Original Article

Vast Potential for Clinical Trial Opportunities; Adipose Tissue Derived Mesenchymal Stem Cells

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Introduction : This is a research innovation that aims to provide an additional therapeutic tool. It will open up a vast panorama of regenerative medicine by application of Adipose Derived Mesenchymal Stem Cells (ADMSCs). ADMSCs are selected since a large amount is available for lipoaspiration and a larger percentage (30%) of Mesenchymal Stem Cells (MSCs) obtainable there from. The applications in clinical practice extend across Mesoderm, Endoderm and Ectoderm layers[1].

Material and Methods : There are three products that can be derived from the lipoaspirate.

They are (1) Stromal Vascular Fraction (SVF), (2) Islet Cell Aggregates (ICAs) Translated from ADMSCs, (3) and ADMSCs with ~95% purity. They are deployed to illustrate the safety and efficacy in clinical trials for (1) Mesoderm Translation as in Osteoarthritis Knee, (2) Endoderm translation to Insulin-producing Cells as applicable to diabetes, and (3) Ectodermal Translation as applicable on Non-healing Indolent Ulcers on the Skin.

Results : All three products are found safe with no adverse side effects. Proof of concept studies along with initial clinical trials for Osteoarthritis, Diabetes Types I and II, and Non-healing ulcer of any aetiology is demonstrated with objective evidence.

Discussion : The evidence based on the results of the clinical trials across all three Germinal Layers is cited along with literature support.

Results are explained based on a plausible scientific hypothesis.

Conclusion : The study enunciates that Autologous SVF and ADMSCs are in futuristic domain for conducting clinical trials across all the three Germinal Layers.

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***Key words* : Research innovation, ADMSCs, SVF, Clinical trial, Regenerative medicine.**

This is a research innovation utilizing the properties of MSCs. MSCs provide a futuristic therapeutic hope for Regenerative medicine. MSCs have fundamental properties such as honing to the site of injury or inflammation[2] and translation to the honed tissue in situ[3]. They are immunoprotective[4] as well. MSCs derived from bone marrow have been the subject of extensive research. However, bone marrow has lower yield of MSCs. Further, there is a limitation to the quantity of bone marrow aspiration. Lipoaspiration of Adipose Tissue contains ~30% MSCs[5] and can easily be obtained up to ~1,000 cm^3 under local anaesthesia.

This article aims at adding some more tools in the armamentarium of medical doctors and corporate Hospitals in pursuit of Regenerative Medicine. The tools are: Stromal Vascular Fraction (SVF) from lipoaspirate, Islet Cell Aggregates (ICA) obtained by transgerminal translation of ADMSCs and purified MSCs. An Autologous cell-based product, Platelet-rich Plasma (PRP) is added as an adjunct since several analgesic and Growth-promoting Cytokines are immediately released by this product[6]. PRP does not have regenerative capability. PRP acts for a short duration and the action does not last more than 3 months[7]. This Stem Cell Research is permissible as per ICMR Guidelines. It also falls in permissible funding research area by Medical Research Council of UK. Stem Cell Research was also recommended by the European Parliament.

Editor's Comment :

- The research innovation based on transgerminal translation of ADMSC is to provide numerous opportunities for deriving products that will constitute a futuristic regenerative medicine.
- Proof of concept is provided.
- Opportunities are doable according to ICMR, DCGI approvals.
- Multi specialty Hospitals/Groups will qualify.

Materials and Method

Enunciation of Concept :

The concept has a fundamental scientific basis which has been established in our laboratory[1]. It has

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Global literature support[8], the clinical trials were presented to the Institutional Ethics Committee of Total Potential Cells. Further approval was obtained with Institutional Committee for Stem Cells Research, Total Potential Cells.

Proof of Concept Study for Diabetes : (n = 7)

A clinical trial comprising Type I Diabetes patients (n = 2) and Type II (n = 5) was registered with DCGI registered no. REF/2013/02/004619. Partly funded by Small Business Innovation Research Initiative, Government of India.

Clinical trial Phase 1 for Osteoarthritis Knee [9] : (n = 6)

•• Six patients of Osteoarthritis Knee (Grade III: 3 and Grade IV: 3) were treated. They were followed up with clinical assessment as per Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Knee Society Score (KSS) for one year with successful subjective and objective improvement.

