, Switzerland) was used to test. Viable mycoplasma in conditioned media were lysed and the released specific enzymes reacted with the substrate, catalyzing the conversion of ADP to ATP. The ATP was then transferred into a light signal via the luciferase enzyme in the kit's reagent. By measuring the level of ATP in a sample both before and after the addition of substrate, a ratio can be determined that indicates the presence or absence of mycoplasma.

A case study

The patient was a 50-year-old male who presented initially with bilateral knee joint pain. His BMI was 24. He sought medical attention after undergoing conventional internal medicine treatments without success. He was diagnosed with Grade II bilateral knee osteoarthritis according to the Kellgren and Lawrence scale. He did not have hepatitis B, hepatitis C or HIV infection; had no malignant tumors; and had no history of allergy to any components of our treatment regimen. He consented to participate in cell therapy treatment, obtained approval from the Ethics Committee of Tam Anh General Hospital, and provided written informed consent to participate in the trial of osteoarthritis treatment using mesenchymal stem cells derived from umbilical cord tissue.

Patients were injected with stem cells two times at one-month intervals. Then, he will be monitored for 6 months, with follow-up visits scheduled at 4 weeks (W4), 8 weeks (W8), 12 weeks (W12), and 24 weeks (W24). Clinical and paraclinical examinations will

be conducted according to the research protocol.

RESULTS AND DISCUSSION

After preliminary screening, seven pregnant women were recruited. They agree and sign the consent form to donate cord tissue for research. According to the screening test results, 6 out of the 7 pregnant women who participated in the study were eligible to donate samples to the stem cell bank. One pregnant woman had a positive CMV IgM test.

During childbirth, no mothers experienced any obstetric complications or adverse events. The collected tissue samples showed no signs of crushing or infection, meeting the criteria for processing.

Isolation and culture of mesenchymal stem cells from umbilical cord tissue

Stem cells obtained from umbilical cord tissue samples adhered to the surface of the culture flask and exhibited an elongated shape similar to that of fibroblast-like cells (Figure 1). This is a characteristic shape of mesenchymal stem cells.

After seven days of culture, the cells inside the tissue fragments migrated and adhered to the surface of the culture flask (Figure 1A). In the same culture flask, cell growth varies among different tissue fragments: most tissue fragments exhibit cell migration relatively quickly (within 5-7 days of culture), while some tissue fragments show slower cell migration, and there were even fragments with no visible cell "crawling out". In the culture process, a sample was observed to be contaminated so the sample was discarded. In this case, the sample was collected during a natural birth in a non-aseptic delivery room, which may be the cause of the tissue sample being contaminated.

Expansion of mesenchymal stem cells

After 12-14 days of primary culture, when the cells in the culture flask were evenly distributed and reached a density of 50-70% confluence in each explant, this was the appropriate time for the secondary passage (from P0 to P1) (Figure 1A, 1B). These cells were transferred to a new culture flask using TrypLE, a recombinant enzyme produced from bacterial expression systems with no risk of viral contamination in animals or humans, to detach the cells from the culture flask surface. The cells were then cultured in a new flask, and the medium was changed every three days until the cells covered the surface of the flask. Subsequently, the cells were harvested and cryopreserved.

MSCs can be successfully isolated using various methods. However, the choice of method can significantly affect the quantity and quality of the cell (Salehinejad *et al.*, 2012). One of the earliest techniques performed in the laboratory for *in vitro* stem cell culture is the tissue culture method. This method is based on the principle of reducing the tissue size enough for gases and nutrients to diffuse freely within the tissue fragment. The success of this isolation method largely depends on the mobility of the cells. Another commonly used method in stem cell isolation is the use of enzymes capable of breaking down tissue structure to release the desired cells. Commercially available collagenase from *Clostridium histolyticum* is a common enzyme used in stem cell isolation by this method (Salehinejad *et al.*, 2012). The processing time of tissue with enzymes is extremely important because it

can disrupt the extracellular matrix and cell membranes, preventing cell attachment to the culture surface during isolation. Therefore, the processing time for enzymes should be minimized.

