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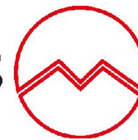

A Histopathological Study of the Therapeutic Effect of Mobilized Intrinsic Stem Cells versus Locally-Injected Stem Cells in Osteoarthritis Knee Joint in White Mice

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Abstract: Background: Osteoarthritis (OA) is a degenerative joint disease involving degradation of articular cartilage and subchondral bone. Mesenchymal stem cells (MSC) are pluripotent cells found in multiple human adult tissues, including bone marrow, synovial tissues, and adipose tissues. Because of their multi-potent capabilities, MSC lineages have been used successfully in animal models to regenerate articular cartilage and in human models to regenerate bone. The number of MSCs that can be isolated from bone marrow is fairly limited. As a result, most research in cartilage regeneration has focused on the use of culture expanded cells. **Aim:** This research was carried out to assess the therapeutic efficacy of the use of growth factors (stem cell factor) as mobilizing factor son stem cells (both intraarticular and intravenous) in white mice in comparison to the effect of direct intra-articular mesenchymal stem cell transplantation (of Wharton's Jelly and umbilical cord blood) on healing of osteoarthritic lesions of knee joint in white mice. **Material and Methods:** This study was performed on 100 adult male rats. OA was induced using insulin syringes for osteotomy in 80 rats bilaterally in both knee joints and 20 rats were left without induction of OA as normal healthy control group. There were 3 groups composed of 80 rats (A, B and C). Group A was 40 rats injected by SCF and subdivided into subgroup A1 composed of 20 rats were injected intra-articular and group A2 composed of 20 rats were injected intravenous. Group B composed of 20 rats were injected intra-articular by the MSC that subdivided into subgroup B1 including 10 rats injected by MSC obtained from human umbilical cord blood and subgroup B2 injected by MSC obtained from human umbilical cord Wharton's jelly. Group C was 20 untreated rats used as untreated control group. 12 weeks later, all rats were sacrificed by intraperitoneal injection of toxic dose of thiopental sodium. Histopathological assessment was done to illustrate pathological changes to induced knee OA and assess response to MSCs and SCF therapies by different routes. **Results:** SC WJ treated group showed best therapeutic response to cartilage cell grade, subchondral bone stage and grade. SC CB treated group show only good response to this therapy as regards cartilage stain grade only, SCF IV treated group showed best therapeutic response to therapy as regards cartilage cell grade and synovium vascular grade. SCF IC treated group responded well to cartilage stage only. **Conclusion:** Improvement to OA with different therapies (MSCs and SCF therapies) occurred in all treated groups with best the rapeutic response to therapy in SC WJ treated group followed by SCF IV treated group.

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Abbreviations: MSCs (Mesenchymal stem cells) CB (cord blood), WJ (Wharton's jelly), SCF (Stem cell factor, IC (intraarticular), IV (intravenous), ADAMTS (A-disintegrin and metalloproteinase with thrombospond in motifs, FGF (fibroblast growth factor).

1. Introduction:

Osteoarthritis (OA) is the most prevalent chronic joint disease and a frequent cause of joint pain, loss of

function and disability. Knee is the most common affected joint. Articular cartilage damage is an important, prevalent and unsolved clinical issue **(Johnstone et al, 2013).** Current treatments for knee OA achieve poor clinical results and fail to modify cartilage with the joint replacement is the last treatment option, bearing enormous effort and expanses**.** Mesenchymal stem cells open new therapeutic perspectives provided their regenerative potential and ability to modulate inflammation. Adult stem cells are stem cells which maintain and repair the tissue in which they are found. Bone marrow transplant is a crude form of stem cell therapy that has been used clinically for many years without controversy. No stem cell therapies other than bone marrow transplant is widely used **(Uth and Trifonov, 2014).**

Mesenchymal stem cells (MSCs) subsequently have been isolated from a variety of other tissues (rather than bone marrow) such as adipose tissue, placenta, umbilical cord and cord blood, dental pulp, and amnion. However, the ability of MSCs isolated from these tissues to form cartilage is currently being examined rigorously **(Hass et al., 2011).** They offer new insights into the biology of progenitor cells and may be relevant in the development of novel therapeutic approaches for a cell-based therapy for late stages of osteoarthritis ***(*Koelling S. and Miosge N. 2009)**.

Transforming growth factor (TGF)-β-induced chondrogenesis of mesenchymal stem cells derived from bone marrow involves the rapid deposition of a cartilage-specific extracellular matrix. ***(Barry et al., 2001)***. Stem cell factor (SCF) (also known as KIT-ligand or steel factor) had a role to keep hematopoietic stem cells (HSCs) remain in the niche by adhering to ECM (extracellular matrix) proteins and to the stromal cells themselves and thus play a large role in ensuring that HSCs remain in the niche ***(Méndez-Ferrer et al., 2008)***. HSCs have also been shown to migrate towards a higher concentration gradient of SCF in vitro, which suggests that SCF is involved in chemotaxis for these cells ***(Bowie et al., 2007)***. Fetal HSCs are more sensitive to SCF than HSCs from adults. In fact, fetal HSCs in cell culture are 6 times more sensitive to SCF than adult HSCs based on the concentration that allows maximum survival ***(Bowie et al., 2007)***. Research is underway to develop various sources for stem cells and to apply stem cell treatments for neurodegenerative diseases, diabetes, heart disease, and other conditions ***(Bubela et al., 2012)***.

Aim: To assess the therapeutic efficacy of the use of growth factors (stem cell factor) as mobilizing factors on stem cells in white mice in comparison to the effect of direct intra-articular mesenchymal stem cell transplantation on healing of osteoarthritic lesions of knee joint in white mice.

