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Pathological Evaluation on Microanatomical Level for the Effect of Xenogeneic Stem Cell-Based Therapy on Replacement of Degraded Articular Cartilage in A Rabbit Model of Osteoarthritis

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Abstract: Background: *Many forms of arthritis, and osteoarthritis (OA) is the most common one, leading to physical disability. The capability of both autologous and allogeneic mesenchymal stem cells to regenerate injured articular cartilage in OA has been proven.* ***Objectives:*** *This work aimed to study the effect of xenogeneic stem cells as a treatment for osteoarthritis based on pathological evidence at the microanatomy level.* ***Materials and Methods:*** *Twenty-four (24) male New Zealand white rabbits were used in this work. They were divided into four groups (n=6); the human stem cell-treated group (HSTG), the sodium hyaluronate-treated group (SHTG), the media stem cell-treated group (MSTG), and the normal saline-treated group (NSTG). OA was induced by a single intra-articular injection of monosodium iodoacetate (MIA) 2.5 mg/0.3 ml normal saline (NS). 4 weeks later of OA induction the (HSTG) was given a single intra-articular injection of human umbilical cord-derived Mesenchymal stem cells (UC-MSCs) at a density of $1.5X10^6$ cells / 0.3 ml media, while the (SHTG) was treated with four injections of 0.3 ml 0.1% sodium hyaluronate at weekly intervals starting 4 weeks post OA induction. Lastly, both the (MSTG) & (NSTG) received an injection of the same volume of medium without cells & normal saline, respectively. Rabbits were euthanized by intravenous injection of sodium phenobarbital (Do lethal) 100mg/kg at 20 weeks post-OA induction then pathology images on microanatomy level were assessed.* ***Results:*** *The results revealed that there were significant differences among all groups in pathological scoring on groundbreaking levels of detail in microanatomy for the stifle joints evaluation at week 20 post-OA induction. The HSTG displayed the best pathological recovery outcomes scoring on the microanatomy level. They were followed by SHTG, which is restricted to pain relief, delayed progression of the disease, and improving general mobility. While the MSTG and NSTG exhibited the worst pathological scores.* ***Conclusion:*** *In brief, a single intra-articular injection of xenogeneic stem cells could stimulate the regeneration of damaged articular cartilage in osteoarthritis, as evidenced by improved pathological findings at the microanatomical level. However, the possibility of immune rejection for this type of cell transplantation requires further exploration, which was not noticed in this study.*

Keywords: Osteoarthritis; Stifle joint; Rabbit; Microanatomy; Pathology; Xenogeneic stem cells therapy; Human umbilical cord-derived stem cells.

1. Introduction

Bones that articulate with neighboring bones possess movable synovial joints. It provides mechanical support during weight bearing. It is primarily comprised of chondrocytes embedded within an avascular extracellular matrix [(1)]. These cells are responsible for the synthesis and maintenance of the extracellular matrix and are arranged in four zones [(2)]. The extracellular matrix provides diverse functions in articular cartilage, including providing biomechanical strength [(3)]. Osteoarthritis (OA) is characterized by the progression of degeneration of articular cartilage, and extracellular matrix as well as changes to the surrounding tissue [(4)], OA and rheumatoid arthritis are the most common inflammatory arthritis and are major causes of disability [(5)], severe pain joint, stiffness and significance reduced mobility are the main symptom of OA [(6)], 10-15% of the global population suffers from osteoarthritis [(7)].

Risk factors of OA include obesity, sedentary lifestyle, chronic postural defect, bone density, occupation injury, trauma, and genetics. For adults over age 60, the prevalence is higher among females than males. There is no cure for OA so treatment is focused on relieving symptoms to improve the quality of life [(8)]. Osteoarthritis diagnosis by physical and radiological examination the loss

of cartilage is beastly visualized via radiology. The physical examination can be used to determine how much pain is presented and to what degree has been compromised [9]. The radiography features of OA include osteophytes, subchondral sclerosis, and reduced joint space [10]. Mesenchymal stem cell (MSC) therapy is a treatment option for knee osteoarthritis (KOA) with high expectations. Stem cells are proposed to have anti-inflammatory and immunomodulatory properties [11-16]. It is hypothesized that stem cells can promote cartilage regeneration and consequently can postpone or avoid the need for Total Knee Arthroplasty (TKA) [17].

According to a systematic review of the efficacy of MSC therapy in KOA, we found a positive effect of MSC therapy (2.1–3.4) point improvement on the Visual Analogue Scale (VAS). However, high methodological heterogeneity across studies, and study outcomes being at high risk of bias did not allow for recommending the use of stem cell therapy in clinical practice. Over the past few years, various new randomized controlled trials (RCTs) have become available, making a thorough analysis of the available evidence valuable [18]. In recent years, various new randomized controlled trials (RCTs) have become available, making a thorough analysis of the available evidence valuable [19-23]. Thus, it is evident that previous studies on the management of degenerative joint diseases using autologous stem cells have shown promising results. The successful use of xenogeneic stem cell therapy to replace degraded articular cartilage will provide the opportunity to reduce the cost, time, and effort currently involved in making osteoarthritis treatment feasible in humans and patients. As well as large animals such as sport horses, are susceptible to joint disease.

2. Objectives

This work aimed to investigate the effect of human umbilical cord-derived mesenchymal stem cells (xenogeneic stem cells) therapies as treatment for osteoarthritis to study the healing of the joints and articular cartilage following experimentally induced OA by pathological evidence on microanatomy level.

3. Materials and Methods

3.1. General Preparation of Experimental Animal

The Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, University Putra Malaysia, approved the use of twenty-four (24) male New Zealand white rabbits, aged six months, weighing between 2.0 and 2.5 kg, and were all deemed clinically healthy after physical and blood profile examinations. The rabbits were kept in individual cages, fed a commercial diet (Cargill), and given unlimited access to drinking water. The study took place on April 9, 2010 (Ref. UPM/FRV/PS/3.2.1.551/AUP-R94).

Throughout the research period, a weekly physical examination was conducted to make sure they were in good health. This included taking their respiration rate, pulse, and rectal temperature. To rule out any potential joint disease, radiographs of both stifle joints were performed prior to the induction of degenerative joint disease.