Phase I Clinical Trial[10]

•• The project was submitted to technical expert Committee, Biotechnology Industrial Research Assistance Council (BIRAC). It was approved. It was funded as Business Ignition Grant (BIG) by BIRAC, Department of Biotechnology and Government of India. It began as Clinical Trial Phase 1 by June, 2015 and was completed by December, 2016. Fifty knees were studied as per well defined Criteria for Clinical Trial.

•• Inclusion and exclusion criteria for treatment were defined.

Inclusion Criteria : Patients of either sex with age 45 to 75 years, near-normal Body Mass Index and Osteoarthritis (OA) diagnosis based on Radiological Evidence were included. Patients with Metabolic Disorders 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 Radiological. Assessment of WOMAC, KSS and VAS Score of each individual enrolled was done.

Exclusion Criteria : Patients with history of taking Corticosteroids, NSAIDs, Glucosamine 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 also excluded.

•• Studies of Indolent Chronic Ulcers (N = 3)

Autoimmune process is attributed as a causative factor to Indolent Ulcer of long duration. Since MSCs have immunoprotective property they were deployed as an addition to ongoing local treatment in 3 cases. Two patients had Autoimmune Disease, one of them had Autoimmune Rheumatoid Disease and other had scleroderma.

Methodology

Lipoaspiration :

Lipoaspiration of Adipose Tissue was done by plastic surgeon under local anesthesia. 500 to 600 mL lipoaspirate was collected with fine cannulae for treatment of Diabetes and osteoarthritis. The amount of lipoaspiration for application to chronic ulcer varied between 50 mL to 250 mL, depending upon the size of the Ulcer.

Preparation of SVF :

Samples of lipoaspirate were studied. 1 ml of lipoaspirate yielded 0.5 million SVF cells with the following protocol:

Lipoaspirate collected was processed in GMP class V laboratory. Lipoaspirate was washed with Phosphate Buffered Saline (PBS) to remove blood. Later the washed lipoaspirate was subjected to enzymatic digestion with collagenase Type 1 to separate the cells from aspirate. With further centrifugation a concentrate of SVF cells was obtained as a small pellet, in about 2ml. The cell pellet obtained was washed twice and subjected to cell counting. Flow cytometry studies (Fig 1) reveal a mixture of MSCs, Preadipocytes, Hemopoietic cells, Endothelial progenitor cells, T cells, B cells, Mast cells, and Macrophages with 30% of MSCs (CD90 positive) and 10% - 12.5% of Hemopoietic cells (CD34 positive).

Culturing of ADMSCs :

Cells obtained from SVF were plated in T25 tissue culture flasks at the density of 2000 cells/cm^2. These were cultured in (Dulbecco's Modified Eagle Medium)

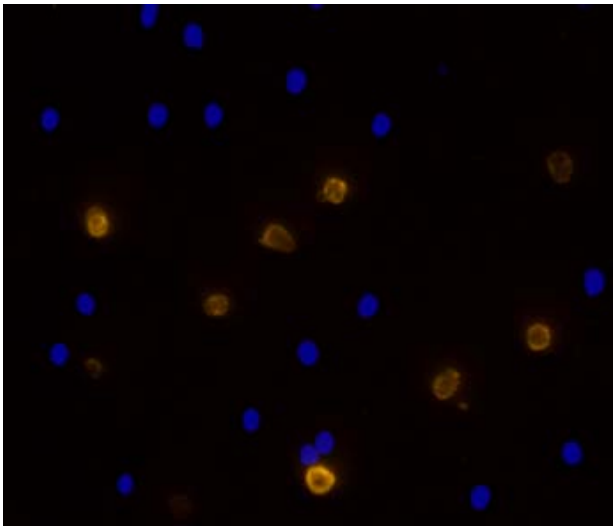

Fig 1 — Flow Cytometry analysis of SVF

DMEM with 10% FBS and 0.1% Antibiotic Antimycotic Solution. Media was changed every third day. Cells expanded in the culture were characterized with Immunocytochemistry. Thus, MSCs with ~95% purity was obtained with 2 passages.

Translation of ADMSCs to ICAs (Islets-like Cell Aggregates) :

The ADMSCs obtained were cultured in Serum Free Media - SFM A, SFM B and SFM C as per Chandra et al (2011)[8]. The ICAs obtained via this process were evaluated for Insulin production, C-peptide presence. These ICAs produced were injected Intrahepatically in patients of Diabetes.

