

## Original Article

# Research Innovation for Osteoarthritis Knee by Adipose Derived Stromal Vascular Factor with Platelet Rich Plasma

Bhaskar Vyas<sup>1</sup>, Pradip Mehta<sup>2</sup>, Rajni B Vyas<sup>3</sup>, Palak Waghela<sup>4</sup>, Anant Marathe<sup>5</sup>

**Purpose :** The study aims to innovate the potential of Adipose derived Mesenchymal Stem Cells to chondrogenic translation so as to establish a less invasive, affordable alternative to knee replacement.

**Background :** Adipose tissue derived Stromal vascular fraction (AD-SVF) is heterogeneous mixture of different cell population. SVF possesses potential to translate into osteogenic and chondrogenic lineages. We designed a study to assess safety and efficacy of AD-SVF in 33 patients (for treating 66 knees) having osteoarthritis.

**Basic Procedure :** 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 Platelet-Rich Plasma (PRP) was given. Clinical outcomes were assessed with WOMAC and KSS scores at the interval of 1, 6 and 12 months. Pre and post MRI studies were done.

**Main Findings :** The safety of AD-SVF was confirmed as there were no adverse reactions. Patients exhibited rapid and progressive improvement of WOMAC & KSS Score by 12 months. For MRI studies, 1.5 tesla machine was deployed. This machine was insufficient to demonstrate new cartilage formation.

**Implications :** Our data demonstrates that AD-SVF therapy in combination with PRP is a safe and valid alternative treatment for knee osteoarthritis in 33 patients (66 knees). The intervention is simple, minimally invasive, requires minimal hospitalization and surgery; provides relief of pain and stiffness and improves physical functioning.

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**Key words :** Osteoarthritis knee, Adipose derived Stromal Vascular Fraction, Stem cell therapy, Regenerative medicine.

In 9.6% of men and 18% of women aged more than 60 years have symptomatic osteoarthritis<sup>1</sup>. Osteoarthritis (OA) of the knee is now commonly treated surgically with total knee replacement when conservative management fails. Cartilage has limited self-renewal ability. This requires hospitalization, major surgery, that may need blood transfusion, has risk of infection and the treatment is expensive. Hence there is a need for development of alternate therapeutic modalities which are less- invasive and less expensive.

Stem cell technology is rapidly developing as a tool for regenerative medicine. Mesenchymal Stem cells [MSCs] possess numerous properties like anti-apoptosis, angiogenesis, growth factor production, anti-fibrosis, anti-inflammatory, immuno protection and chemo-attraction; in addition to translation to

<sup>1</sup>MS, Plastic Surgeon, Stem Cell Scientist, Total Potential Cells (P) Ltd, Parimal Majumdar Wada, Raopura, Vadodara 390001 and Corresponding Author

<sup>2</sup>MS, Orthopedic Surgeon, Samanvaya Trust, Parimal Majumdar Wada, Raopura, Vadodara 390001

<sup>3</sup>MD, Stem Cell Scientist, Total Potential Cells (P) Ltd, Parimal Majumdar Wada, Raopura, Vadodara 390001

<sup>4</sup>MD (Radiodiagnostics), Consulting Radiologist, MRI Centre, Sir Sayajirao General Hospital, Vadodara 390001

<sup>5</sup>Ph D, Technical Director, Total Potential Cells (P) Ltd, Pranav Lab, Raopura, Vadodara 390001

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### Editor's Comment :

- Article is original research in Indian Innovation.
- There is substantial clinical evidence for ADSVF application to be considered as a substitute for joint replacement for osteoarthritis knee.

chondrocytes. Numerous preclinical trials have shown promising results in osteoarthritis using SVF in various animal models<sup>2-4</sup>.

SVF isolated from human subcutaneous adipose tissue is an extract representing a heterogeneous collection of cells. Its composition is Mesenchymal stem cells (30-40%) having chondrogenic potential<sup>5,6</sup>, endothelial precursor cells, T regulatory cells, macrophages, smooth muscle cells, and pre-adipocytes. Cells other than ADMSCs having CD 34+ cells in SVF also support angiogenesis<sup>7</sup>.

Highly biologically active molecular substances obtained following the disintegration of platelets not only cause an immediate relief from pain but also potentiate the action of MSCs. The effect is immediate but is not long lasting and cannot regenerate the cartilage singularly. Therefore PRP was used in combination with SVF.

Autologous SVF was injected intra-articularly along with PRP to investigate safety & efficacy in 33 patients (66 knees) with Osteoarthritis.