3.2. Experimental Design

Based on the type of treatment they had received, the twenty-four (24) male rabbits were divided into four groups at random: the groups treated with human mesenchymal stem cells (HSTG), stem cell media (MSTG), sodium hyaluronate (SHTG), and normal saline (NSTG) (negative control). There were six animals in each group (n = 6). A single intra-articular injection of monosodium iodoacetate (MIA) was administered to each animal's left stifle joint to cause OA, while the right joint was left unaltered to serve as the uninduced control. According to their groups, the rabbits began therapy four weeks after OA induction and were slaughtered twenty weeks later (sixteen weeks of treatment). Following that, samples were collected for pathological comparisons.

Weekly general medical examinations were performed on the rabbits during the trial, which included checking their respiration rate, pulse, and rectal temperature. Throughout the trial, daily observations were also made on their hunger, skin health, hair, and feces. Following sixteen weeks of therapy, all rabbits were put to death with sodium pentobarbital (Do lethal), which was administered intravenously at a dosage of 100 mg/kg (24). After making incisions in the left and right stifle joints, samples of articular cartilage were taken for histological analysis.

3.3. Isolation of Mesenchymal Stem Cells from Human Umbilical Cord Blood (UC-MSC)

At the Immunology Laboratory of the Faculty of Medicine and Health Science, University Putra Malaysia, human umbilical cord-derived mesenchymal stem cells (UC-MSCs) were generated (25).

From the Britannia Women and Children Specialist Centre, human umbilical cords were gathered. With the help of obstetrics and gynecology experts, the samples were taken at the time of delivery from a full-term pregnancy. According to the guidelines set out by the Faculty of Medicine and Health Sciences University Putra Malaysia's ethics committee, all samples were acquired with written, informed permission. The newly acquired samples were kept and transported at 40°C in a sterile specimen tube filled with DMEM/F12 medium supplemented with 0.5% Fungi-zone (Gibco, Invitrogen), 0.1% Gentamicin (Gibco, Invitrogen), and 1% Penicillin and Streptomycin (Gibco, Invitrogen). Before being processed further, human umbilical cord samples (1-3 inches) were removed, sterilized with 70% alcohol for 30 seconds, and then soaked and cleaned in 1X PBS to get rid of extra blood. The

remaining Wharton's Jelly tissues were chopped and finely minced into paste-like tissues after the blood vessel in the umbilical cord samples was removed. Enzymatic-mechanical dissociation techniques developed at the Immunology Laboratory of the Faculty of Medicine and Health Sciences, University Putra Malaysia, were used to the paste-like tissues of umbilical cord samples (25).

In summary, to break down the collagen matrix, the tissues were combined with 15–20 milliliters of enzyme mixes that included 0.4% Collagenase Type II (Worthington, New Jersey, USA) and 0.01% DNase I (Worthington, New Jersey, USA) in 1X PBS. For 30 minutes, mixtures were incubated at 370C while being stirred at 200 rpm. To terminate the enzymatic process after incubation, an equivalent amount of MSC complete culture medium was added to the mixtures. For a higher yield of single cell suspension, the mixtures were subsequently mechanically homogenized using a hand-held cell homogenizer (Hassen Wagger) at 9000 rpm for ten to fifteen minutes. To remove extra tissue, the homogenized mixtures were filtered through Becton Dickinson 70 µm and 40 µm cell strainers. After collecting the supernatants, they were centrifuged for ten minutes at 1500 rpm. After being resuspended, the cell pellet was once again cleaned in 1X PBS for ten minutes at 1500 rpm. The cell pellet was resuspended in two milliliters of MSC complete medium after the supernatant was disposed of.

Following the application of trypan blue dye exclusion cell count to the single-cell suspensions, around 1.0 x106 cells were seeded onto a T25 tissue culture flask (Nunc Brand Products). After adding 40 ng/ml of basic fibroblastic growth factor (bFGF) (Promega, USA) to the initial cultures, they were incubated for at least a week at 370C with 5% CO2 humidification. Later, non-adherent cells were eliminated by swapping out the medium. Every three days, the medium was replaced and the main cultures' progress was tracked. Primary adherent cultures at P0 were subcultured into a T75 flask after being subjected to a 0.05% trypsin-EDTA solution (Invitrogen, BRL, Canada) for five minutes at 370C when they had reached 80–90% confluence. The extra cells were then cryopreserved in freezing medium (10% DMSO, 90% fetal bovine serum) and kept for further passages or further downstream studies.

3.4. Experimental Study

3.4.1. Induction of Osteoarthritis

Prior to the trial, all rabbits were acclimated for two weeks. Based on blood indicators and physical tests, the rabbits were deemed healthy. The results of the radiological and physical exams indicated that the stifle joints were normal. Ketamine hydrochloride, xylazine hydrochloride, and acepromazine were injected intramuscularly into rabbits to induce anesthesia at doses of 40 mg/kg, 5 mg/kg, and 1 mg/kg, respectively. Heart rate, respiration rate, ocular and intradigital reflexes, and stimulus response were used to gauge the level of anesthesia. As is customary, the skin was aseptically treated with tincture iodine, 70% alcohol, and chlorhexidine scrub after the hair above the left stifle joint was trimmed. 2.5 mg MIA and 0.3 ml NS were injected intra-articularly using a 26-gauge, 1½-inch hypodermic needle (26,27).

The needle was advanced between the menisci and femoral epicondyles after being positioned in the midline (Figures 1 & 2). Before trying to deliver the MIA, the needle was repositioned if there was any resistance to injection, which was seen as proof that the needle was not entering the joint cavity. When the injection was finished, the needle was removed. Care was taken to ensure that the needle tract showed no signs of leaking.