2. Material and Methods:

This study was carried out on 100 adult male albino rats of Wistar strain housed in the Animal Facility of Medical Research Center, Faculty of Medicine, Ain Shams University. At the start of the experiment, the animal house staff detected the approximate age and weight of the rats (4 to 6 months age and 200 to 250 gm weight) respectively. The animals were housed in groups of ten in stainless steel cages (34×47×18 cm^3) with soft wood shavings as bedding under constant laboratory conditions (temperature 24-28°C, relative humidity 60-70 % and 12 hours dark-light cycles). They were fed regularly with no specific diet and with free access to water and libitum. All rats were apparently healthy at the time of starting the experiment with no evident joint problem (no swollen joints, limping, deformities or aggressive behavior denoting pain). They were left 2 days before starting induction of arthritis to be adapted to the place.

Induction of arthritis:

Arthritis was induced bilaterally in the knee of80 rats (with 20 / 100 rats used as healthy control) by mechanical trauma (Arthrotomy) done by using needle of insulin syringes inserted in knee joints of rats under anesthesia that is induced by ether **(Erdem et al., 2011)**. The left knee is considered as untreated self-clinical control for each rat. The Right Knee is affixed as target for research where intra-articular injections of growth factors and MSCs, histopathological preparation of paraffin sections, are examined in this knee. Clinical assessment was done 3 weeks after induction of trauma in all rats to ensure induction of arthritis.

Injection of the animals:

The rats were divided into three groups as follows: **(Erdem et al., 2011)**. Group (A): Composed of 40 Westar strain, albino, adult male rats with mechanically-induced osteoarthritis. Stem Cell factor (SCF) was injected according to weight of the mouse (dose of SCF is 50ug/Kg). This group is subdivided into 2 subgroups: Subgroup A1: composed of 20 rats that were injected intra-articular with SCF in the right knee Subgroup A2: composed of 20 rats that were injected intravenous with SCF in tail **(Herodin et al., 2003).** Follow up of SCF intravenously injected rats will be carried out by detection of CD34+ stem cells in peripheral blood sample, by flowcytometry done from K3-EDTA anti-coagulated blood obtained from retro-orbital venous plexus". **(Horie et al., 2009)**. Group (B): Composed of 20 white rats with induced osteoarthritis subjected to injection of mesenchymal stem cells cultured in vitro with TGF-beta

(Transforming Growth Factor-beta). This group is subdivided into 2 subgroups: Subgroup B1: composed of 10 rats that were injected intra-articular with stem cells obtained from human umbilical cord blood's buffy coat (vein blood) and Subgroup B2: composed of 10 rats that were intra-articular injected with stem cells obtained from human umbilical cord Wharton's Jelly. **(Horie et al., 2009). Group (C):** Composed of **20** white rats with induced osteoarthritis by arthrotomy, left without interference as control group.

Preparation of Stem Cells: Umbilical cord blood (UCB) samples collection:

50 ml hUCB were withdrawn by milking from the umbilical vein and collected in sterile 15 ml Falcon tubes containing 2 milliliters of Acid Citrate Dextrose (ACD) anticoagulant **(Eichler et al., 1999)** were collected from placenta of full-term deliveries by caesarean sections. The samples were stored at 22 ±4 °C before processing **(Eichler et al., 1999).** Under complete asepsis within the laminar air flow, each cord blood sample was diluted 1:1 with phosphate buffered saline (PBS) and was carefully loaded onto Bicol separating solution 2:1 ratio. After density gradient centrifugation at 2000 rpm for 30 minutes at room temperature, mononuclear cells (MNCs) were carefully aspirated with a Pasteur pipette from the interphase using fishing technique and were washed three times with PBS; each time were centrifuged at 1500 rpm for five minutes, a clear cell pallet was formed in the bottom of the tube **(Mackay et al., 1998).** The viability of the isolated mononuclear cells was determined and counted by a trypan blue exclusion test **(Greish et al., 2012).**

Culture of mesenchymal stem cells

The cells were cultured in complete culture medium; Dulbecco's modified Eagle's medium (DMEM) containing 12% fetal calf serum, 1% antibiotics – antimycotic; 100 units/ml of penicillin, 100 ug/ml of streptomycin and 250 ug/ml Amphotericin B. Cells were placed in 25 ml Falcon flasks**.** The flasks were incubated at 37 °C in 5% CO_2**.** After 2 weeks of culture, the adherent cells were almost confluent. Adherent cells were harvested using 0.25% trypsin for 5 min at 37 °C, 5 ml of medium was added to deactivate it. Cells were then counted with a haemocytometer. Cells were then collected in a 15 ml Falcon tube and centrifuged at 2000 rpm for 10 min. The supernatant discarded and the sediment washed twice with PBS, and then centrifuged at 1500 rpm for 5 min **(Can and Balci, 2011).**

Umbilical cord Wharton's Jelly samples collection:

A twenty centimeters long portion of the umbilical cord is obtained in a 50 ml Falcon tube containing 12.5 U/ml collagenase. The umbilical cord was cut into 5 cm pieces. The tissue sample was washed with Dulbecco's Phosphate Buffer Saline (DPBS) thoroughly. The cord was cut along the horizontal axis and the vein and arteries were removed. The remaining tissue sample was minced mechanically. The minced was washed twice DPBS and subjected to sequential digestion with collagenase **(Can and Balci 2011).** The tissue was incubated with 12.5 U/mL collagenase for 2 hours at 37°C in shaking water bath. The enzyme-digested tissue samples were passed through 250 µm sterile disposable plastic sieve. The retained material was washed with DPBS and subjected to RBC lysis. Later, the tissue samples were thoroughly washed DPBS containing 1% Penicillin/Streptomycin and 2.5 µg/mL Amphotericin B. Mesenchymal stromal cell colonies were visible by 7-14 days. By the end of 2nd week the confluent flask was subjected to passaging by 0.05% trypsin. Further passaging of the cells was carried out using T75 cm^2 flask once in three days **(Can and Balci 2011).**

The isolated MSCs from both sources (UCB and Wharton's Jelly) will be cultured in-vitro on DMEM in presence of TGF-B3 for amplification of cultured cells. Verification of MSCs' (Mesenchymal Stem Cells) proliferation was done by immunohistochemistry for CD 44.