Research by Salehinejad and colleagues on isolating MSCs from Wharton's jelly tissue of the umbilical cord showed that MSCs obtained from WJ-MSCs using tissue culture methods had greater consistency of cellular morphology than those obtained using a combination of collagenase and trypsin enzymes. However, according to this study, it took approximately four weeks from the start of primary culture to the first passage of the tissue culture method, with the enzyme-using group taking 10 days (Salehinejad *et al.*, 2012). The primary culture period of the collagenase/trypsin method is shorter, hence yielding a greater number of cells in a shorter time. Therefore, the enzyme-based method is more commonly used overall. However, the use of enzymes such as collagenase, trypsin, and hyaluronidase can degrade the extracellular matrix and cell membranes, leading to reduced cell viability and proliferative capacity.

Since there is still no standardized and uniform procedure for isolating mesenchymal stem cells from Wharton's jelly tissue of the umbilical cord, in this study, we used the tissue culture method. The aim was to obtain a more homogeneous cell population. Our results also showed superiority compared to the same method in other studies. With this method, explants are cultured without previous cellular dissociation by enzymatic treatment. This method fixes some drawbacks associated with enzyme-based culture methods, such as proteolytic stress, which can result in cellular damage and decreased cell viability (Todtenhaupt *et al.*, 2023). In addition, enzymatic treatment methods also have additional limitations, including the degradation of cell surface receptors, changes in cellular function, and an increase in population doubling time (PDT), which signifies cellular aging (Chatzistamatiou *et al.*, 2014). Specifically, using the tissue culture method, the total time for culture was only 20 days.

Cell Quality Assessment

Viability

Cell viability was assessed using the trypan blue staining method and read using the specialized image system Countess II FL (Thermo, USA). On the Countess machine's screen, live cells are highlighted in green, while dead cells appear in red (Figure 1C). After the expansion culture process, from 5 donated tissue samples meeting the criteria, we obtained a total of 650 cell vials, each containing approximately 1 million cells stored in the master bank (Table 1). These cryopreservation cells will be thawed for use in assessing cell quality and cell therapy in further studies.

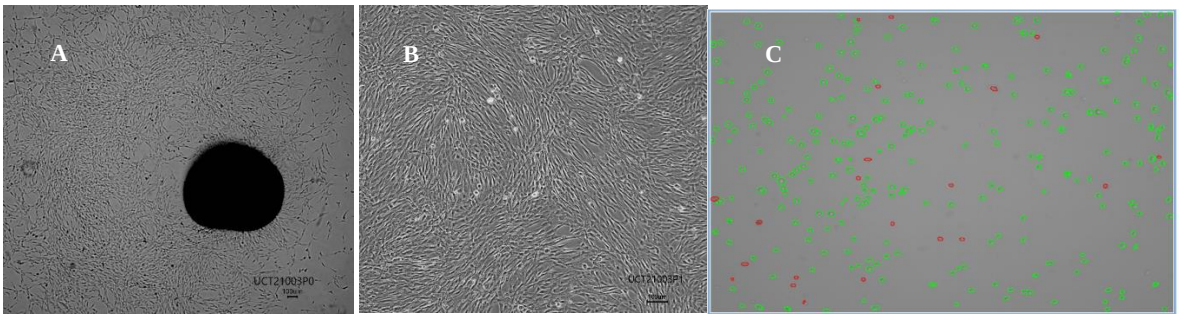

Figure 1. The expansion of MSCs (1A: Primary cell – the cell "crawling out" from explants; 1B – the cell in passage 1; 1C – the cell on the countess screen, the green cell as the live cell, and the red cell as the dead cell).

Table 1. The total number of cells obtained after cultivation.