Figure 1: Intra-articular injection of MIA into the rabbit stifle joint

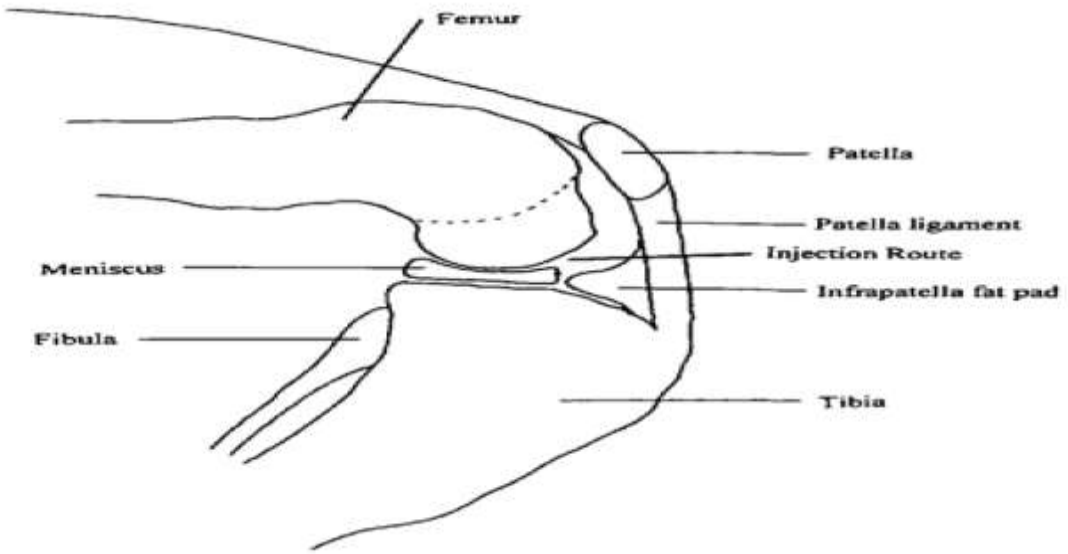

Figure 2: Stifle joint diagram illustrating the intra-articular injection route

3.4.2. Protocol of Treatment

In an MIA-induced model of OA in a rabbit's stifle joint, the potential of the current therapeutic approaches employing xenogeneic MSCs to repair OA-damaged tissues was investigated. In this investigation, four treatment groups were employed. A single intra-articular injection of human umbilical cord-derived MSCs at a density of 1.5X106 cells/0.3 ml medium was administered to the first group, the human stem cells treatment group (HSTG), four weeks after OA induction (the cells were in the fourth passage). The osteoarthritic stifle joints of the second group, known as the media stem cell-treated group (MSTG), were injected with an identical volume of cell-free medium.

Beginning four weeks after OA induction, the third group, known as the sodium hyaluronate-treated group (SHTG), received four weekly injections of 0.3 ml 0.1% sodium hyaluronate. Finally, a single intra-articular injection of 0.3 ml NS was administered to the afflicted stifle joints to the fourth group, the NS-treated group (NSTG). Note: Throughout the whole research period, all rabbits had 10 minutes of exercise each day, with the exception of the first month following the start of treatments, during which time they were given a break.

3.5. Histopathology Evaluation

Both legs' stifle joints were decalcified using EDTA + 12% hydrochloric acid for approximately one month after being preserved in 10% formalin for around two months. Twice a week, the decalcification solution was replaced. Both tibia and femur samples were initially divided into medial and lateral sections before being further split into two sections. Using an automated tissue processing device, the samples were dehydrated using a succession of increasing ethanol concentrations, followed by xylene clearing and paraffin impregnation. Hematoxylin-Eosin (H and E) and Safranin O stains were applied to the slides after they had been sectioned using a microtome following paraffin blocking. After that, a microscope image analyzer (OLYMPUS) was used to take histology pictures.

Following staining with H and E dye, histopathological alterations of the articular cartilage and subchondral bone were assessed using the scoring method outlined by Kobayashi et al.28. The following was a brief breakdown of the degree of change: 0 for no change (normal), 1 for little change (mild), 2 for moderate change (moderate), and 3 for severe change (severe or very severe). Chondrocyte cell loss, cloning and hypertrophy, disorganization, articular cartilage surface irregularity, articular cartilage surface fibrillation, decrease in Safranin O stain, degeneration/necrosis, marginal osteophyte formation, and subchondral changes were among the items graded (Table 1).

Table 1: Histological Grading Scale

Grading Scales Histological OA feature	Grade (0)	Grade (1)	Grade (2)	Grade (3)
Chondrocyte Loss	Normal	Mild	Moderate	Severe
Chondrocyte Cloning & Hypertrophy	Normal	Mild	Moderate	Severe
Chondrocyte Disorganization	Normal	Mild	Moderate	Severe
Surface Irregularity of Articular Cartilage	Normal	Mild	Moderate	Severe
Fibrillation of Cartilage Surface	Normal	Mild	Moderate	Severe
Safranin O Stain Reduction	Normal	Mild	Moderate	Severe
Degeneration/Necrosis	Normal	Mild	Moderate	Severe
Marginal Osteophyte Formation	Normal	Mild	Moderate	Severe
Subchondral Changes	Normal	Mild	Moderate	Severe
Total Histological OA Score	0 – 27			

Normal (no alterations) means that there are no OA lesions in the subchondral bone and articular cartilage. Mild alterations indicate that less than 50% of the articular cartilage or subchondral bone is altered. About 50% of the articular cartilage or subchondral area was impacted, according to moderate alterations. Significant alterations revealed histological alterations in over 50% of the subchondral or articular cartilage.

3.6. Statistical Analysis

Utilizing SPSS version 16 for Windows, the study's data was analyzed utilizing both parametric and nonparametric statistical techniques as well as descriptive statistics. It was deemed statistically significant when the P value was less than 0.05. The data was presented as either the standard deviation (SD) of the mean or the mean ± standard error (SEM). Weight measures, cytokine levels, mass, and the size of the quadriceps femoris muscles were all subjected to a one-way ANOVA and a post hoc test (Tukey). All groups were subjected to Kruskal-Wallis and Mann Whitney (one tail) testing for histological, radiological, and gross lesion assessment.

4. Results

4.1. Histopathology Assessment

Twenty weeks following OA induction (16 weeks after the administration of various therapies), a histopathological study was performed on stifle joints. Under a 200µm powered objective, samples of the right (normal) joint revealed a smooth articular cartilage surface with a layer of flattened chondrocytes beneath in the tangential zone. While the subchondral bone showed a normal distribution of trabeculae made up of osteocytes and canaliculi enclosing bone marrow packed with blood-forming factors, chondrocytes were typically distributed in parallel rows in the transitional and radial zones of the articular cartilage. Furthermore, Safranin O fast green stain was applied evenly and deeply to the intercellular matrix in the non-calcified portion (the area between the articular surface and the tidemark) and less so in the calcified portion (figure 3).