Transforming Growth Factor beta-3 (TGF- B3):

The kits were brought from Sigma-Aldrich where TGF- B3 is human, recombinant, expressed in HEK 293 cells with catalog number H8791. The product is lyophilized from a solution of 50 mM sodium acetate, pH 4.5. TGF- B3 vial was briefly centrifuged before opening according to the pamphlet. It was reconstituted in sterile 4mM HCl containing 0.1% endotoxin-free recombinant human serum albumin. Storage of the product was at -20°C, where lyophilized product remains active for one year at -20°C. Upon reconstitution, the cytokine can be stored at 2-8°C for short term only, or at -20°C to -80°C in aliquots for long term with avoidance of repeated freezing and thawing cycles. Dose of TGF- B3 that is used for in-vitro amplification of cultured MSCs on DMEM, is equivalent to 0.5-10 ng/ml **(Xu et al., 2007).**

Preparation of Growth Factors: Stem Cell Factor (SCF):

The kits were brought from Sigma-Aldrich where SCF is human, recombinant, expressed in Escherichia coli, cell culture tested with catalog number S7901. The product is lyophilized from a 0.2 µm filtered solution in phosphate buffered saline containing 50 µg bovine serum albumin per µg cytokine. The contents of the vial were reconstituted according to the pamphlet, to a stock solution containing sterile phosphate buffered saline (PBS) and 0.1% bovine serum albumin (BSA) to a final concentration of not less than 10 µg/ml of SCF. The stock solutions were apportioned into working aliquots and stored at -20 °C

or at -70 °C for extended storage of working aliquots. Repeated freezing and thawing is not recommended. Dose of SCF injected in each rat's right knee is equivalent to 50µg/Kg **(Herodin et al., 2003).**

Preparation of histological sections of the knee joints:

Rats in all groups were sacrificed by intraperitoneal injection of 50 mg/kg thiopental sodium. The right knee of all rats was taken from skinned sacrificed animals and placed in 10% buffered phosphate formalin for 24 hours before being subjected to acid decalcification. The joints were decalcified by immersion in 3% nitric acid solution and maintained at room temperature for an average of 5-7 days. Decalcified joints were longitudinally sectioned and processed in ascending grades of alcohol for dehydration, xylene for clearing, then tissue sections were infiltrated and embedded in paraffin with one cell block made from each joint, then these blocks were sectioned on 5 microns with 2 sections submitted on each slide for the staining. All slides were stained with Hx & E and toluidine blue stain with one slide from each group stained with Masson's trichrome stain. Toluidine blue stain was used for the detection of cartilage glycosaminoglycan (GAG). Although, it is not the best stain used for this issue as it had been compared to those of Safranin O (as it was not available), and found to be inferior, many studies use the toluidine blue stain to illustrate the GAG content in the articular cartilage affected by OA including the studies done by **Pritzket et al, 2005 and Little et al, 2010.** Some sections will also be stained with Masson's trichrome stain for better illustration of the sub synovial fibrosis compared to hematoxylin and eosin stain **(Greish et al., 2012).**

The scoring system that were applied to our studied cases:

Cartilage loss is the hallmark of osteoarthritis (OA) that is why grading systems concerning cartilage damage as the most important parameter had been developed, with the **Mankin** system which developed **in 1971**, is the most widely used system. Over the last four decades, several "modified Mankin scores" have been developed **(Little et al, 2010).** Thus the following parameters were considered: A1- Cartilage Structure: 0,1,2,3,4,5,6,7,8 (normal, minor surface irregularity, surface fibrillation at superficial zone, clefts affecting mid zone, clefts reaching the deep zone, clefts reaching the calcified cartilage, loss of cartilage at superficial zone, loss of cartilage at mid zone, and finally loss of cartilage at deep zone respectively), A2- Cartilaginous Cells: 0,1,2,3 (normal, diffuse hypercellularity/hypertrophy, cloning/clustering, and hypocellularity respectively) A3- Toluidine blue stain for glycosaminoglycan depletion: 0,1,2,3,4,5 (normal, mild reduction of the stain, moderate reduction of the stain, sever reduction of the stain, pericellular stain and total absence of the stain respectively), A4- Tide Mark Integrity: 0 or 1 (normal or showed duplication, irregularity and crossed by blood vessels respectively). The final cartilage grade was 0 to 17. Then B- assessment of subchondral bone affection include both articular bone plate and subchondral trabecular bone grades (0,1,2,3) and stage (extent) (0, 1,2,3,4): Regarding the grades, B1, they include; (no affection, microfracture with fibrocartilaginous tissue affecting articular bone plate, microfracture with fibrocartilaginous tissue affecting subchondral bone and osteophyte formation respectively). Then, the extent B2: including (0,1,2,3,4): No affection, less than 10%, 10 to 25%, 25 to 50% and more than 50% respectively). Then C- the Pannus is assessed as follow (0,1,2,3,4): (no pannus, fibrovascular tissue in superficial cartilage zone, fibrovascular tissue in mid cartilage zone, fibrovascular tissue in deep cartilage zone, and finally fibrovascular tissue in calcified cartilage). Then D-the cartilage stage (extension of the cartilage destruction concerning the extent of both cartilage structure and the pannus) (0,1,2,3,4): (no affection, less than 10%, 10 to 25%, 25 to 50%, and more than 50%). Finally, E- Synovial inflammation that was scored by **Gerwin N et al, 2010** and include: E1- synovial cell lining (0,1,2,3) (one cell, 2 to3 cells,4 to5 cells and more than 5 cells respectively). E2- synovial fibrosis (0,1,2,3) (no fibrosis, mild, moderate and marked fibrosis respectively). E3- Synovial inflammation (0,1,2,3) (no inflammation, mild peri-vascular, moderate diffuse with occasional lymphoid aggregates, sever diffuse with frequent lymphoid aggregates respectively). E4- Synovialvascularity: (0 or 1) (no significant increase vascularity or significant increased vascularity respectively) with total synovial inflammation score: 0 to1, 2 to 4, 5 to 10: (no inflammation, low inflammation and high inflammation score respectively).