## MATERIAL AND METHODS

A pilot study of 6 patients was conducted with satisfactory results<sup>8</sup>. The pilot study was carried out between 2011- 2013 with prior approvals from Institutional Ethics Committee. The study was enrolled as a clinical trial CTRI No 2013/02/004619.

### Regulatory Approvals :

Indian Council of Medical Research (ICMR) has issued several editions of guidelines regarding stem cell research and therapy. The latest edition was in 2017.

In April 2018, Govt. of India published a notification in the Gazette to clarify about Stem Cell therapeutics. Accordingly, products such as Stromal Vascular Factor derived from Adipose Tissue come under the category of minimal manipulation.

A pilot study of 6 patients was conducted and results are published, whereby both safety and efficacy were provided with a proof of concept. The research was submitted for funding by Biotechnology Industry Research Assistance Council (BIRAC), Department of Biotechnology, Government of India and technical expert committee headed by Prof G Padmanabhan granted approval for funding. The research was conducted at Department of Scientific and Industrial Research (DSIR) certified research laboratory by Total Potential Cells Pvt Ltd (TPCPL) Research underwent scrutiny by Ethics Committee registered with DCGI by TPCPL. Institutional Committee for Stem Cell Research and Therapy under the Chairmanship of Dr Ramesh Bhonde at TPCPL further scrutinized the research and granted its approval. This was forwarded to ICMR.

Biotechnology Ignition Grant Scheme (BIG) funding was received with a time-limit to complete the project by 18 months. No: BIRAC/CCAMP0130/BIG -03/13. DCGI was informed. Clinical trial was registered at Clinical Trial Registry of India by their no. CTRI No 2013/02/004619. DCGI and ICMR representatives visited TPCPL by invitation and verified documents concerning Institutional Ethics Committee and ICSCRT approval in 2017. They noted that there were no adverse side effects and there was proven efficacy within the specified time limit by BIRAC. BIRAC in 2018 published the research in their book "BIRAC Innovations : Propelling the Bio-Economy"

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Following clinical examination of 138 patients, 33 patients with osteoarthritis of both the knees were selected as per the Inclusive & Exclusive criteria. Follow up of clinical and radiological assessments were

done up to 18 months.

### Clinical Assessment was done as follows :

**Western Ontario and McMaster Universities Arthritis Index (WOMAC)Score :** This index evaluates pain with 5 criteria, stiffness with 2 criteria and physical function with 17 criteria. The scores are summed for items in each subscale. The possible ranges are; pain=0-20, stiffness=0-8, physical function=0-68. The statistical decrease in the score will indicate improvement.

**Knee Society Score (KSS) :** In KSS pain parameter, range of motion, walking, and stairs climbing were assessed. The scoring for pain point was 0-50; range of motion is 105-120; walking 0-50; stairs climbing 0-50. The statistical increase in the score will indicate improvement.

Total 33 patients with radiological evidence for osteoarthritis of both knees, satisfying selection criteria are included. MRI details in additions to Loss of Cartilage in patients are described in Table 2.

The patients underwent blood investigations including complete blood count, blood sugar, blood urea, serum creatinine, SGPT, HIV (I and II) antibody test, hepatitis B & C, Prothrombin time, thyroid stimulating hormone estimation.

### Surgical Protocol :

The surgical procedures for lipoaspiration and intra-articular injection into the knee joints were performed at Premdas Jalaram Hospital, Spandan Hospital Vadodara Gujarat India. 800 – 1000 cc of lipoaspirate was obtained from lower abdomen. Under expert hands, collection of such huge amounts of lipoaspirate was routinely done under local anaesthesia with sedation. Lipoaspirate was processed at Total Potential Cells (P) Ltd, a GMP Class V research lab, Department of Science & Industrial Research approved in-house lab.

### Isolation of Stromal Vascular Fraction :

Adipose tissue was aspirated with specially designed aspiration cannulae, with diameter ranging from 3mm, 4mm, 5mm and 6mm under local anesthesia. Lipoaspirate, with uniform viscosity like honey, was collected from lower abdominal subcutaneous fat. The samples were transferred to a GMP class V laboratory for cell isolation.

The isolation of adipose tissue derived stromal vascular fraction (AD-SVF) was performed with density gradient centrifugation technique. ADSVF from lipoaspirate were isolated after digestion with 0.2 % collagenase were incubated in CO<sub>2</sub> Incubator at 37° C. Cells obtained were made into a pellet at 1500 g in centrifuge.