Lot	Number of the cell	Live cell
UCT21001	$97x10^6$	93%
UCT21003	$107x10^6$	95%
UCT21005	$112x10^6$	96%
UCT21006	$122x10^6$	97%
UCT23001	$216x10^6$	96%

Assessment of the immune phenotype of MSCs: In this study, we conducted an assessment of the immune phenotype of MSCs by flow cytometry with a Beckman Coulter system. The CD marker panels were designed according to the standards of the International Society for Cellular Therapy (ISCT). The results indicated that more than 99% of the mesenchymal stem cells (MSCs) expressed the markers CD73, CD105, and CD90, while less than 0.1% of the cells were expressed for the markers CD34, CD11b, CD19, CD45, and HLA-DR (Table 2). This result complies with the ISCT standards for the immunophenotypic characterization of MSCs (Dominici *et al.*, 2006). In a 2009 publication by Schugar, mesenchymal stem cells were isolated from Wharton's jelly by collagenase, and the percentages of the markers CD105, CD73, CD44, and CD90 were reported to be 35%, 42%, 48%, and 65%, respectively (Schugar *et al.*, 2009). On the other hand, the percentages of CD44- and CD90-positive cells in samples isolated from Wharton's jelly using an enzyme cocktail of hyaluronidase, trypsin, and collagenase were 96% and 91%, respectively, according to Weiss's publication in 2006 (Weiss *et al.*, 2006). In another study conducted in 2012, Yongan Xu and colleagues used the collagenase type II enzyme method for MSC isolation and reported positivity rates for the CD44, CD90, and CD105 markers of 99.24%, 93.34%, and 99.47%, respectively (Xu *et al.*, 2012). This demonstrates that our cells fully meet the criteria defined for stem cells by the ISCT and are eligible for application in regenerative treatments.

Table 2. Cell identity and purity at the final harvest of 5 batches.

	UCT21001	UCT21003	UCT21005	UCT21006	UCT23001
CD90	99.9%	99.7%	99.6%	99.7%	99.8%
CD73	99.8%	99.7%	99.4%	99.4%	99.6%
CD105	99.5%	99.8%	99.5%	99.7%	99.7%
CD34, CD11b, CD19, CD45, HLA-DR	0.1%	0.01%	0.1%	0.04%	0.03%

The multilinage differentiation of UC-MSCs

The differentiation results were evaluated in each environment by staining the cells with specific dyes. In the cartilage differentiation environment, we observed the formation of cell clusters with a structure resembling cartilage. These cell clusters appeared blue after staining with alcian blue (Figure 2A). Similarly, lipid droplets that formed during adipogenic differentiation appeared red when stained with oil red O (Figure 2B), and calcium formation during osteogenic differentiation was detected by staining with alizarin red (Figure 2C). This demonstrated that our stem cells meet the ISCT-defined standards for MSCs regarding their differentiation potential. Adherence to these standards is not only a guarantee for the reliability of the product but also a crucial step in ensuring safety and effectiveness in medical treatments and applications.

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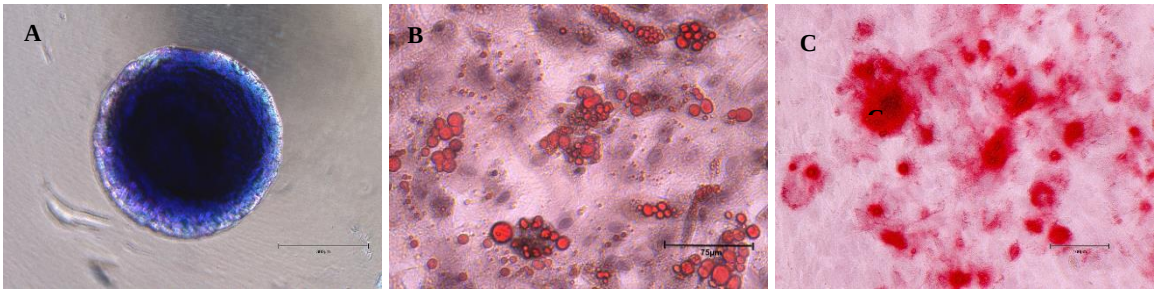

Figure 2. Images of differentiated cells after staining (2A - Chondrogenic differentiation – 10x; 2B - Adipogenic differentiation – 20x; 2C - Osteogenic differentiation – 20x).