Thus, the total joint score was 0 to 42 with mild affection till 14, moderate affection from 15 to 28 and sever joint affection was from 29 to 42.

Statistical methods:

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative data as minimum & maximum of the range as well as mean±SD (standard deviation) for the quantitative parametric data. Inferential analyses were done for quantitative variables using ANOVA Test for more than two independent groups with parametric data, and Tukey's post hoc test to find homogenous groups. The level of significance was taken at P value < 0.050 is

significant, otherwise is non-significant. The p-value is a statistical measure for the probability that the results observed in a study could have occurred by chance.

3. Results:

All treated groups showed statistical improvement compared to the untreated OA group in all examined histopathological parameters with some variation among the groups in the degree of the response.

Table (1): Comparison between study groups regarding cartilage grade including structure, cellularity, stain grade and tide mark:

Group	N	Mean±SD	Range	HG	Mean±SD	Range	HG	Mean±SD	Range	HG	Mean±SD	Range	HG
		Cartilage structure			Cartilage Cellularity			Cartilage Stain			Tide Mark		
SCF IV	20	1.3±1.1	0.0–4.0	A, B	0.4±0.6	0.0–2.0	A, B	1.3±1.6	0.0-4.0	B	1.0±0.2	0.0-1.0	B
SCF IC	20	1.8±1.8	0.0–6.0	B	0.9±1.1	0.0–3.0	B, C	1.3±1.4	0.0-4.0	A,B	1.0±0.2	0.0-1.0	B
SC CB	10	1.3±0.8	0.0–2.0	A, B	0.8±0.8	0.0–2.0	B,C	1.1±1.4	0.0-4.0	A,B	0.8±0.4	0.0-1.0	B
SC WJ	10	1.3±1.5	0.0–4.0	A, B	1.2±0.9	0.0–3.0	C	1.3±1.3	0.0-3.0	A,B	1.0±0.0	1.0-1.0	B
OA control	20	5.3±2.4	2.5–8.0	C	2.2±0.4	2.0–3.0	D	3.2±1.2	2.0-5.0	C	1.0±0.0	1.0-1.0	B
Healthy control	20	0.0±0.0	0.0–0.0	A	0.0±0.0	0.0–0.0	A	0.0±0.0	0.0-0.0	A	0.0±0.0	0.0-0.0	A
^P		<0.001*											

ANOVA test, *Significant, HG: Homogenous groups by post hoc Tukey's test. Homogenous group means that there is no statistically significant difference between its members as regards the parameter under study.

Table (1) showed that: **Cartilage structure grade** (depth of cartilage destruction) was significantly highest among.

SCF IC treated group with the P value <0.001 compared to other treated groups, So they give the least therapeutic response, **cartilage cells grade (Cellularity)** was significantly highest among SC WJ treated group compared to other treated groups (least response) and lowest among SCF IV treated group (best response), **cartilage stain grade** was significantly highest among SCF IV treated group (least response), lowest among SC CB treated group (best response) and cartilage stain grade was significantly higher than healthy control group and significantly lower than OA control group, however, **tidemark integrity** grade was not significantly different among treatment groups as well as between them and OA control group, but was significantly higher than healthy control group. **(Figure 1,2).**

Table (2): Comparison between study groups regarding pannus grade and regarding cartilage stage (considering the extent of cartilage structure destruction and extent of pannus).:

Group	N	Mean±SD	Range	HG	Mean±SD	Range	HG
		Pannus Grade			Cartilage Stage (Extent of cartilage destruction and Pannus)		
SCF IV	20	1.4±0.9	0.0–3.0	B	1.1±0.8	0.0-3.0	B,C
SCF IC	20	1.0±0.6	0.0–2.0	B	1.0±0.6	0.0-2.0	B
SC CB	10	1.5±0.8	0.0–3.0	B	1.9±0.7	1.0-3.0	C
SC WJ	10	1.6±0.9	1.0–3.0	B	1.8±1.2	1.0-4.0	B,C
OA control	20	3.2±1.0	2.0–4.0	C	3.2±1.2	1.0-4.0	D
Healthy control	20	0.0±0.0	0.0–0.0	A	0.0±0.0	0.0-0.0	A
^P		<0.001*					

^ANOVA test, *Significant, HG: Homogenous groups by post hoc Tukey's test.

Table (2) showed that: Pannus grade was not significantly different among treatment groups, but was significantly higher than healthy control group and significantly lower than OA control group. Also cartilage stage was significantly highest among SC CB treated group (least response to therapy) and lowest among SCF IC treated group (best response to therapy). Also cartilage stage was significantly higher than healthy control group and significantly lower than OA control group. **(Figure 3).**

Table (3): Comparison between study groups regarding subchondral bone grade and subchondral bone stage

Group	N	Mean±SD	Range	HG	Mean±SD	Range	HG
		Subchondral Bone Grade			Subchondral Bone Stage		
SCF IV	20	2.2±0.9	0.0–3.0	C	1.2±0.7	0.0-2.0	B,C
SCF IC	20	2.4±0.7	1.0–3.0	C	1.5±0.7	0.0-3.0	C
SC CB	10	1.7±1.3	0.0–3.0	B, C	1.1±1.1	0.0-3.0	B,C
SC WJ	10	1.2±1.4	0.0–3.0	B	0.8±0.8	0.0-2.0	B
OA control	20	2.6±0.8	1.0–3.0	C	3.8±0.4	3.0-4.0	D
Healthy control	20	0.0±0.0	0.0–0.0	A	0.0±0.0	0.0-0.0	A
^P		<0.001*					

^ANOVA test, *Significant, HG: Homogenous groups by post hoc Tukey's test.