Degeneration, necrosis, marginal osteophyte formation, cloning and hypertrophy of chondrocytes, chondrocyte disorganization, surface irregularity of articular cartilage, fibrillation of articular cartilage surface, decreased intensity of Safranin O stain, and loss of chondrocyte cells were all observed in the histopathological observations for the left OA stifle joint (articular cartilage and subchondral changes). The following is a description of these observations based on the various groups: (1) HSTG: After staining with H and E stain, seen under a 200µm powered objective, the left (OA) articular cartilages and subchondral bones of the femoral condyle and tibial plateau showed mild to moderate fibrillation and chondrocyte loss in the tangential zone. Along with modest to moderate chondrocyte colonies, hypertrophy, necrosis, and disarray, it also had mild to moderate cellular loss in the tangential and radial zones. In addition, no pathological alterations were found in the subchondral bone. Additionally, the intercellular matrix's staining with Safranin O fast green stain showed a considerable decrease. The combination of the femoral condyle's 11.17 ± 0.36 and the tibial plateau's 12.66 ± 0.30 histopathological scores for this group came to 23.83 ± 0.66 (figure 4; Tables 2 and 3).

(2) SHTG: Viewed using a 200µm powered objective, the left (OA) articular cartilages and subchondral bones of the femoral condyle and tibial plateau showed moderate to severe fibrillation and chondrocyte loss in the tangential zone following staining with H and E stain. Along with moderate to severe chondrocyte colonies, hypertrophy, necrosis, and disarray, there was also moderate to severe cellular loss in the tangential and radial zones. In addition, subchondral structures displayed certain alterations, such as the substitution of fibrous tissue for bone marrow components and the development of cysts in some joints. Additionally, it showed a

moderate to severe loss of intercellular matrix staining with Safranin O fast green stain. The combination of 19.00 ± 0.22 (femoral condyle) and 20.83 ± 0.23 (tibial plateau) yielded a total histopathological score of 39.83 ± 0.45 for this group (figure 5; Tables 2 and 3).

(3) MSTG: Following staining with H and E stain, visible at a 200µm powered objective, the left (OA) articular cartilages and subchondral bones of the femoral condyle and tibial plateau showed severe to very severe fibrillation and chondrocyte loss in the tangential zone. Additionally, it had severe to very severe chondrocyte colonies, hypertrophy, necrosis, and disarray along with a considerable cellular loss in the tangential and radial zones. In addition, there was marginal osteophyte formation, minor subchondral cyst formation, and mild subchondral fibrosis in the subchondral bone. Additionally, the intercellular matrix's Safranin O staining was severely to extremely severely lost. The sum of 24.51 ± 0.13 (femoral condyle) and 25.65 ± 0.09 (tibial plateau) yielded a total histopathological score of 50.16 ± 0.22 for this group (figures 6 and 8; Tables 2 and 3).

(4) NSTG: Severe to very severe fibrillation and chondrocyte loss in the tangential zone were identified in the left (OA) articular cartilages and subchondral bones of the femoral condyle and tibial plateau under a 200µm powered objective following staining with H and E stain. Additionally, it had severe to very severe chondrocyte colonies, hypertrophy, necrosis, and disarray along with a considerable cellular loss in the tangential and radial zones. In addition, there were certain alterations in subchondral structures, such as the substitution of fibrous tissue for bone marrow components. In several joints, there was marginal osteophyte production along with concomitant cyst formation. It also had a significant to extremely severe loss of intercellular matrix Safranin O staining. The sum of 24.66 ± 0.16 (femoral condyle) and 26.32 ± 0.11 (tibial plateau) yielded a total histopathological score of 50.98 ± 0.27 for this group (figures 7 and 9; Tables 2 and 3). Overall, the average histological findings showed significant differences ($P < 0.05$) between the various treatment groups.

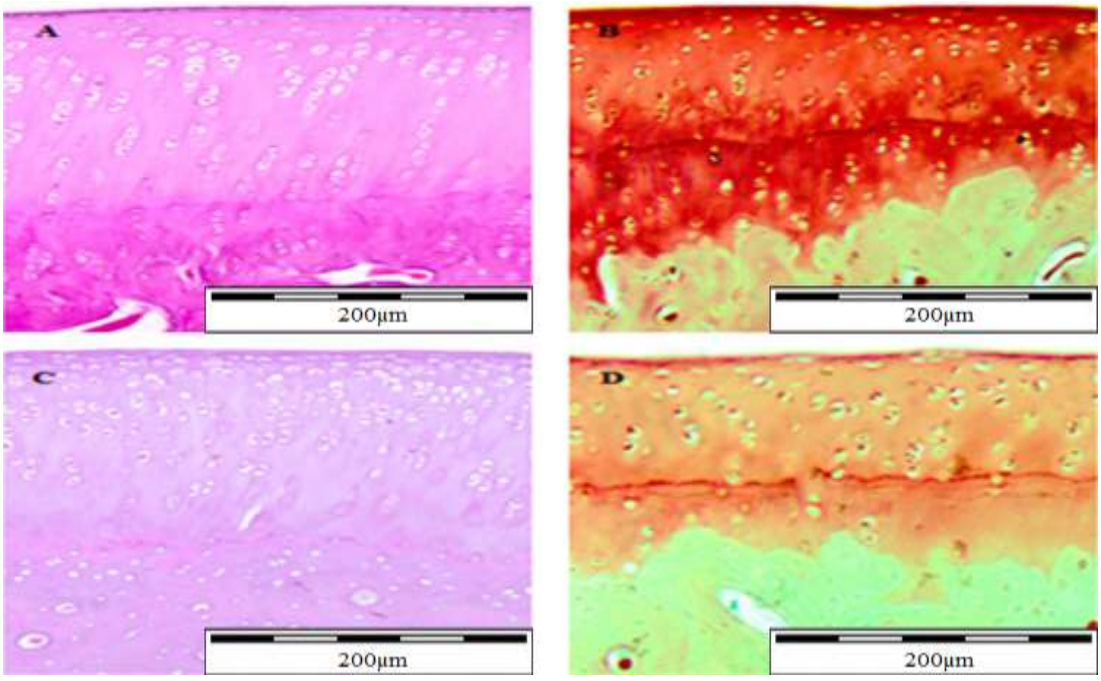

Figure 3: Under a 200 µm powered objective, the right (normal) articular cartilages and subchondral bones of the tibial plateau and femoral condyle were visible. (A) Femoral condyle H and E staining; (C) Tibial plateau H and E staining. In both A and C, the tangential zone's chondrocytes were flattened beneath a smooth articular cartilage surface. In the transitional and radial zones, chondrocytes were arranged in parallel rows. The subchondral bone showed no signs of pathological alteration. (B) Femoral condyle staining with Safranin O; (D) Tibial plateau staining with Safranin O. The extracellular matrix staining in B and D was consistently normal.