### Standard Operating Procedure for obtaining Concentrated Platelet Rich Plasma (ConcPRP) :

Concentrated PRP is obtained from 25cc

autologous blood for 1 joint with centrifugation 3 times at 200 x g for 20 min, 100 x g for 10 min and 800 x g for 15min at room temperature.

After the first spin 3 distinct layers will be observed –

**Bottom layer :** Red blood cells (accounting for 50–80% of the total volume)

**Middle layer :** Very thin band of white blood cells – buffy coat

**Top layer :** Straw-colored PRP

After second spin, buffy coat will be well defined. Supernatant plasma will be pipetted out leaving buffy coat undisturbed.

After third spin, a pellet containing most of the platelets will be generated. Keeping ~2 cc, the remaining supernatant will be discarded. When the pellet is dissolved in ~2 cc, it will yield concentrated platelet rich plasma to be injected into the knee joint.

**Characterization of ADMSCs :**

SVF was characterised to flow Cytometry (Fig 1). (Toprani Labs, Vadodara and C-CAMP, Bangalore) Isolation, culture and characterization with immuno cytochemistry of MSCs were done. Characterization was performed with 4 positive markers and 3 negative markers (Figs 2 & 3).

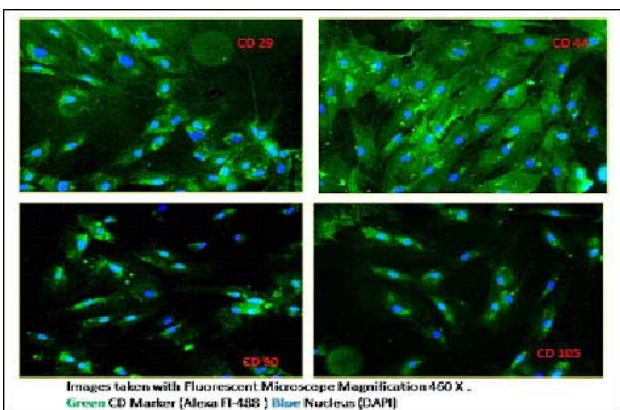


Fig 2 — Characterization of the ADMSCs (Positive markers)

**Statistical Analysis** was done by Prism software (version 6). All data are expressed as mean ±SD. Data was further analysed with ANOVA followed by Turkey’s multiple comparison test for relevant inferences.

**Proportion of AD-SVF & Concentrated PRP :**

2cc of AD-SVF will be mixed with 3cc of Concentrated PRP to be injected into knee joint.

**OBSERVATION**

Sl.No.	Sex (M/F)	Age (yrs)	Weight (kg)	Amount of lipoaspirate	No of SVF cells million
1.	F	58	55	250	110
2.	F	68	62	200	124
3.	M	79	59	270	118
4.	F	67	62	400	248
5.	M	63	60	470	237
6.	F	58	67	450	268
7.	F	45	75	700	250
8.	F	60	68	700	210
9.	F	56	104	800	322
10.	F	56	78	700	260
11.	F	77	46	400	209
12.	M	64	56	500	287
13.	M	67	82	480	189
14.	F	49	71	1000	240
15.	M	62	82	650	206
16.	F	61	57	800	390
17.	F	65	42	420	157
18.	M	72	63	550	254
19.	M	71	66	700	290
20.	F	56	64	470	253
21.	F	59	65	550	436
22.	F	62	63	525	230
23.	M	53	65	550	240
24.	F	61	85	600	279
25.	F	55	68	650	220
26.	M	60	87	700	579
27.	F	60	70	700	298
28.	M	56	70	600	329
29.	F	62	57	650	259
30.	F	66	71	800	264
31.	F	60	90	800	469
32.	M	76	68	600	224
33.	F	68	70	850	610