Table (3) showed that both Subchondral bone grade and stage were significantly highest among SCF IC treated group and lowest among SC WJ treated group. Also subchondral bone grade was significantly higher than healthy control group and significantly lower than OA control group**. (Figure 3).**

Table (4): Comparison between study groups regarding synovium lining cell grade, synovial fibrosis, synovial inflammation and synovial vascularity.

Group	N	Mean±SD	Range	HG	Mean±SD	Range	HG	Mean±SD	Range	HG	Mean±SD	Range	HG
		Synovial Lining Cell Grade			Synovial Fibrosis			Synovial Inflammation Grade			Synovial Vascularity		
SCF IV	20	0.2±0.6	0.0–2.0	A	0.9±0.2	0.5-1.0	B	0.4±0.4	0.0-1.0	A	0.2±0.3	0.0-0.5	A,B
SCF IC	20	0.3±0.5	0.0–2.0	A	0.7±0.5	0.0-2.0	B	0.4±0.4	0.0-1.0	A	0.3±0.4	0.0-1.0	A,B
SC CB	10	0.5±0.7	0.0–2.0	A	1.0±0.7	0.0-2.0	B	0.5±0.7	0.0-2.0	A	0.3±0.5	0.0-1.0	A,B
SC WJ	10	0.7±1.0	0.0–3.0	A	0.9±1.0	0.0-3.0	B	0.6±0.8	0.0-2.0	A	0.4±0.5	0.0-1.0	B
OA control	20	1.4±1.0	0.0–3.0	B	2.4±0.5	2.0-3.0	C	2.2±0.8	1.0-3.0	B	0.4±0.8	0.0-1.0	C
Healthy control	20	0.0±0.0	0.0–0.0	A	0.0±0.0	0.0-0.0	A	0.0±0.0	0.0-0.0	A	0.0±0.0	0.0-0.0	A
^P		<0.001*											

^ANOVA test, *Significant, HG: Homogenous groups by post hoc Tukey's test

Table (4) showed that: Synovium lining cell grade was not significantly different among treatment groups as well as between them and healthy control group, but was significantly lower than OA control group. However, synovium fibrosis grade was not significantly different among treatment groups, but was significantly higher than healthy control group and significantly lower than OA control group. Regarding Synovium inflammatory cell infiltration grade was not significantly different among treatment groups as well as between them and healthy control group, but was significantly lower than OA control group. On the other hand, Synovium vascular grade was significantly highest among SC WJ treated group (least response to therapy) and lowest among SCF IV treated group (best response to therapy). Also synovium vascular grade was significantly higher than healthy control group and significantly lower than OA control group. **(Figure 4).**

Table (5): Comparison between study groups regarding total cartilage grade, Total synovial score and total joint score

Group	N	Mean±SD	Range	HG	Mean±SD	Range	HG	Mean±SD	Range	HG
		Total Cartilage Grade			Total Synovial Score			Total Joint Score		
SCF IV	20	4.0±2.3	1.0–8.0	B	1.7±0.9	1.0-4.0	B	11.4±3.5	6.0-18.0	B
SCF IC	20	4.9±3.8	1.0–14.0	B	1.7±1.1	0.0-4.0	B	12.4±4.6	7.0-21.0	B
SC CB	10	4.0±1.9	1.0–7.0	B	2.3±1.8	0.0-5.0	B	12.5±4.2	6.0-18.0	B
SC WJ	10	4.3±3.0	0.0–9.0	B	2.4±2.5	0.0-7.0	B	11.5±6.8	0.0-20.0	B
OA control	20	11.7±3.5	7.5–16.0	C	6.8±1.5	5.0-8.0	C	31.3±6.0	23.0-39.0	C
Healthy control	20	0.0±0.0	0.0–0.0	A	0.0±0.0	0.0-0.0	A	0.0±0.0	0.0-0.0	A
^P		<0.001*								

^ANOVA test, *Significant, **HG**: Homogenous groups by post hoc Tukey's test

Table (5) showed that total cartilage grade, total synovial score and total joint score were not significantly different among treatment groups, but was significantly higher than healthy control group and significantly lower than OA control group.

4. Discussion:

Articular cartilage damage is an important, prevalent and unsolved clinical issue of osteoarthritis (OA). In the absence of vascularity, the cartilage does not have access to progenitor cells that may support and participate in regenerative processes. Clinical interventions such as marrow stimulation techniques that support cell invasion from the bone marrow do not restore the original cartilage structure and function in the lesions. Instead, these options lead to the formation of a poorly organized, mechanically in adapted fibrocartilage made of type-I collagen instead of type-II collagen with proteoglycans that are normally found in the hyaline cartilage **(Johnstone et al., 2013).**

Mesenchymal Stromal Cells (MSC) opened new therapeutic perspectives provided their regenerative potential and ability to modulate inflammation **(Uth and Trifonov, 2014).** Umbilical cord is the easiest obtainable biological source of MSCs and the Wharton's jelly of the umbilical cord is a rich source of fetus-derived stem cells. However, the use of MSCs for therapeutic application is based on their subsequent large-scale in vitro expansion. Generation of large quantities of MSCs is required to meet the clinical demand and biomedical research needs. The conditions have been optimized, for scaling up of WJ-MSCs.

