Innovation of a Novel Therapeutic Modality for Treatment of Osteoarthritis of Knee with Autologous Adipose Derived Stromal Vascular Fraction (AD-SVF)

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Abstract

Background: Adipose tissue derived stromal vascular fraction is crude heterogeneous mixture of different cell population. SVF possess potential to translate into oesteogenic and chondrogenic lineage. We designed a pilot study to assess safety and efficacy of autologous adipose derived stromal vascular fraction (AD-SVF) in 25 patients (50 knees) with both the knees having osteoarthritis.
Methods: Patients were selected based on selection criteria. Lipoaspiration from the lower abdomen was done. The lipoaspirate was processed to obtain SVF. The intra-articular injection of SVF and PRP was given. Clinical outcomes were assessed with WOMAC, KSS, and VAS scores and radiologically at the interval of 6 weeks, 6 months and 1 year.
Results: The safety of AD-SVF was confirmed as there were no adverse reactions related to therapy. Patients exhibited rapid and progressive improvement of WOMAC, KSS and VAS Score by 1 year. MRI studies did not show radiological evidence of cartilage.
Conclusions: AD-SVF therapy is safe & efficacious alternative treatment for knee osteoarthritis. The intervention is simple, minimally invasive, require minimal hospitalization and surgery.

Keywords: Osteoarthritis, Adipose derived Stromal Vascular Fraction, Stem cell, alternate therapeutic intervention.

Introduction

Osteoarthritis of knee joint is a major clinical problem with occurrence of ~15 % in above 50 years of age.

Knee joint is a complex joint. It has medial and lateral cartilages in the form of two menisci within the joint. Additionally, there are cruciate ligaments that traverse the joint. There is an additional patello- femoral compartment too. Also there is a large synovial membrane.

This study is designed to access the safety and efficacy of Stromal Vascular Fraction (SVF) derived from adipose tissue. It is aimed at a composite tissue repair across all the above structures.

At present joint replacement surgery is the therapeutic modality of choice for Grade 2, 3 & 4 Osteoarthritis of knee joints. This is a major surgical procedure often requiring blood transfusion and is not without complication. Biological drugs are the subject for research in 21st century. Cellular and stem cells therapy are emerging as a preferred mode of research & innovation for several conditions. Successful results with the application of MSCs for the treatment of Osteoarthritis knee joint are being reported.[1,2] With mesenchymal stem cells derived from adipose tissue[3,4] and bone-marrow.[5,6] The regeneration of the damage tissues in the osteoarthritis of knee joint would need multi lineage stem cells that will have synergy between cells and their microenvironments.

The present study is designed with the use of SVF derived from Adipose Tissue (AT). The preference for AT is because of easy accessibility & availability of large no. of mononuclear cells in the SVF, derived there from. SVF contains ~ 30 % mesenchymal stem cells. MSCs have multipotency, capable of multi lineage translation and, provide protection against inflammation. They have immuno-suppressive property and beside this they also have chondrogenic potential.[7]

Stromal Vascular Fraction (SVF) is a crude extract, heterogeneous collection of cells contained within adipose tissue. The composition of SVF consists of mesenchymal stem cells, hematopoietic stem cells, endothelial precursor cells, T regulatory cells, macrophages, smooth muscle cells, pericytes and pre-adipocytes. The cells other than ADMSCs also support angiogenesis and there is trans- lineage translation of haemopoietic stem cells- CD 34 – to MSCs.[8] Therefore, the study is conducted with injection of SVF without further isolation of ADMSCs and their cultures.

Pain relieving property of Platelet Rich Plasma (PRP) is well established. PRP immediately releases several cytokines. They acts synergistically with MSCs to enhance the release of cytokines by MSCs.[9] Therefore, combination of SVF with PRP were utilized.

Thus the study is designed on the basis of well-established basic science principle. This will be step forward for innovation in safe and efficacious alternate therapeutic mode for the treatment of OA knee. The study design is in total compliance with ICMR guidelines 2013: 6.1.6.[10]

A pilot study was conducted with approvals from Institutional Ethics Committee (IEC) and Institutional Committee for Stem Cell Research (IC-SCR). The randomized clinical trial was registered with CTRI ref no: 2013/02/004619.

In the present report, we provide validation for proof of concept by treating 25 patients with 50 knees using autologous adipose derived stromal vascular fraction.