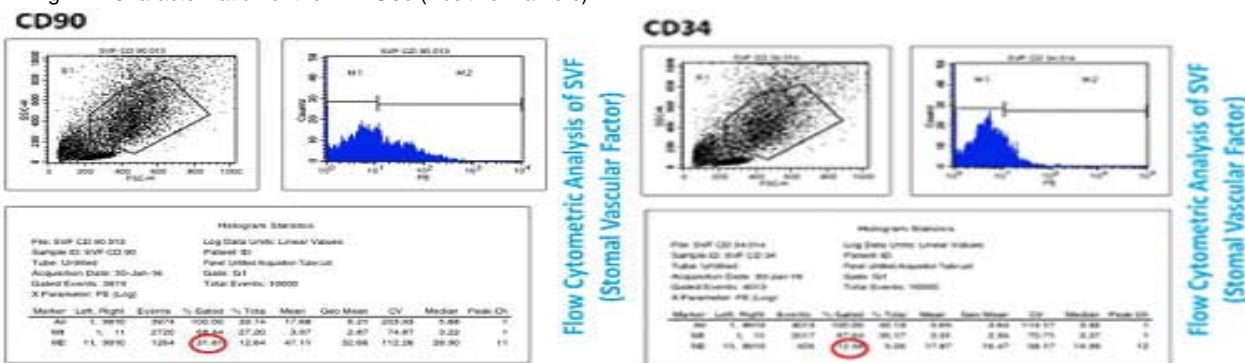
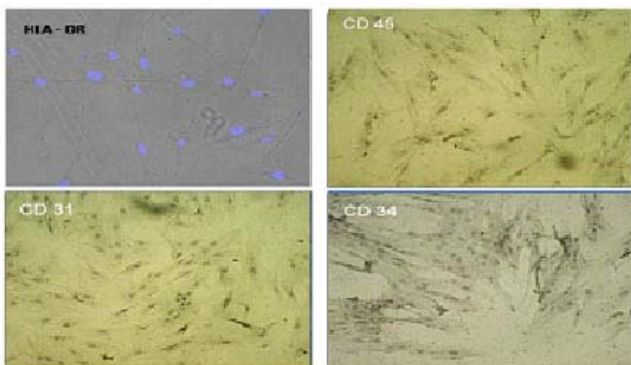


Fig 1 — Flow Cytometric Analysis of cell population in ADSVF: (a) Cells Positive for CD90 in Flow cytometric analysis of SVF CD90 n=31.81 – 39%, (b) Cells Positive for CD34 in Flow cytometric analysis of SVF CD34 n=12.58%





Images taken with Fluorescent /Light Microscope Magnification 460 X . HLA Marker (CY-3) Nucleus(DAPI/ Hematoxyline) Blue / Pararub

Fig 3 — Characterization of the ADMSCs (Negative markers)

**Flow Cytometry Analysis :** SVF in our samples contain 39% MSCs and 12.5% hematopoietic cells.

**WOMAC Score :**

Post Operative WOMAC Score of 66 knees before and after administration of SVF and PRP were as follows \*p<0.05, \*\*p<0.01, \*\*\* p<0.001 for Pre vs Follow-up groups; #p<0.05, ##p<0.01, for comparison of 1month follow up vs 6 and 12 month follow up groups (Tables 1,2 & Figs 1,2 ).

The WOMAC score for right knee and left knee significantly (P<0.001) dropped with respect to pre treatment. In 31 of 33 the subjects, the score dropped at very first month and continued to improve till 12 months, 1 patient had prolapsed meniscus in the joint space and was advised excision. One patient was lost to follow up.

**KS Score :**

The increase in score indicates improvement. KS Score in 33 patients are shown in tabular representation. There was overall improvement in all the four criteria as per statistical analysis (Table 3).

KS Score of Osteoarthritis patients (total 66 knees) before and after administration of SVF and PRP \*p<0.05, \*\*p<0.01, \*\*\* p<0.001 for Pre vs Follow-up groups; # p<0.05, ##p<0.01, for comparison of 1month follow up vs 6 and 12 month follow up groups.

One patient developed the symptoms of pain. He continued to do cycling for ~15 kms a day against the advice. On examination with MRI he was found to have prolapse of lateral and medial cartilage into the joint. This was removed with arthroscopy. Another patient required to be treated with a repetition of the same procedure after 17 months. He was walking ~7-8 kms a day and was repeatedly going up and down staircases upto 4<sup>th</sup> storey.

The physical therapy outcomes in

terms of range of motion, subjective pain, and functional status were all improved.

**DISCUSSION**

Present study confirms the cell population in Stromal vascular factor as reported by previously by Rada *et al*, 2010<sup>9</sup> Preponderance of Mesenchymal stem cells in SVF derived from adipose tissue to the extent of 39% in our samples would have significantly contributed to better results than obtained from other sources.

MSCs exhibit different levels of osteogenic and chondrogenic differentiation properties. The regenerative property of SVF primarily depends on MSCs.