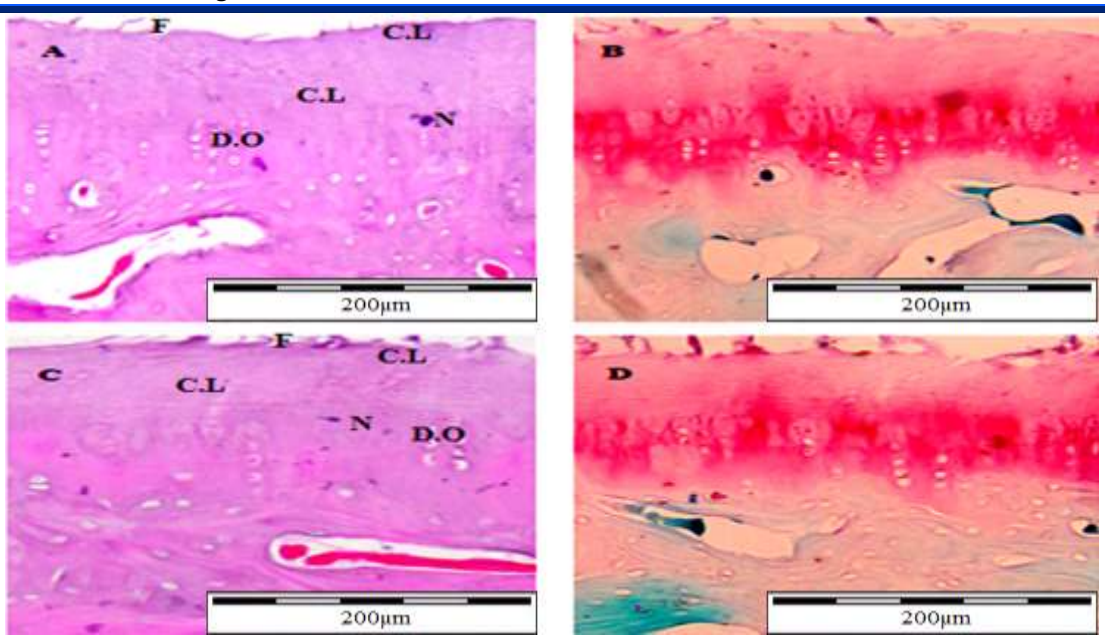

Figure 4: The femoral condyle and tibial plateau's left (OA) articular cartilages and subchondral bones were seen using a 200 µm powered objective on the HSTG. The tangential zone showed modest to moderate fibrillation (F) and chondrocyte loss (C.L) in both A and C. (A) Femoral condyle H and E staining; (C) Tibial plateau H and E staining. They also displayed mild to moderate necrosis (N), chondrocyte disorganization (D.O.), and mild to moderate cellular loss (C.L.) in the radial and transitional zones. Aside from it, the subchondral bone showed no signs of pathological alteration. Both B and D showed a moderate decrease of intercellular matrix staining. (B) Femoral condyle staining with Safranin O; (D) Tibial plateau staining with Safranin O.

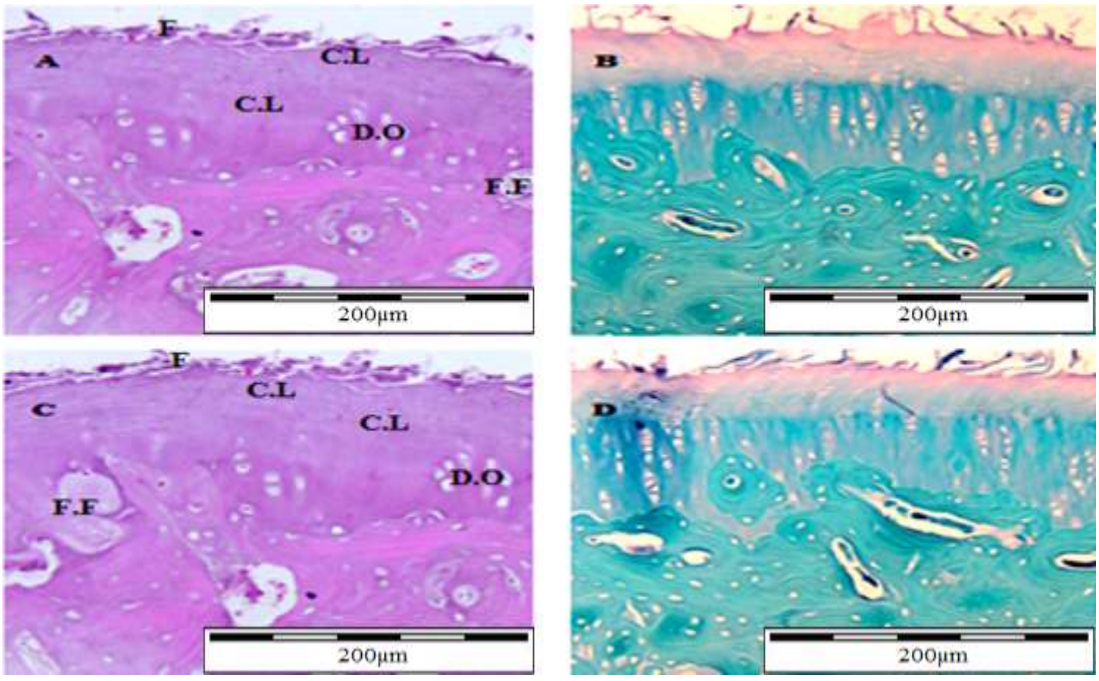

Figure 5: Under a 200 µm powered objective, the left (OA) articular cartilages and subchondral bones of the femoral condyle and tibial plateau of the SHTG were visible. Both A and C showed moderate to severe fibrillation (F) and chondrocyte loss (C.L) in the tangential zone. (A) Femoral condyle stained with H and E; (C) Tibial plateau stained with H and E. Additionally, they displayed considerable chondrocyte disorganization (D.O.) and cellular loss (C.L.) in the radial and transitional zones. In addition, there was minimal subchondral fibrosis development (F.F.) in the subchondral bone. (B) Femoral condyle staining with Safranin O; (D) Tibial plateau staining with Safranin O; both B and D showed moderate to severe intercellular matrix staining loss.