Material & Methods

Patient selection

The patient selection was done on the basis of following criteria;

Inclusion criteria: Patients with either sex with age between 45 to 75; near normal body mass index and Osteoarthritis (OA) diagnosis based on radiological evidence were included. Patients with metabolic disorder such as hypothyroid, diabetes or abnormal blood pressure level were controlled before the initialization of treatment. Proofs of cartilage damage in the joint(s) were obtained radiologically. Assessment of WOMAC, KSS and VAS Score of each individual enrolled was done.

Exclusion criteria: Patients with history of taking corticosteroids or NSAIDs, glycosaminoglycan, or suffering from active cardiac or respiratory disease; patients positive for markers for Hepatitis B, C, or HIV; and patients with history of allergic reactions were excluded from the study. Patients with loose bodies in the joint were excluded.

In this trial 138 patients were consulted and 25 patients were selected based on the above inclusion and exclusion criteria. The 25 patients were selected with both knees affected (Table 1). The grading of 50 knees was calibrated as per Kellgren Lawrence grading scale. Grading for 50 knees joints; wherein 10 knee joints was of grade 2, 36 knee joints were of grade 3 and, 4 knee joints were of grade 4.

Radio logically the patients were subjected to x-ray and MRI. Apart from erosion of the cartilage on the joint surfaces, MRI studies revealed the additional pathology in the joints such as medial meniscus tear (n=22), lateral meniscus tear (n=8), anterior cruciate ligament (n=19), posterior cruciate ligament (n=6), cyst (n=14), osteophytes (n=20), Infusion in supra-patellar bursa (n=20) and, foreign bodies (n=4).

Total 25 patients with radiological evidence for osteoarthritis knee, advised for joint replacement therapy elsewhere and satisfying above selection criteria were selected for the study (Table 1). The patients underwent blood investigation before commencement of therapy. This included complete blood count, blood sugar, blood urea, serum creatinine, SGPT, HIV (I and II) antibody test, hepatitis B, surface Antigen & HCV antibody test, Prothrombin time, Thyroid Stimulating Hormone estimation were done to ascertain fitness of treatment.

Table 1: Demographic characteristics of patients enrolled for the study

Age	Height	Weight	BMI(kg/m2)	Sex	Grade Right	Grade Left	Amount of lipoaspirate	No. of SVF cells injected
45	5.2	75	30.4 - Ob	F	3	3	700	300 x 10^6
60	5.3	68	26.56 - N	F	2	2	700	272 x 10^6
56	5.3	104	40.6 - Ob	F	3	3	800	416 x 10^6
56	5.6	78	27.63- N	F	2	3	700	312 x 10^6
77	4.11	46	20.44- N	F	3	3	400	184 x 10^6
64	5	56	24.23- N	M	3	3	500	224 x 10^6
67	5.5	82	30.11- Ob	M	2	2	480	328 x 10^6
49	5.4	71	26.72- N	F	3	3	1000	284 x 10^6
62	5.5	82	30.11- Ob	M	4	4	650	328 x 10^6
61	4.11	57	25.33- N	F	3	3	800	228 x 10^6
65	4.11	42	18.66- N	F	3	3	420	168 x 10^6
72	5.3	63	24.60- N	M	3	3	550	252 x 10^6
71	5.5	66	24.24- N	M	2	2	700	264 x 10^6
56	5	64	27.70- Ow	F	3	3	470	256 x 10^6
59	4.11	65	26.22- Ow	F	3	2	550	260 x 10^6
62	5.5	63	23.14- N	F	3	3	525	252 x 10^6
53	5.4	65	24.46- N	M	2	2	550	260 x 10^6
61	5.8	85	28.40- Ow	F	3	3	600	340 x 10^6
55	5.2	68	27.58- Ow	F	2	2	650	272 x 10^6
60	5.8	87	29.06- Ow	M	3	3	700	348 x 10^6
60	5.02	70	28.39- Ow	F	2	3	700	280 x 10^6
56	5.5	70	25.71- Ow	M	4	4	600	280 x 10^6
62	5	57	24.67- N	F	3	3	650	228 x 10^6
66	5	71	30.73- Ob	F	3	3	800	284 x 10^6
60	5.3	90	35.15- Ob	F	3	3	800	360 x 10^6

PRP – Platelet Rich Plasma, BMI – Body Mass Index;0-18.5 -> underweight (UW), 18.5-25 -> Normal (N), 25-30 -> Overweight (OW) and >30 – Obese (O).