Nguyen *et al* 2016<sup>10</sup> reported that autologous adipose derived SVF and PRP was injected to treat Osteoarthritis knee. Their study design was comparable to the present study. Nathan *et al* 2016<sup>11</sup>,

Table 1 — Details of Lipoaspirate of the Patients undergoing treatment

MRI Findings In OA Patients	No of Knees
MMT = Medial Meniscus tear	26
LMT = Lateral Meniscus Tear	9
EMJ = Extrusion of meniscus joints	0
ACL = Anterior cruciate ligament	21
PCL = Posterior cruciate ligament	6
MCL = Medial Collateral Ligament	0
LCL = Lateral Collateral Ligament	0
CYST	16
OSTEO = Osteophytes	23
ISB = Infusion in suprapatellar bursa	22
SFTJ = Subluxation in femur-tibial joint	6
FB = Foreign bodies	4

Table 2 — The Western Ontario and McMaster Universities Arthritis Index (WOMAC) score in Osteoarthritis Patients Pre and Post treatment with Stem cells and PRP

	Pre	1 month	6 month	1 Year
Right Knee	67.84±13.21	42.75±20.54***	16.56±12.26***	9.09±9.49***
Left Knee	64.31±14.27	37.34±17.07***	15.13±13.45***	8.19±8.96***

Table 3 — The Knee Society Score in Osteoarthritis Patients Pre and Post treatment with Stem cells and PRP

Knee Society Score - Pain Point				
Right Knee	12.59±7.47	29.53±12.85***	38.13±8.59***	40.47±9.87***
Left Knee	14.38±7.16	32.97±10.99***	40.47±7.76***	42.19±10.16***
Knee Society Score - Range of Motion				
Right Knee	103.91±4.71	105.94±4.83	107.66±5.08	106.72±20.85
Left Knee	107.03±6.2	108.75±5.96	109.69±7.06	107.66±21.51
Knee Society Score - Walking				
Right Knee	21.88±11.48	30±8.42**	37.19±5.23***	36.88±8.59***
Left Knee	21.88±10.61	32.5±6.22***	37.5±4.4***	37.5±8.42***
Knee Society Score - Stairs Climbing				
Right Knee	25.31±10.92	35.47±8.74**	37.19±9.58***	37.5±10.78***
Left Knee	25.47±11.17	34.38±8.4**	38.13±8.96***	38.44±11.1***

reported 4 cases with autologous AD-SVF and PRP followed with subjective scoring of 12 months post therapy with favorable results. The present study reports 33 patients with age of 45-75 years, a larger group to assess safety and efficacy with internationally accepted WOMAC and KSS score for 12 months.

PRP has shown to contain 1507 protein based bioactive factors, Qureshi *et al*, 2009<sup>12</sup>. These factors like TGF B and FGF2 help in formation of new cartilage<sup>13</sup>. Combination of PRP with MSCs in intra-articular injections increases collagen type II expression and reduces chondrocytes apoptosis as reported by Koh *et al*, 2013<sup>14</sup>.

Repair and regeneration of cartilage tissue in the meniscus is explained by Barry *et al*, 2013<sup>15</sup>. They concluded that adult mesenchymal stem cells stimulate the regeneration of meniscus tissue and delayed the progressive destruction of cartilage.

Hurne *et al*, 2012<sup>16</sup> observed in C57BL6J mice where in ADMSCs thickened synovial lining and formation of associated ligaments and reduced destruction of ligaments. Favourable results in our study, even in patients with cruciate ligament rupture can be explained on this basis.

Favourable results in our study need to be buttressed further with (1) increasing the post-operative follow up with MRI studies with higher powered machine such as 7.5 Tesla (2) post treatment follow up with arthroscopic observation of the joint (3) follow up of the patient with longer duration, of 5 years.

We have shown that SVF along with PRP improved WOMAC as well as KSS score. We have observed improved score corresponding in OA patients without use of major invasive methods.

### Limitations of the study :

The study has the following limitations:

(1) Translation of MSCs to chondrocytes/promoting chondrogenesis needs to be established with higher power of MRI machine and arthroscopy.

(2) The follow up of longer the 12 months is needed.

### CONCLUSION

Intra-articular injections of autologous adipose SVF in combination with autologous PRP present a promising, minimally invasive option of treating OA knees.

### ACKNOWLEDGEMENT

The M S University, Baroda has been incubator for the study. Manipal Institute of regenerative medicine, Bengaluru and Toparani Advance Lab, Vadodara for providing necessary facilities for Flow cytometry.

Research innovation is available in open domain. Approximate cost will be around Rs. 50,000.

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