Preparation and Culturing for Obtaining MSCs :

Cells were cultured for 7 days to obtain pure ADMSCs. These were cells that showed characteristics of MSCs. They were adherent to plastic surface of flasks, positive for CD29, CD44, CD90 and CD105 cell surface markers (Fig 2) and were negative for CD34, CD31 and CD45 Cell surface markers (Fig 3).

Translation to ICAs :

Approximately 500 ICAs were obtained in each plate. These showed glucose-dependent insulin release in in vitro tests.

Islets were positive for C-peptide marker.

Clinical Data :

Osteoarthritis —

SVF was mixed with 4 mL of PRP and were injected intra-articular in the patients via Supra and Medial portal in the Knee joint by an Orthopaedic surgeon—3 million cells/kg of body weight were injected. Patient was kept under observation for 24 hours and was advised rest for 8 days. Pre- and post- treatment WOMAC and KSS scores were taken to access efficacy of the SVF.

Diabetes —

Initially, SVF amounting to 3 million cells/kg of Body Weight were injected Intramuscularly divided into 4 injections. After 21 days, the second injection of ICAS was given Intrahepatically via Percutaneous Route with Sonography control. Approximately 500 ICAs were injected. Patient was kept under observation for 24 hours.

Non-healing Ulcers —

Wound site was cleaned and disinfected with Topical Polyvinyl Iodine 5%. The dead tissue was removed and later 15 - 150 Million ADMSCs were injected in the wound periphery with Hypodermal needle; also, 50 million cells were sprayed over the granulating surface of the wound. The wound was lightly covered with tulle-graze dressing.

Results

There was no adverse reaction recorded in any of the treatment procedures. Thus, safety is well established.

Osteoarthritis —

Pain relief was obtained after 2 to 7 hours of injection in each knee. Post WOMAC and KSS scores improved as shown in Figs. 4 & 5. WOMAC score decreased significantly in both left and right knee with Brown Forsythe Statistical Analysis Test ($p = 0.0208$, 0.0058) in all the subjects after the treatment with SVF.