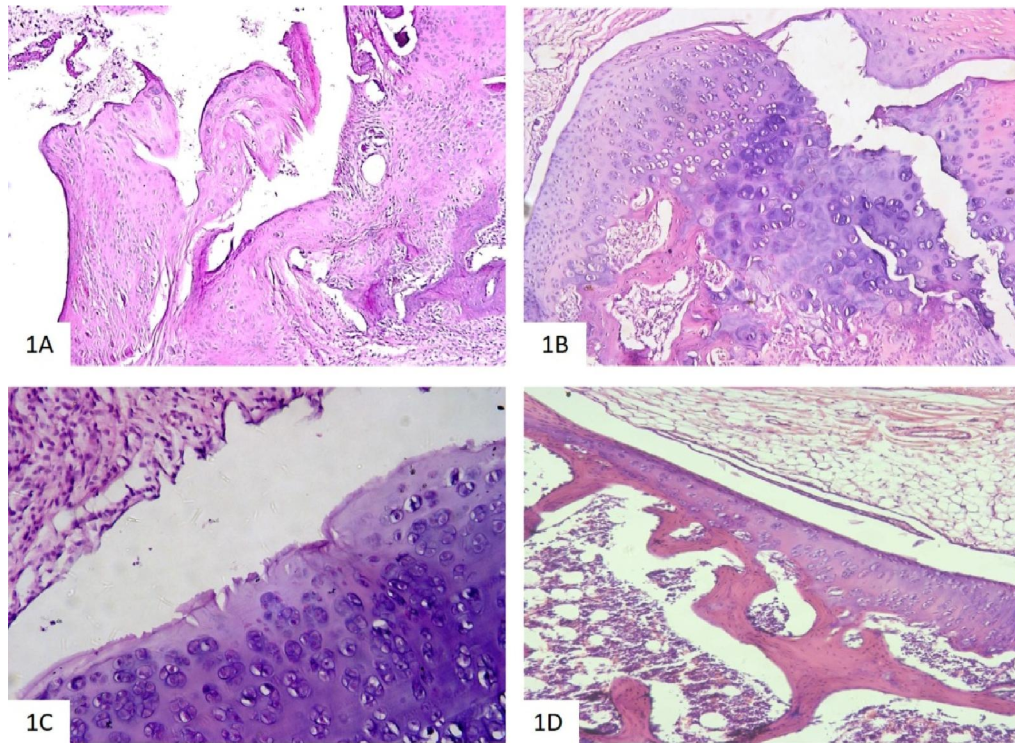

Fig. 1: A: Grade 7 cartilage structure affection in osteoarthritis-induced rat without treatment, H & E, x100. B: Grade 5 and grade 7 cartilage structure affection in osteoarthritis induced rat without treatment, H & E, x100. C: Grade 2 cartilage structure affection in rats treated with intraarticular injection of Wharton's jelly stem cell, H & E, x200. D: Marked joint improvement in rats treated with intraarticular injection of stem cell factor SCF, H & E x100.

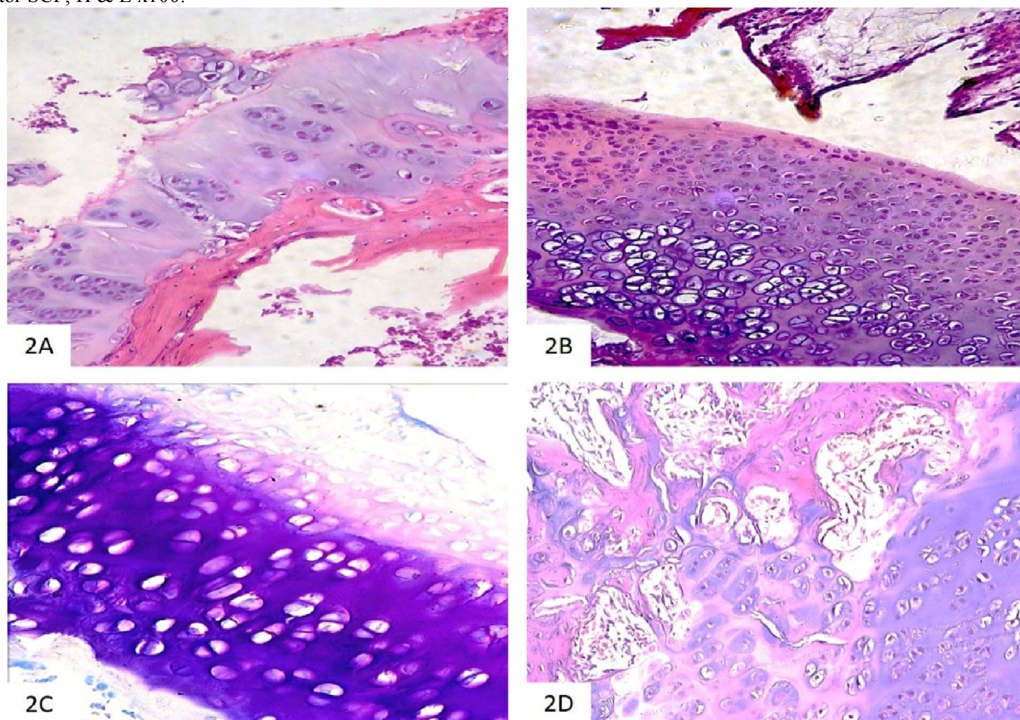

Fig.2: A: Cartilage hypocellularity along with grade 6 cartilage structure affection in osteoarthritis induced rat without treatment, H & E, (A) x200. B: Diffuse hypercellularity and cloning of the cartilaginous cells along with grade 1 cartilage affection in intravenous stem cell factor treated rat H & E, x100. C: Grade 2 stain in rat treated with intravenous injection of stem cell factor, toluidine blue stain, x200. D: Grade 1tide mark affection in rat treated with intraarticular injection of Wharton's jelly stem cell, H & E, x200