In pathogenesis, bone remodeling involves the degradation of old or damaged bone by osteoclasts (bone resorption) and the subsequent deposition of new bone by osteoblasts (bone formation). In OA, bone resorption is faster than bone formation. Under normal physiological conditions, MSCs promote the formation of osteoclasts by producing key osteoclastogenic cytokines, such as RANKL and M-CSF. However, in the presence of inflammation, MSCs inhibit osteoclast formation and activity, partially through the secretion of osteoprotegerin (OPG), a crucial anti-osteoclastogenic factor.

Under laboratory conditions, when MSCs are cocultured with osteoclasts in the presence of high levels of factors that induce osteoclast formation, they mimic the inflammatory environment found in vivo and trigger MSCs to suppress osteoclastogenesis. MSCs play dual roles, either by stimulating or inhibiting osteoclast formation, depending on the inflammatory context. This behavior of MSCs toward osteoclasts mirrors their interactions with other immune cells and holds promise for utilizing MSCs therapeutically in inflammatory conditions associated with bone loss (Sharaf-Eldin *et al.*, 2016).

Karyotype

All 5 donated samples underwent a karyotype test and the results show that they did not exhibit an abnormal chromosome. According to a recently published study by a group of Pakistani authors, MSCs derived from umbilical cord tissue maintain their stemness up to the 15th passage (Shafiqa Naeem Rajput, 2024). In our study, a cytogenetic test was performed on the cells at passage 5, and the results showed that the phenotypes of all the samples remained unchanged (Figure 3). Research by de Witte and colleagues has shown that MSCs from umbilical cord tissue, after prolonged passaging (P4, P8, P12), still maintain stable morphology and expression of immune properties similar to those of cells at lower passage numbers (de Witte *et al.*, 2017). However, the immunomodulatory characteristics of these MSCs are diminished. Within the scope of our project, we utilized MSCs at the 3rd passage (P3) for transplantation into patients to maintain the genetic stability of the cellular therapy product.