Table 2: MRI findings (Note: The most affected knee was investigated for MRI)

MRI findings	No. of joints
MMT= Medial Meniscus tear	22
LMT= Lateral Meniscus Tear	8
ACL= Anterior cruciate ligament	19
PCL= Posterior cruciate ligament	6
CYST	14
OSTEO= Osteophytes	20
ISB= Infusion in suprapatellar bursa	20
SFTJ= Subluxation in femur-tibial joint	6
FB= Foreign bodies	4

Surgical Protocol

Approximate 1000 cc of lipoaspirate was aspirated by a trained plastic surgeon with cannulae under local anaesthesia and sedation. Lipoaspirate was collected from lower abdominal subcutaneous fat in the operating room in a sterile container. The samples were transferred to a GMP class V laboratory for cell isolation under strict aseptic conditions.

Laboratory Protocol

The isolation of adipose tissue derived stromal vascular fraction (AD-SVF) was performed with modification to[11] Michael et.al, 2010 protocol.

Proof of concept was established for the isolation of MSCs from adipose tissue obtained by plastic surgeons for indications other than the present study. It was established that AT contains at least 0.5 million SVF/ 1 cc of lipoaspirate. SVF was then taken to 2 passages to isolate pure ADMSCs population. They were further translated to cartilage.

Quantification of MSCs in SVF through Flow Cytometry

SVF was subjected to flow cytometry studies at two different laboratories.

Characterization of ADMSCs

The ADMSCs cells were verified with four positive markers; CD 29, CD – 44, CD - 90 and CD – 105; three negative markers; CD 31, CD 34, CD 45.[12]

Preparation of cells for injection

The dosage for SVF cells was determined based on above criteria (Flow Cytometry) are as follows;

1. 1000 cc adipose tissue yields ~ 500 million SVF
2. Dosage for intra –venous administration of MSCs is between 1-4 million MSCs / Kg body weight[13]
3. SVF at our lab contains ~ 30 % MSCs and to reiterate MSCs are the most effective cells for cartilage regeneration
4. The MSCs injected into the knee joint remain localise.
5. Therefore SVF containing ~ 250 million cells are suspended in 2 ml of Isolyte M (Clarias Ostuka, Ahmedabad, India). PRP was obtained by the standard protocol by Graham, 2002.

About 14 cc of autologous PRP was obtained after centrifugation of 50 cc heparinised blood. 4 ml PRP (approximately 2, 00,000 platelet count/µl) was added to the isolated cell suspension. This was injected in each knee joint intra articularly at 1- 4 million cells/ kg body weight.

Following aseptic procedure, the cell suspension was injected intra-articularly at 3 points uperolateral, inferomedial and inferolateral.

Results

Laboratory Results

Quantification of MSCs in SVF through Flow Cytometry

SVF was subjected to flow Cytometric studies at two different laboratories. The results indicated that SVF contains ~ 30% of cell population as MSCs (Table 3).

Table 3: Quantification of MSCs positive and negative cell population

Fluorescence Tag	Markers	% Gated (M2)	Result
PE	Isotype Control	0.39	
	CD 90	31.81	Positive
	CD 34	12.58	Positive (very few cells)
FITC	Isotype Control	1.37	
	CD105	1.37	Negative

MSCs characterization with positive & negative markers

The characterization of MSCs derived from AD-SVFis shoen in Fig. 1 & 2.

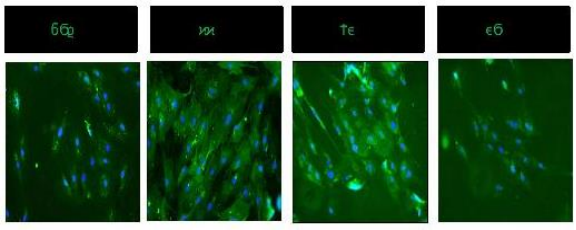


Fig. 1: Positive Markers

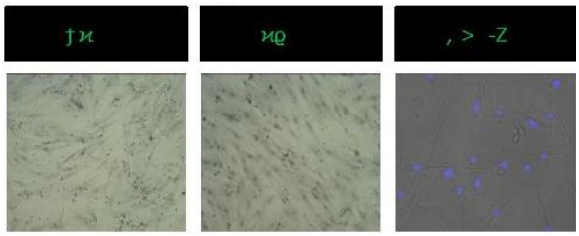


Fig. 2: Negative Markers

Clinical Results

Safety:

All 25 patients were comfortable and none of them had any adverse reaction.