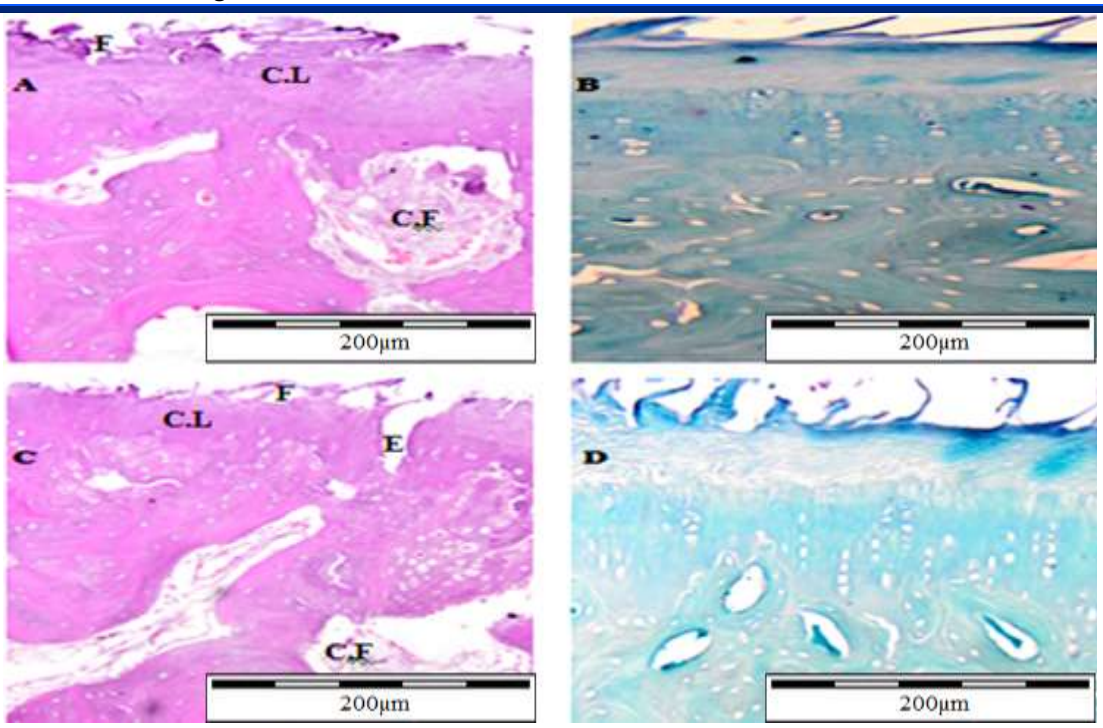

Figure 6: Under a 200 µm powered objective, the left (OA) articular cartilages and subchondral bones of the femoral condyle and tibial plateau of the MSTG were visible. (A) Femoral condyle H and E staining; (C) Tibial plateau H and E staining; both A and C showed extremely severe fibrillation (F). Additionally, they had tangential, transitional, and radial zones of severe to total cellular loss. In addition, there was significant fibrosis and cyst development in the subchondral bone (C.F). (B) Femoral condyle staining with Safranin O; (D) Tibial plateau staining with Safranin O; both B and D showed a significant to total reduction of intercellular matrix staining.

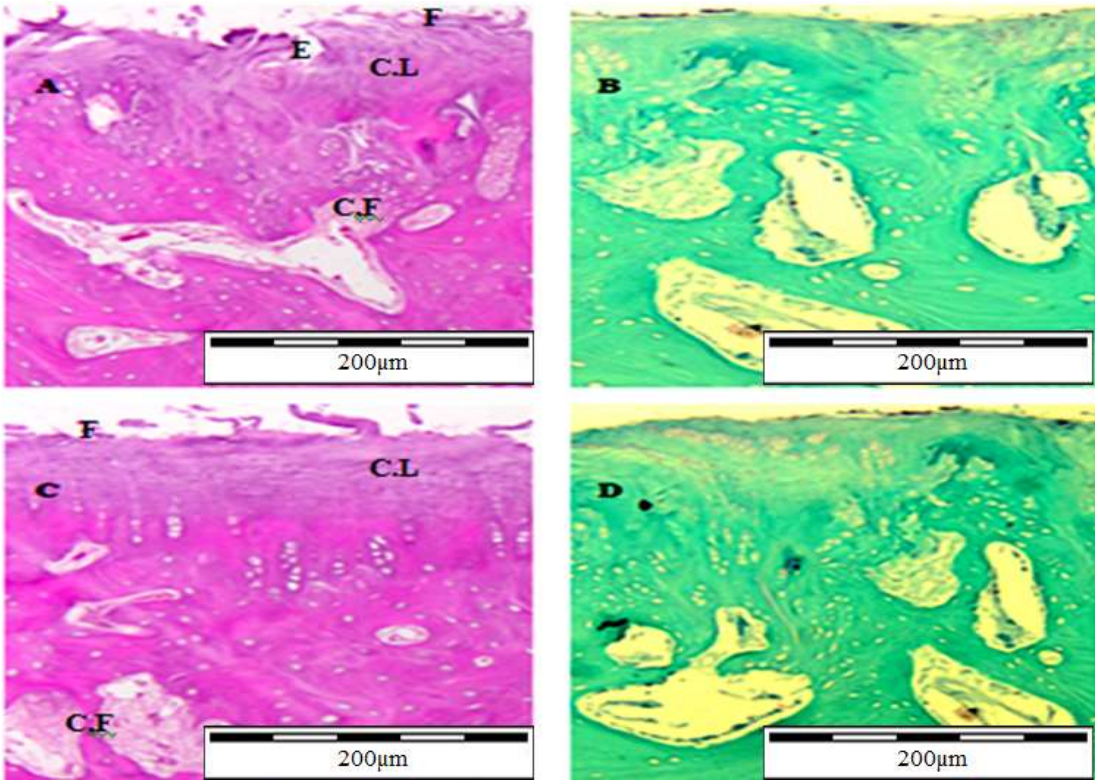

Figure 7: The NSTG's left (OA) articular cartilages and subchondral bones of the tibial plateau and femoral condyle were seen using a 200 µm powered objective. (A) Femoral condyle stained with H and E; (C) Tibial plateau stained with H and E; both A and C showed extremely severe erosion (E) and fibrillation (F). Additionally, they had tangential, transitional, and radial zones of severe to total cellular loss. In addition, there was significant fibrosis and cyst development in the subchondral bone (C.F). (B) Femoral condyle staining with Safranin O; (D) Tibial plateau staining with Safranin O; both B and D showed a significant to total reduction of intercellular matrix staining.