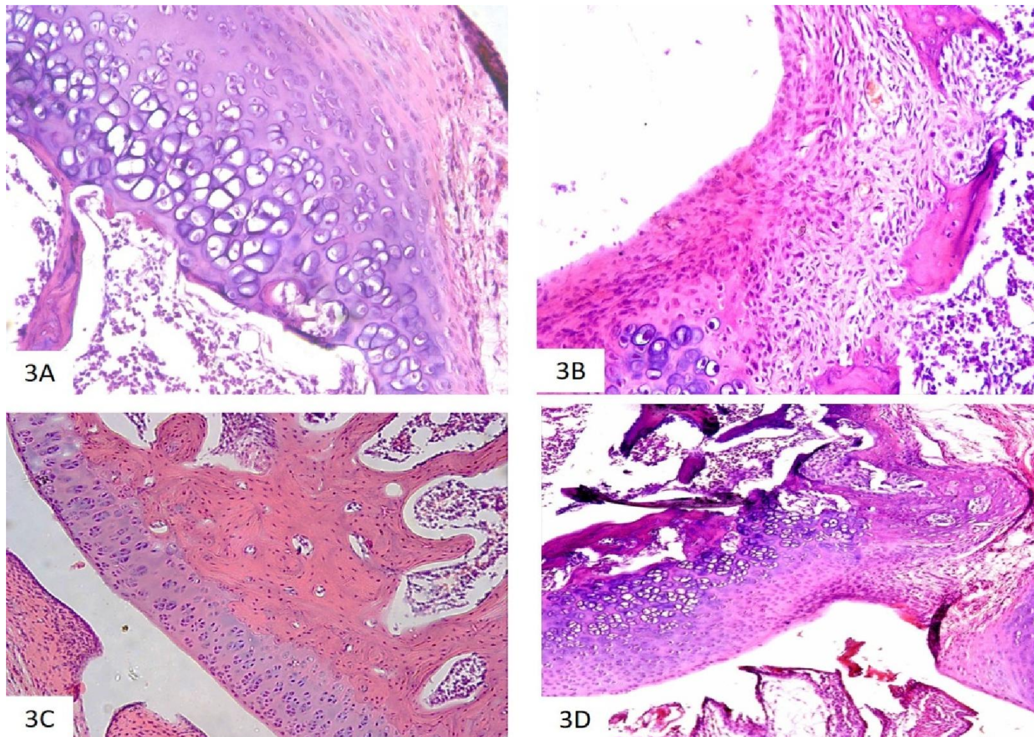

Fig. 3: A: Grade 1 to 2 pannus in rat treated with intraarticular injection of stem cell factor, H & Ex200. B: Grade and stage 4 pannus in osteoarthritis-induced rat without treatment associated with articular bone plate microfracture, H & E x200. C: Grade 1 subchondral bone affection in rat treated with intra articular injection of stem cell factor, H & E x100. D: Grade 3 subchondral bone affection in rat treated with intra articular injection of stem cell factor as evident, H & E x100.

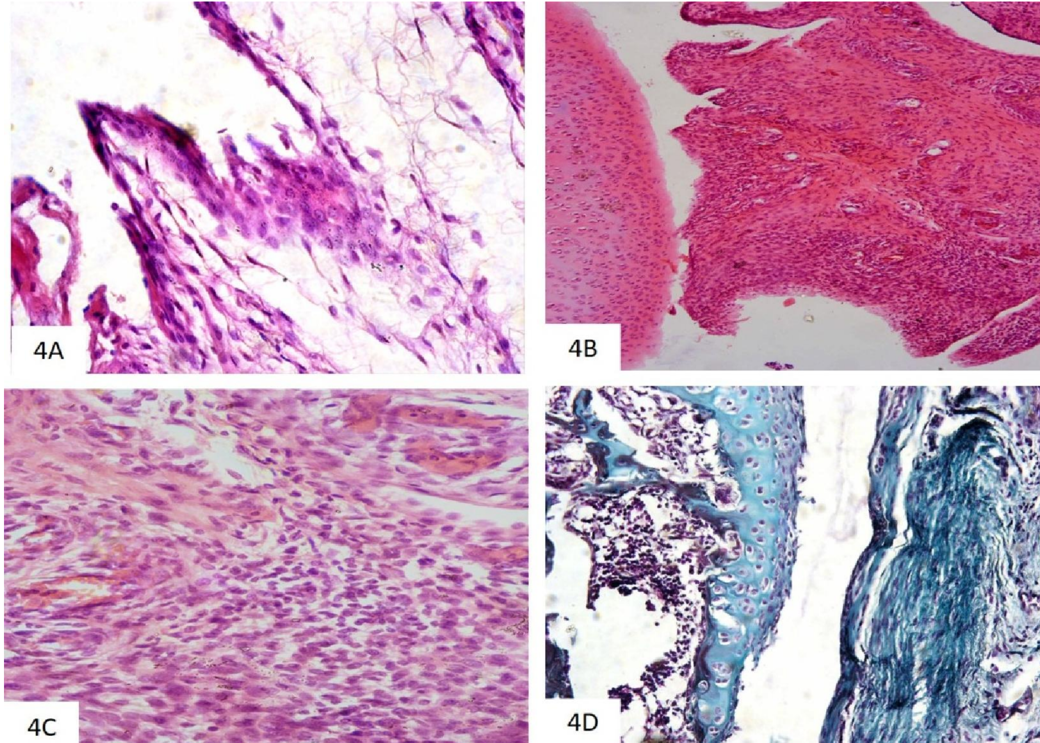

Fig. 4: A: Grade 2 synovial cell proliferation in rat treated with intra venous injection of stem cell factor, H & E x 400. B Grade 3 synovial inflammation with grade 1 vasculature in osteoarthritis-induced rat without treatment, H & E x200. C: Higher magnification of previous image x400. D: Grade 2 synovial fibrosis in osteoarthritis-induced rat without treatment, Masson's trichrome stain x 100.