Efficacy:

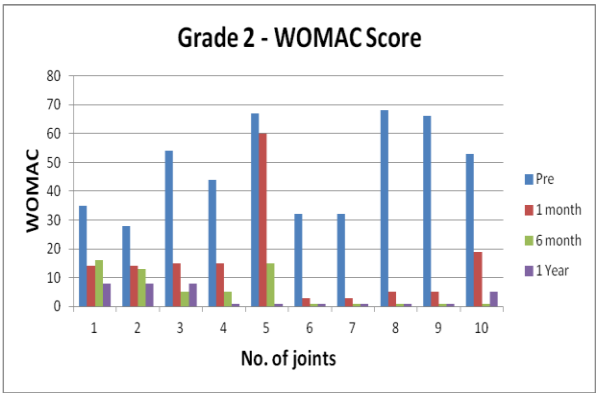
All 25 patients had pain relief within 24 hours. They were given bed rest with the permission to use only western toilet. They were discharged on second day after injection. Physiotherapy in bed was started on 8th day and by about a month they were allowed to walk up to 1 km. The clinical assessment was done at end of 1, 6 & 12 months. Arthroscopic inspection of joint was contemplated. However, the Ethics Committee denied the permission for an invasive procedure that may affect the outcome as well.

The clinical results were assessed by WOMAC, KSS, Visual Analogue Score (VAS) and radiological evaluations.

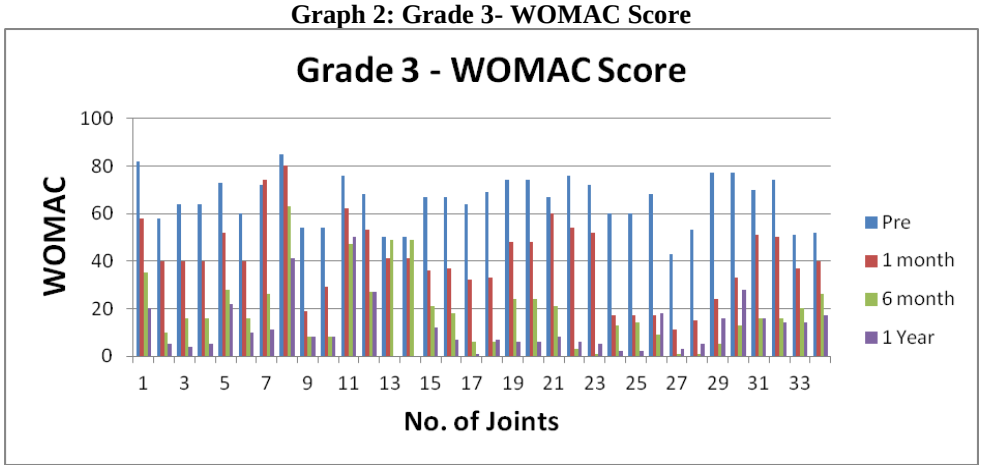
WOMAC Score:

The graphs (Graph 1, 2 & 3) represents that n=10 no. of joints were of grade 2, n=34 of grade 3 and n=4 of grade 4. The pre-operative WOMAC score is highest than post-operative 1, 6 and 12 month assessment. The WOMAC score gradually decreased.

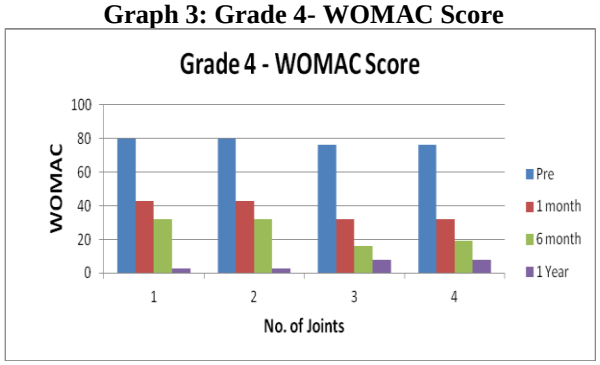
Graph 1: Grade 2- WOMAC Score



Graph 2: Grade 3- WOMAC Score



Graph 3: Grade 4- WOMAC Score



KS Score:

The increase in score of KSS indicates improvement. This was noted in all 24 patients (48 knees).