Table 2: Histopathology scoring of left (OA) stifle joint (Femur) for different groups at week 20

Observations	HSTG	SHTG	MSTG	NSTG
Average Chondrocyte Loss	1.67	2.17	2.67	2.83
Average Chondrocyte Cloning & Hypertrophy	1.33	2.33	2.83	2.50
Average Chondrocyte Disorganization	1.83	2.17	2.67	2.83
Average Surface Irregularity of Articular Cartilage	1.17	2.00	2.50	2.67
Average Fibrillation of Cartilage Surface	1.50	1.83	2.67	2.83
Average Safranin O Stain Reduction	1.17	2.33	3.00	3.00
Average Degenerative/Necrosis	1.00	2.17	2.83	2.67
Average Marginal Osteophyte Formation	0.67	2.33	2.67	2.50
Average Subchondral Changes	0.83	1.67	2.67	2.83
Total Averages Pathology Scores ± Std	11.17± 0.36	19.00± 0.22	24.51± 0.13	24.66± 0.16

1: Mild, 2: Moderate, 3: Severe

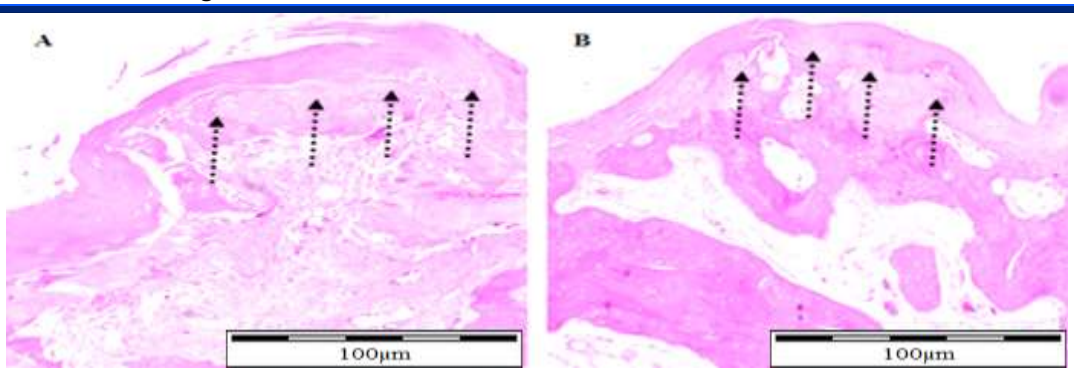

Figure 8: Picture of marginal osteophyte formation on the femoral condyle (A) and tibial plateau (B) of the MSTG observed under 100 µm powered objective.

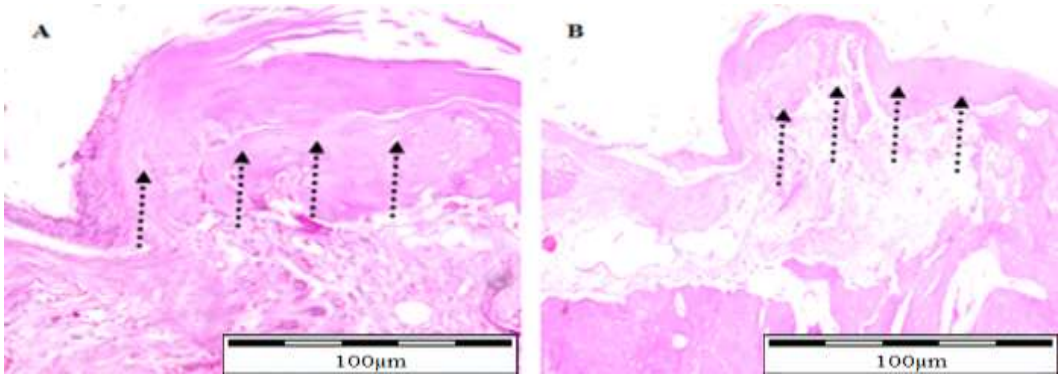

Figure 9: Picture of marginal osteophyte formation on the femoral condyle (A) and tibial plateau (B) of the NSTG observed under 100 µm powered objective.

Notes: Osteophytes are non neo plastic osteo-cartilaginous protrusions growing at the margins of OA joints (black arrows).

Table 3: Histopathology scoring of left (OA) stifle joint (Tibia) for different groups at week 20

Observations	HSTG	SHTG	MSTG	NSTG
Average Chondrocyte Loss	1.83	2.33	2.83	3.00
Average Chondrocyte Cloning & Hypertrophy	1.33	2.50	3.00	2.83
Average Chondrocyte Disorganization	2.17	2.33	2.83	3.00
Average Surface Irregularity of Articular Cartilage	1.33	2.17	2.67	2.83
Average Fibrillation of Cartilage Surface	1.67	2.17	2.83	3.00
Average Safranin O Stain Reduction	1.33	2.50	3.00	3.00
Average Degenerative/Necrosis	1.17	2.33	2.83	2.83
Average Marginal Osteophyte Formation	0.83	2.67	2.83	2.67

Average Subchondral Changes	1.00	1.83	2.83	3.00
Total Averages Pathology Scores ± Std	12.66± 0.30	20.83± 0.23	25.65± 0.09	26.32± 0.11

1: Mild, 2: Moderate, 3: Severe

5. Discussion

Osteoarthritis (OA) is a condition characterized by cartilage deterioration and synovitis, 10-15% of the global population suffers from this condition [7].

The present work on the histopathological observations in stifle joints indicated that there were significant differences among the different groups after 20 weeks of OA induction (16 weeks after the start of treatments). The right normal joints revealed no detectable histopathological changes in the joint structures (articular cartilage and subchondral changes), and there were no significant differences among the different groups. Histopathological changes in the left OA-induced stifle joint were detected and were significantly differences among different groups after 20 weeks of OA induction.

The results of the current study showed that the xenogeneic stem cell-treated rabbits (HSTG) demonstrated slow progression of OA compared to controls, and showed mild to moderate histopathological changes in the joint structures. This is consistent with previous studies reported that mesenchymal stem cells (MSCs) have great potential for bone tissue engineering and regenerative therapy due to their capacity for self-renewal and differentiation [29, 30]. MSCs also restrain the onset of inflammation, which leads to reduced cartilage lesions. MSCs were found to limit the breakdown of proteoglycan in the cartilage of a rabbit arthritis model by decreasing the production of tumor necrosis factor (TNF-α) and MMP-1 [31]. MSCs release growth factors such as IL6 and TGF beta leading to the suppression of apoptosis and fibrosis [32].