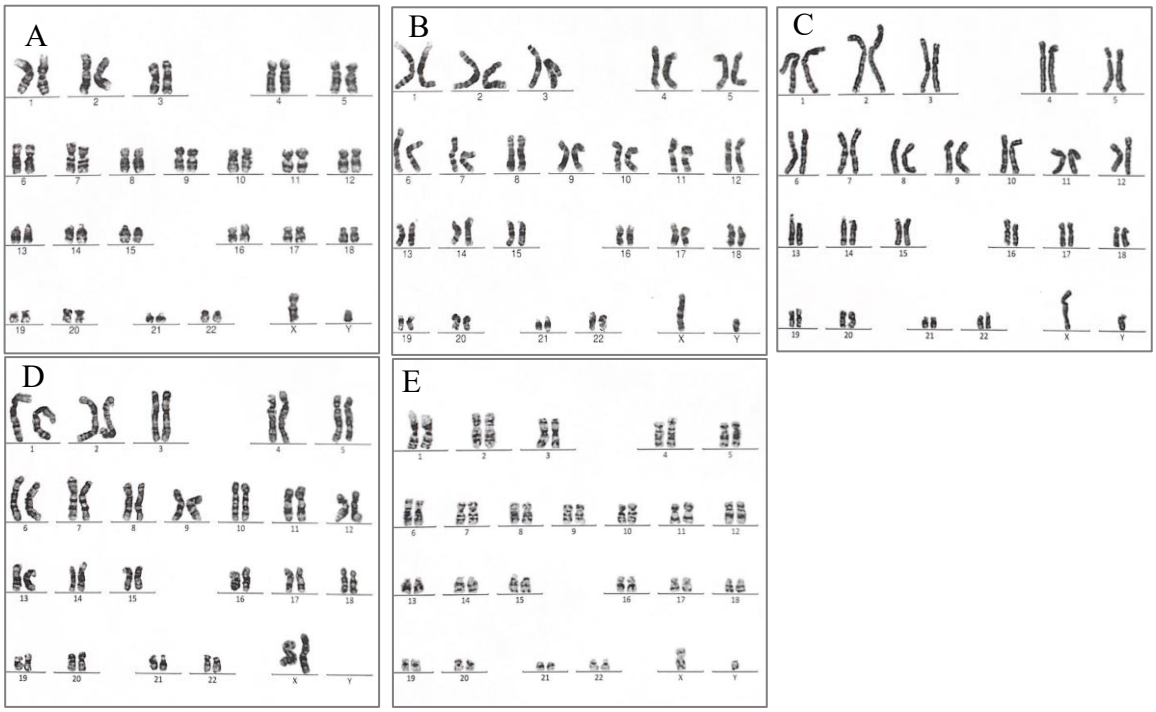

Figure 3. Karyotyping results of 5 donated samples (3A –UCT21001; 3B – UCT21003; 3C – UCT21005, 3D – UCT21006; 3E – UCT23001).

Biological testing

Sterility tests are conducted to determine whether a pharmaceutical product or medical device is free from viable microorganisms. These tests are crucial for ensuring the safety and efficacy of products intended for patient use, particularly those administered via injection, implantation, or other means that could introduce microbes into the body. The sterility test typically involves exposing the product to a suitable culture medium that supports the growth of microorganisms. If any viable microorganisms are present in the product, they will grow in the culture medium, allowing for detection. The test was conducted under strict aseptic conditions to prevent contamination. Sterility testing is a critical part of quality control in pharmaceutical and medical device manufacturing, helping to ensure that products meet regulatory requirements and are safe for patient use.

The microbiological test results demonstrated that our cells are completely sterile and suitable for banking and further study.

A case study using MSCs from the umbilical cord to treat knee osteoarthritis

Features of the MSCs used in the case study

The randomly selected cryovial cells in the master bank were thawed rapidly and seeded into T-flasks at a density of 5000 cells/cm². The total number of cells in the tube at the time of thawing was 1.11 x 10^6 cells/mL, with a cell viability rate of 96%.

After 10 days of culture, a total of 47.2 x 10^6 cells were harvested, with a viability rate of 96%. The cells were suspended in 0.9% NaCl at a concentration of 8.10^6 cells/mL. The suspension was injected into each joint at 2.5 mL. This suspension is also used for quality control. During sample harvesting, conditioned media was collected for bacterial and fungal testing.

The patient received intra-articular stem cell injections at a dosage of 20 million cells per joint.

Table 3. Quality control of MSCs for two doses.

Criteria	First dose	Second dose
Mycoplasma	Negative	Negative
Endotoxin	Negative	Negative
CD marker	Negative marker (0.8%) CD73 (98.7%) CD90 (99.945%) CD105 (99.9%)	Negative marker (0.34%) CD73 (99.32%) CD90 (99.9%) CD105 (99.97%)

Quality control of MSCs

After the first injection, the patient experienced mild joint effusion. Clinical physicians performed joint fluid aspiration for the patient, and there was pain at the injection sites; however, the pain significantly improved after each follow-up

visit. No signs of infection around the joints, joint inflammation, or joint bleeding were noted. No other adverse reactions were recorded.

Clinically, the reactive protein C (CRP) concentration was slightly elevated but not significantly elevated, and the erythrocyte sedimentation rate was normal. A mildly elevated erythrocyte sedimentation rate and slightly elevated CRP concentration are indicators of synovial inflammation on laboratory tests.

At various times from W0 to W24, the patient did not exhibit any systemic symptoms, such as headache, dizziness, itching, or anaphylactic shock. According to the Kanofski scale, the number of points increased from 70 (at W0) to 90 (at W24). Regarding motor function, the flexion angle of the right knee was 90 degrees at W0, which improved to 135 degrees after stem cell injection. The extent of motion of the right knee at W0 was 15 degrees, which improved to 0 degrees at 6 months post-injection. For the left knee, similar improvements were observed in the flexion and extension ranges, with the flexion angle increasing from 60 degrees to 135 degrees and the extension range decreasing from 15 degrees to 0 degrees. The VAS score at the time of injection was 3/10, which increased to 5/10 post-injection. However, the patient's pain gradually decreased, and by W8, the patient no longer felt joint pain, maintaining this status until W24.