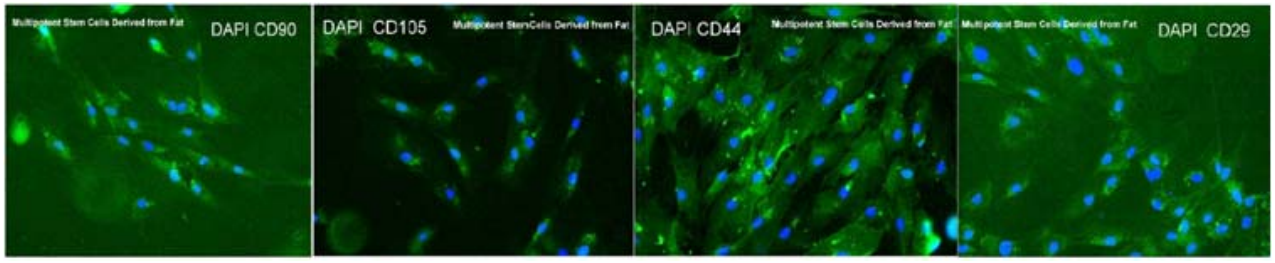

Fig 2 — Positive cell markers – CD29, CD44, CD105, CD90

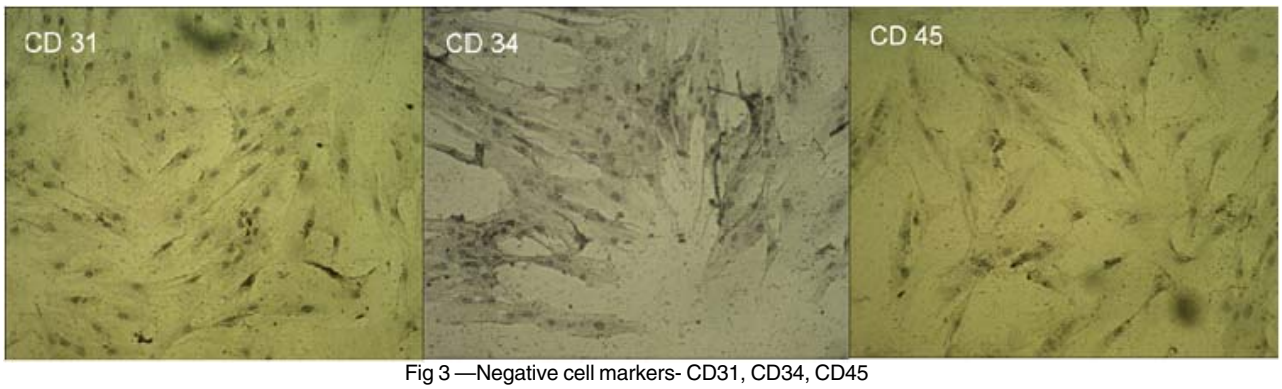

Fig 3 —Negative cell markers- CD31, CD34, CD45

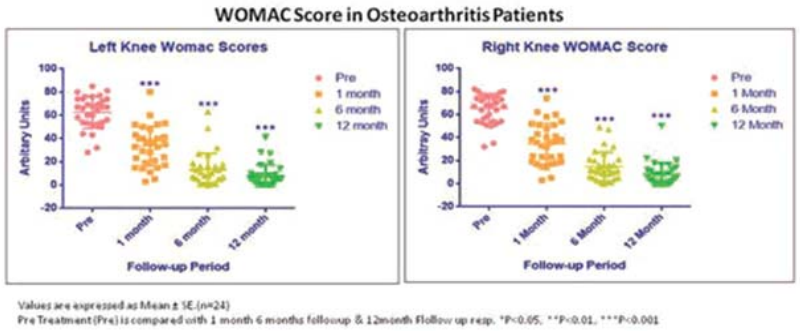

Fig 4 — WOMAC score of osteoarthritis subjects before and after treatment with SVF

KSS score showed increase in all categories, that is, as applied to pain relief, walking and climbing (p = 0.0111, 0.0005; 0.0054 < 0.0001; 0.8194, 0.9266). One patient was lost to follow-up during the treatment.

Diabetes —

There was no adverse reactions post injection of SVF. There was mild improvement as seen by decrease in Blood Glucose levels. However, after ICA injection the patient showed remarkable improvement in Blood Glucose levels both in fasting and Post Prandial Blood Sugar States and were even advised to decrease their Insulin dosage or Oral Drug Therapy as shown in the Table 1.

Non-healing Ulcers —

SVF as well as simultaneous application of cultured MSCs have proved highly efficacious in cases of indolent Ulcers that heal within a month or so. We have treated a case of Chronic Indolent Ulcer in Scleroderma. The patient had a Non-healing Ulcer on fingers following the breakdown after manipulation. It healed within a month with injection of MSCs on the peripheral edges of the ulcer. A decubetous Ulcer on the heel in a paraplegic patient, 5 cm in diameter, extending in depth up to Calcenium was treated. Following debridement and gauging out necrotic bone, SVF was locally applied. Approximately, 150 Million ADMSCs were injected on the periphery of the wound. There was no irritation or adverse reaction. The wound came to surface with obliteration of cavity by 15 days. The Ulcer got completely healed with Scar Tissue remaining as visible in 30 days (Fig 6).

A patient with Rheumatoid Disease had Chronic Non-healing Ulcer on medial side of the lower leg, 10 cm by 3 cm in diameter, extending up to medial maleolus. Following debridement, it was treated with local application of SVF. On the peripheral edges of the Ulcer, injection of MSCs, approximately 200 million were given Subcutaneously. The interval between two injections was 7 days. The healing occurred in about a month.

Coincidental Finding for Tissue Regeneration :

Grey hair turning to black was observed in 4 aged patients.[11] This published research has generated insights into Melanin Metabolism. It is being pursued for further Fundamental Research.

Improvement of vision in a Diabetic patient having retinopathy was observed.

Cure for widespread Peripheral Neuropathy in a diabetic patient was observed.

Discussion

Regenerative medicine has catapulted into the Surgical domain recently with the seminal publications by Nguyen *et al* (2016) and Guo *et al* (2016)[12,13]. Thus a Clinical Trial Platform is created for all Medical Doctors and Corporate Hospitals. The study has combined PRP with SVF. PRP does not have any of the properties of the Stem Cells for Regeneration[7]. However, it is a cluster of ~150 Cytokines that are known to have analgesic properties as well as Growth-promoting Factors.