A previous study conducted that Rabbit synovial fluid and joints treated with Xenogeneic (human) umbilical cord blood MSCs (HUCB-MSCs) showed reduced inflammation and improved proteoglycan and collagen type 2 production and structure [33].

A study was done on the rat model of kOA induced by ACLT to investigate the effects of therapies of Human adipose-derived MSCs (hADSCs) and human umbilical cord-derived MSCs (hucMSCs) on KOA. The results showed that hADSCs and hucMSCs exerted chondrogenic potential and significantly attenuated the development of kOA in rats [34].

Clark, *et al* reported that a patient with severe knee OA showed positive results after a single-dose injection of BM-MSCs in the absence of supplemental drugs [35].

Another previous studies, also consistent with the present work, MSCs the combination therapy of MSCs and chondrogenic factors as Small molecule drugs, including kartogenin (KGN) which could induce MSC chondrogenesis in a dose-dependent pattern, and could also improve the production of the chondrogenesis-related protein of MSCs, including Col II and aggrecan [36], and Cartilage-inducing factors as bone morphogenetic proteins (BMPs) demonstrated fascinating roles in MSC-based therapies for cartilage regeneration and recruit endogenous MSCs to the injured area to stimulate the repair process of cartilage [37], the transforming growth factor (TGF) superfamily are also a member of Cartilage-inducing factors as TGF-β is essential for the regulation of MSC differentiation in the process of cartilage repair [38].

However, many engineering pathways are used to enhance cell retention and survival, MSCs can be implanted in a scaffold, encapsulated, or injected in combination with other anti-inflammatory and pro-chondrogenic factors [39], and can regulate the chondrogenic differentiation of Stem Cells by miRNAs [37].

In the present study, sodium hyaluronate (SHTG) showed reduced OA progression compared with control and revealed moderate to severe histopathological changes in the joint structures likely as a result of sodium hyaluronate's ability to decline OA progression in affected OA stifle joint structure. Recently, a study explored the effect of growth hormone in OA therapy compared to the effect of hyaluronic acid in rabbit models of OA. GH showed better clinical, macroscopic, and microscopic results as compared to HA and placebo [40]. Another study reported that intra-articular growth hormone injection and hyaluronic acid have demonstrated encouraging outcomes in cartilage regeneration and repair in knee osteoarthritis [41]. Intra-articular injections of hyaluronic acid are as effective as NSAIDs with fewer systemic adverse events; this therapy has a delayed onset of action in comparison with intra-articular corticosteroids, but a longer-lasting benefit [42]. Tusoun, *et al* suggested that a single injection of sodium hyaluronate-chondroitin sulfate in patients with lateral epicondylitis offers better pain benefits for 6 months after injection than intra-articular corticosteroids [43].

The main aim of non-modifying osteoarthritis drugs is controlling symptoms, especially pain [44], a medico-economic evaluation of Intra-articular injections of hyaluronic acid showed that, together with clinical benefits, costs of knee osteoarthritis decreased due

to the decreased need for other treatments. Intra-articular injection of exogenous HA would increase the viscosity of synovial fluid and improve lubrication by forming a layer on the surface of cartilage, in addition to having a function of shock absorption for trauma directed towards the joint and protective cartilage [(42)]. However, Lang *et al*, reported that BMSCs plus HA had a more prominent therapeutic effect on cartilage defects in canines when compared to the HA alone [(45)].

In the current study, the left stifle joint induced with OA from the MSTG or NSTG showed increased severity of OA progression and revealed severe to very severe microscopic appearance in joint structures. Likely, both treated groups did not respond to the treatments, which resulted in the progression of OA with severe consequences. These findings are consistent with previous studies in which severe histopathological lesions of OA were observed in the stifle joint injected with normal saline only [(45, 46, & 47)].

In general, the histopathology results for articular cartilage and subchondral bone from the current study indicate that the xenogeneic therapy compared to other treatments has the best therapeutic effect on OA, followed by sodium hyaluronate, which showed delayed progression of OA, while the media of stem cells and normal saline showed the most severe histopathological changes in the articular and subchondral cartilage. These appear to mirror findings from previous research [(47)].

Generally speaking, with the continued development of xenogeneic stem cell transplantation, many studies have reported that it has considerable therapeutic effects in many diseases [(48, 49)], although the potential problems associated with xenogeneic stem cell transplantation as immunological incompatibility, cell death, abnormal cell differentiation and proliferation, viral transmission from animals to humans, research carried out to resolve these problems [(49)].

6. Conclusions

Cell approaches and tissue engineering as therapies for different diseases will raise hope for using xenografts for the treatment of incurable diseases soon. The results of the current study will likely add to this growing pool of potential.

The histopathological evaluation of the stifle joints after 20 weeks of OA induction (16 weeks after the start of different treatments) revealed that there were no histopathological changes detected in the right (normal) joints, but they were detected in the left OA stifle joints (articular cartilage and subchondral changes of the femoral condyle and the tibial plateau). There was a significant difference in histopathological scoring among all the groups.

Overall, in our experimental study, the histopathological scores for articular cartilage and subchondral bone of the stifle joints indicated that the treatment with human UM-MSCs was the most effective therapy, followed by evidenced by significant improvements in the histopathological scores. Sodium hyaluronate therapy did not do as much as the latter 2 and showed improvements in rabbit body weight and sizes of the joints while other parameters showed delayed progressions of OA. Both media without cells and normal saline treatments showed the most severe pathological changes in the key parameters and proved that these agents had no remedial effect on degenerative joint disease (OA).

7. Recommendations

Based on the current study that was carried out to evaluate the usefulness of human UM-MSCs therapy (xenogeneic stem cells) in the treatment of OA, there was evidence of enhanced replacement of the degraded articular cartilage due to the stem cells therapies but the probability of immune rejection for xenogeneic cell transplantation needs more exploration. Further studies can be conducted to elucidate the mechanisms of the effects of stem cells in cartilage repair and to determine the fate of the injected stem cells in vivo, as well as to provide broader interpretations for the effects of cell-based therapies on OA.

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