The scoring for individual of KSS was as follows;

The range 0-50 for pain points in grade 2 (n=10), grade 3 (n=34) and grade 4 (n=4) joints; range of motion 100-110 in grade 2 (n=10), grade 3 (n=34) and grade 4 (n=4) joints; range 0-50 for walking in grade 2 (n=10), grade 3 (n=34) and grade 4 (n=4) joints and, range 0-50 for stairs climbing in grade 2 (n=10), grade 3 (n=34) and grade 4 (n=4) joints are shown in Table 3,4,5.

Table 4: Grade 2 – KSS Score

	Pain Point				Range of Motion				Walking				Stairs Climbing			
No. of Joints	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**1 Year**
1	10	20	45	45	100	100	100	120	30	40	40	40	50	50	50	40
2	10	20	45	45	110	110	110	120	30	40	40	40	50	50	50	40
3	10	45	45	45	100	100	110	105	40	40	40	40	30	40	40	30
4	10	45	45	45	110	110	110	105	40	40	40	40	30	40	40	30
5	10	10	30	45	110	110	100	100	20	20	40	40	40	40	50	40
6	20	50	50	45	100	110	105	105	20	40	40	40	30	50	30	40
7	20	50	50	45	100	110	105	105	20	40	40	40	30	50	30	40
8	10	45	50	45	100	110	105	105	20	40	40	40	30	30	40	40
9	10	45	50	45	110	110	105	105	20	40	40	40	30	30	40	40
10	10	45	50	45	110	110	110	105	40	40	40	40	0	50	50	40

Table 5: Grade 3 – KSS Score

	Pain Point				Range of Motion				Walking				Stairs Climbing			
No. of Joints	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**1 Year**	**Pre**	**1 month**	**6 month**	**1 Year**
1	10	10	30	40	110	110	120	120	10	20	30	40	15	15	30	40
2	20	30	45	45	120	130	130	130	20	30	30	40	30	40	40	50
3	20	30	40	45	110	110	110	120	20	30	40	50	30	30	40	50
4	20	30	40	50	120	120	120	130	20	30	40	50	30	30	40	50
5	1	10	30	40	100	100	110	120	10	20	30	40	15	30	40	50
6	10	20	40	45	110	110	120	130	20	30	40	50	30	40	40	50
7	10	20	40	40	110	100	100	120	10	20	40	40	15	30	40	40
8	10	20	40	40	110	110	110	110	10	20	40	40	15	30	40	40
9	30	45	45	45	100	100	100	110	40	40	40	40	40	40	50	50
10	30	45	45	45	100	100	100	110	40	40	40	40	40	40	50	50
11	10	20	30	30	100	100	110	110	10	20	20	20	15	30	30	30
12	10	30	40	40	110	110	120	110	10	30	30	30	15	30	40	40
13	10	20	20	0	100	110	110	0	30	30	30	0	30	30	30	0
14	10	20	20	0	100	110	110	0	30	30	30	0	30	30	30	0
15	30	30	30	45	100	110	110	105	30	30	40	40	30	30	30	30
16	30	30	30	45	100	110	110	105	30	30	40	40	30	30	30	30
17	10	20	45	45	100	100	100	100	40	40	40	40	30	40	50	40

18	10	20	45	45	110	110	100	100	40	40	40	40	30	40	50	40
19	10	20	35	45	110	110	110	110	10	40	40	40	15	40	40	40
20	10	20	35	45	120	110	110	110	10	40	40	40	15	40	40	40
21	10	10	30	45	100	100	100	100	20	20	40	40	40	40	50	40
22	10	45	45	45	105	105	105	100	40	40	40	40	30	40	30	50
23	10	45	45	45	105	105	105	100	40	40	40	40	30	40	30	50
24	10	30	30	45	100	110	110	100	10	30	40	40	15	30	30	40
25	10	30	30	45	100	110	110	100	10	30	40	40	15	30	30	40
26	10	45	45	45	110	110	110	105	30	20	40	30	40	40	50	30
27	10	45	45	45	110	110	110	105	30	20	40	30	40	40	50	30
28	10	45	50	45	110	110	110	105	40	40	40	40	0	50	50	40
29	10	45	45	20	100	110	105	110	10	30	40	30	30	30	30	30
30	10	45	45	20	100	110	105	110	10	30	40	30	30	30	30	30
31	10	45	30	20	100	110	105	105	10	30	30	30	30	20	10	10
32	10	45	30	20	100	110	105	105	10	30	30	30	30	20	10	10
33	30	30	30	45	100	100	110	105	30	30	30	40	30	30	30	30
34	30	30	30	45	100	100	110	105	30	30	30	40	30	30	30	30