Regarding the improvement in quality of life, as assessed using the Lequesne Index, the severity of osteoarthritis pain changed from 22 points at W0 (severity) to 2.5 points at W24 (mild) (Figure 4D). The patient expressed satisfaction with the treatment therapy and a significant improvement in quality of life. Specifically, regarding the patient's pain level, there was a marked reduction from pain even while lying down or immobile to no pain at W24, even during movement. Positive changes in therapy were also reflected in the patient's maximum walking distance. Initially, the patient only walked approximately 100-300 m with the assistance of a cane or family member, but post-treatment, the patient could walk longer distances independently without assistance.

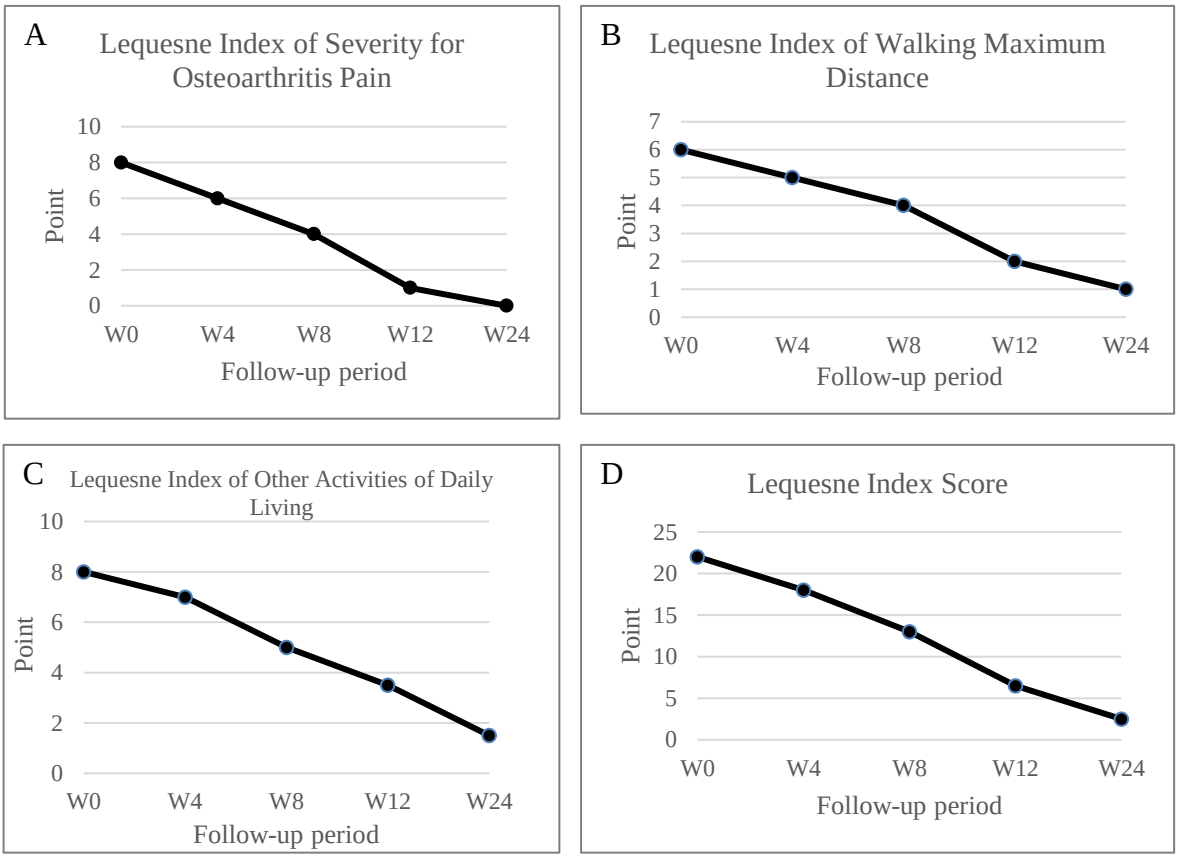

Figure 4. Lequesne score during the follow-up period.