Low seeding density along with basic fibroblast growth factor (bFGF) supplementation in the growth medium, which is DMEM, resulted in propagation of more than $1x10^8$ cells within a time period of 15 days from a single umbilical cord. The up scaled WJ-MSCs retained their differentiation potential and immunosuppressive capacity. They expressed the typical hMSC surface antigens and the addition of bFGF in the culture medium did not affect the expression levels of HLA-DR and CD 44 according to ***Nekanti et al., (2010).***

Embryonic stem cells have already been used to enhance the healing of cartilage defects in vivo and to allow for the production of a cartilage matrix capable of integrating with defects in human arthritic joint cartilage. However, use of embryonic stem cells remains largely controversial for ethical reasons to do with the harvesting of cells from human embryos, and due to safety issues because their use is associated with immune rejection problems and with the formation of teratomas ***(Lee et al., 2014).***

This work was designed to assess the therapeutic efficacy of the use of growth factors (stem cell factor or SCF administrated by both intra-articular and intravenous routes) as mobilizing factors on autologous stem cells in osteo-arthritic white mice in comparison to the effect of direct intra-articular stem cell transplantation (after in vitro culture of human umbilical cord blood stem cells on DMEM with TGF beta3) on the process of healing of induced osteoarthritic lesions (by scratching) of knee joints in white mice. Human Umbilical cord MSCs (obtained following Caesarian sections) were derived from two sources, cord blood buffy coat and Wharton's Jelly. This was followed by in vitro culture on DMEM in presence of TGF beta3. All hUC-MSCs were injected intraarticular into osteoarthritic knee joints of white mice. ***Marijnissen et al., (2002).***

In this study, the response to the treatment was assessed only by application of a histopathological scoring system comparing the joints of OA induced rats without treatment and the treated groups with the different modalities **(Little et al., 2010).** The total joint score was out of a mark of 42. A score up to 14 indicates mild joint affection, scores of 15-28 indicate moderate joint affection and scores of 29-42 indicate severe joint affection. Initial scores in white mice with induced osteoarthritis that were left without treatment ranged from 23-39. This indicated high degree of knee joint affection.

Liu et al., (2010) found that the majority of joints from mice injected with UC-MSCs had normal morphology with a smooth articulation cartilage surface, and an absence of inflammatory cell infiltrate and pannus formation. Also **Watson, (2013)** reported that mesenchymal stem cells are being used for their therapeutic potential to enhance the regeneration of cartilage and bone to prevent or modify disease progression and bone marrow concentrate, with its mesenchymal stem cell and hematopoietic stem cell populations, along with abundant growth factors, exhibits anti-inflammatory, immunosuppressive, osteogenic, and chondrogenic qualities.

In this study, In vitro cultured hUC-MSCS that were in vitro amplified on DMEM (according to **Nekanti et al., (2010)**) in presence of TGF beta3, showed that SC WJ treated group of osteoarthritic white mice showed the best therapeutic response among treated groups, to cartilage cell grade, Sub-chondral bone grade, but showed the least therapeutic response among treated groups as regards synovial vascular grade. They achieved a joint score of 3-20 for SC WJ treated mice including 10 mice with induced osteoarthritis by scratching. In this work, we found that SC CB treated group of osteoarthritic white mice showed best therapeutic response among treated groups as regards Cartilage stain grade, but showed the least therapeutic response among treated groups as regards cartilage stage. **Calvo et al., (2004)** pointed out the change in articular cartilage matrix staining in addition to the histopathological changes corresponding to early OA represented as a significant reduction in articular cartilage cellularity.

In the current study, the response to intra-articular injection of UC-MSCs achieved joint scores of *7-18* for SC CB treated group of white mice with induced osteoarthritis. Regarding the 20 healthy control, all had a joint score of zero whereas in 20 rats that were used as OA control group with induced OA by scratching, the score ranged from 23-39.

Paul et al., (1991) reported an MRI study in advanced cartilage degeneration in the rabbit knee and found a correlation between cartilage thinning and the loss of proteoglycans. Cartilage thinning has been therefore correlated with tissue destruction, which finally was to be detected radiographically as joint space narrowing. Other MRI investigations performed in early OA have identified an initial phase of cartilage hypertrophy followed by degeneration and loss. In this study, unfortunately, no radiological facilities were available to assess the rat joints.

SCF in this study, was introduced by two routes which are: Intraarticular (IC) into right knee joint cavity and Intravenous (IV) route into rats' tail veins. Both routes aim at mobilizing rats' own autologous MSCs towards osteoarthritic knee joint with induced OA by scratching for healing. It was found that SCF IV treated group showed best therapeutic response among treated groups as regards cartilage cell grade and synovium vascular grade, but showed the least therapeutic response among treated groups as regards cartilage stain grade. Whereas this work showed SCF

IC treated group showed the best therapeutic response among treated groups as regards cartilage stage and showed the least therapeutic response among treated groups as regards subchondral bone grade and subchondral bone stage. As regards the number of improved parameters in response to therapy, it's concluded that UC-MSCs WJ therapy showed improvement in cartilage cell grade, subchondral bone grade and subchondral bone stage. Whereas SCF IV therapy showed improvement in cartilage cell grade and synovial vascular grade. Also, SCF IC therapy improved cartilage stage only and UC-MSCs CB therapy improved cartilage stain grade only. These findings need to be further confirmed and analysis of the possible causes for the variation in treatment response to different SC by different modalities need to be further investigated.

5. Conclusion:

This study showed the best therapeutic response to knee OA therapy is achieved with UC-MSCs WJ therapy followed by response to SCF-IV therapy that mobilizes autologous stem cells towards site of inflammation". Both SC CB and SCF IC improved only one parameter.

Limitations and future recommendation:

Our study face limitation as no combination of histopathology, radiology and clinical for follow up of response to growth factors and stem cell therapies was done. Also, there was limited number of studied rats and short time of follow up duration (3 months only) and thus more studies on therapy with different types of growth factors administrated by different routes with follow up for longer durations among larger number of rats is needed not only to assess the therapeutic response but also to investigate the possible side effects of autologous mobilized stem cells, hUC-MSCs & SCF therapies. Finally, many studies are targeting stem cells therapy in animals with a good success so more efforts are needed to approve stem cells studies in humans.

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Authors declare any conflict of interest.

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