Table 6: Grade 4 – KSS Score

	Pain Point				**Range of Motion**				**Walking**				**Stairs Climbing**			
No. of Joints	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**12 month**	**Pre**	**1 month**	**6 month**	**1 Year**
1	10	30	45	45	110	100	100	120	10	30	40	40	15	40	50	40
2	10	30	45	45	110	100	100	120	10	30	40	40	15	40	50	40
3	10	40	30	40	100	100	105	105	20	40	40	40	15	40	30	40
4	10	40	30	40	100	100	105	105	20	40	40	40	15	40	30	40

Range of Motion: Improvement of KS score for Range of motion criteria was seen in all patients at 12 month follow up compare to pre-treatment. However, grade 3 patients shows improved score.

Pain: There was significant increase in pain point score in all subjects. Grade 2 patients showed improved score.

Walking: The walking score was improved from 30- 50 in patients with grade 3. The rest patients with grade 2 and 4 increased their walking limits pain free.

Climbing: The stairs climbing ability has improved in almost all subjects as compared to pretreatment.

Visual Analogue Score:

VAS score, though being entirely a subjective is highly indicative of patient's satisfaction. At the end of 1 year 24 out of 25 patients scored between 9-10.

Radiological evaluations

MRI studies at the interval of 1, 6 & 12 months did not show the cartilage regeneration. Further study with cartilogram and 2 –DESS also did not show evidences of cartilage regeneration.

Discussion

Stromal Vascular Fraction (SVF) from bone marrow and adipose tissue are the two cell lines emerging as a substitution for pure MSCs.[14,15] They contain mesenchymal stem cells. MSCs from both the sources are having identical properties. The limitation of bone marrow derived SVF is mainly its low yield of MSCs.[16] This is indeed low as compared to adipose tissue derived stromal vascular fraction.

The bone marrow aspiration is usually limited to 250-300 cc. Such a volume will not provide 1 to 4 million MSCs per kilogram body weight. We therefore preferred the adipose tissue as the source for SVF.

The conservative management which sustains physiotherapy & analgesic may suffice for grade-1 patients. The surgical intervention is indicated for grade 2, 3 and 4. Joint replacement has emerged as a standard surgical treatment since it is a major expensive surgical procedure and alternative is being sought.

Injection of PRP has advantage of an immediate pain relief; but, platelet does not have regeneration properties. The pain relief is short lived.[17]

Cytotherapy with chondrocytes obtained after translation from bone marrow derived mesenchymal stem cells as well as adipose tissue is also used.[18] This mode as per the ICMR guidelines comes into the category of substantial manipulation. The translation of cartilage would take about 2 to 3 weeks in the lab there is a remote chance of contamination. In that case, the entire culture needs to be thrown away. Additionally translated cartilage cells loose there stemness. Therefore, there anti- inflammatory property is lost along with their capacity to hone to the site of lesion. Thus implementation of cartilage cells may need a scaffold. For exact application to the eroded condoyle arthroscopy may also be needed. Cultured MSCs from bone marrow and adipose tissue are also in use. Some researchers have advocated multiple injection- upto 3 times intra articularly.

The present innovation over rides the disadvantage of above methods. Additionally there is an added advantage of synergistic action by hematopoietic and other cell types.

This is the one of the first few report for the use of SVF in osteoarthritis. For establishing the dose we relied on Chris et.al 2013.[19] They have used 1 million MSCs / kg body weight. The intraarticular administration of SVF is unlikely to go in circulations: The stem cells, instead will hone to the inflamed joint surfaces. For better results we deployed up to 4 million MSCs/ kg body weight that may have been contained in the quantity of SVF injected. This is most likely to be from ~250 million SVF cells injected.

The absence of the demonstrable cartilage regeneration on MRI even after 1 year is explained by the fact that regenerated cartilage is suppose to be embryonic like cartilage.[20] That does not cast a shadow. Most researchers have reclined for efficacy on the clinical criteria as decided by WOMAC, KSS and VAS.[21] We therefore believe that it is safe and efficious to treat grade 2, 3 and 4 osteoarthritis patients with minimal invasive procedure. The series needs to be expanded to 100 cases to complete phase II clinical trial.

Conclusion

The study establishes the safety and the efficacy for a novel therapeutic mode for the treatment of osteoarthritis.

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