Our research results were similar to those of previous studies. These include clinical studies utilizing mesenchymal stem cells from umbilical cord tissue for the treatment of knee osteoarthritis in South Korea (Park *et al.*, 2017). Patients with grade 3 knee osteoarthritis according to the Kellgren-Lawrence scale and grade 4 according to the ICRS were selected to participate in the clinical trial. Over the 7-year follow-up period, researchers assessed safety levels, VAS scores, International Knee Documentation Committee (IKDC) scores, magnetic resonance imaging (MRI) results, and histological evaluations. Seven participants were enrolled. Cartilage repair was observed at the 12-week arthroscopic evaluation. The VAS and IKDC scores improved at 24 weeks. Clinical outcomes remained stable after 7 years of follow-up. MRI after 3 years demonstrated the presence of regenerated cartilage. Only mild to moderate treatment-related adverse effects were observed in five patients. No cases of bone degeneration or tumor formation occurred over the 7-year period (Park *et al.*, 2017). The application of this novel stem cell-based therapeutic product appears to be safe and effective for durable knee joint cartilage regeneration. Another trial utilizing mesenchymal stem cells from umbilical cord tissue was conducted at the University of Los Andes in Santiago, Chile. A randomized, triple-blind, single- or double-dose trial

comparing the traditional treatment methods of injecting hyaluronic acid (HA) was performed. The trial group consisted of 9 patients who received either a single dose of 20 million cells or a repeat of the initial dose after 6 months. The control group comprised 8 patients who were injected with HA at baseline and after 6 months. Clinical scores and MRI data were evaluated throughout the 12-month follow-up period. No serious adverse effects were reported. Only patients treated with umbilical cord tissue-derived mesenchymal stem cells experienced significant improvements in pain and function from baseline. No significant differences in MRI findings were detected. The research team concluded in a phase I/II trial that repeated treatment with umbilical cord tissue-derived mesenchymal stem cells is safe and superior to other treatment methods after one year of follow-up (Matas *et al.*, 2019).

Our research confirmed the characteristics and functions of mesenchymal cells that have been studied for decades. The population of mesenchymal stem cells has immune regulatory properties, and do not express MHC class I or II, making them amenable to allogenic transplantation without host rejection (Klyushnenkova *et al.*, 2005). Moreover, these cells contain cytokines and chemokines that have immunosuppressive effects on many immune cells, thereby reduceing inflammation (Kwon *et al.*, 2022) and resulting in a noticeable elevation of pain. The major therapeutic efficacy of MSCs is based on their paracrine effect; the cells produce various growth factors, including VEGF, HGF, FGF, and TGB-β, that prevent apoptosis during OA progression (Gupta *et al.*, 2020) while enhancing proliferation and angiogenesis. Chemokines and cytokines also support the process of tissue repair and regeneration, as well as modulating tissue remodeling and inflammation (Mancuso *et al.*, 2019). This is the major difference and the foremost advantage of MSC-based therapy in OA treatment: while the other treatments of corticosteroid and HA injection only soothe inflammation and pain without any impact on OA pathology, the use of MSCs is a regenerative medicine for the restoration of bone tissues and blood vessels. A main concern about MSC therapy is the inconsistency among donors, and cell quality can vary due to the processes of donor screening, sample collection, and cell culture. Our team strictly followed the guidelines of AABB, EC, ISCT, and GMP to manufacture clinically graded MSCs, proving that with a manufacturing standard and a good *in vitro* quality control process, MSC-based therapy is reproducible. Thus, this success is a further quality confirmation of our cell processing method at the clinical level: our MSCs were collected via an explant method that minimizes cellular stress while maximizing the purity and quantity of the cell population, expanded ex vivo and cryopreserved to satisfy all the criteria for clinical-grade MSCs and possess therapeutic power in OA treatment, and potentially in many other cell-based therapies.

CONCLUSIONS

We have successfully established a master stem cell bank that meets the standards for cell-based therapy. Additionally, initial applications for treating knee osteoarthritis have shown promising results. We also propose large-scale treatments and longer evaluation periods to assess the effectiveness of therapy more accurately.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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