The cost of finding a new molecular drug is steadily increasing from $ 1.2 billion in 2001 to ~ $ 3.0 billion

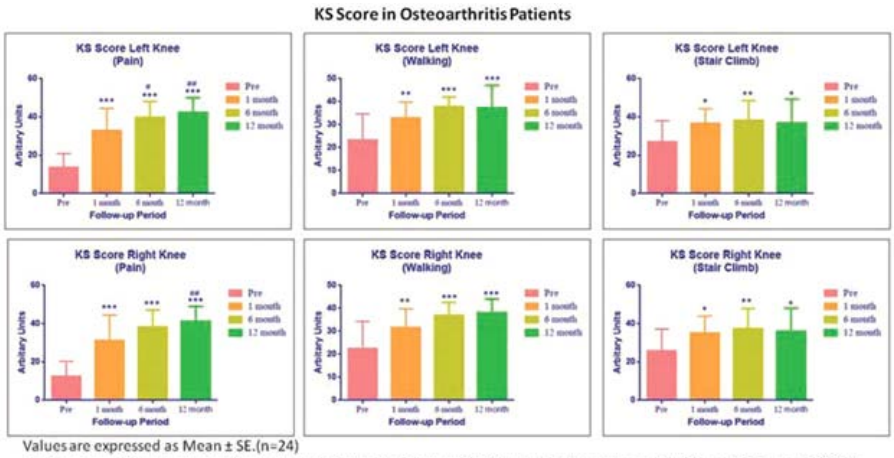

Fig 5 — KSS score of the Osteoarthritis subjects before and after treatment with SVF

Table 1 — *Lowering of Blood Sugar Levels and Insulin requirement reduced after Stem Cell Therapy*

Subject	Gender	Glucose levels (before induction)		Glucose levels (after induction)		Insulin dose (before induction)		Insulin dose (after induction)	
		Fasting	PPBS	Fasting	PPBS	Morning	Evening	Morning	Evening
1	F (14)	330	140	330	140	20 U	12 U	10 U	6 U
2	F (21)	180	360	110	260	24 U	12 U	16 U	10 U
3	M (46)	217	215	163	170	Tab	Tab	Tab	Tab
4	M (64)	140	206	120	160	Tab	Tab	Tab	Tab
5	M (58)	127	201	100	150	Tab	Tab	Tab	Tab
6	M (47)	249	198	130	160	Mixtard 50 U	Crystalline 40 U	Mixtard 40 U	Crystalline 20 U
7	M (78)	195	267	110	130	22 U	18 U	Tab	No drug

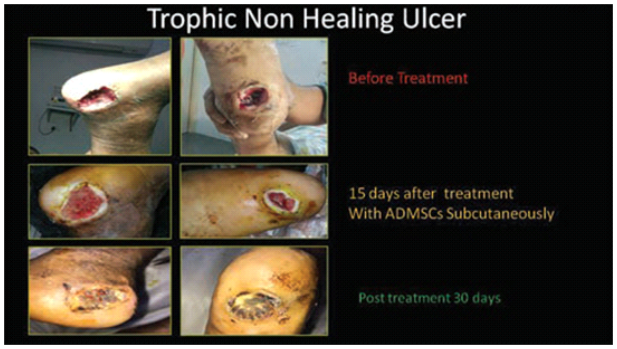

Fig 6 — Wound healing of full-thickness nonhealing trophic ulcer at different time points after autologous MSCs transplantation

in 2017[14]. The focus has shifted to Biological Resources. In the first decade of the present millennium, Obstetricians discovered a gold mine in the waste product that they routinely throw away; this was by way of Fetal Cord Blood.

Simultaneously, MSCs derived from bone-marrow underwent extensive research and application for regeneration. In the present decade this cell line is being replaced by yet another easily accessed and more abundant source of MSCs - this is Adipose Tissue. Refinement of laboratory methods has yielded an easier to obtain product, that is, SVF. SVF is easier to obtain since it does not require purification to obtain, culture, expand, or for translineage translation. This study reflects on a huge potential for Medical Doctors and corporate hospitals to utilize the skills so as to extend to a super specialty of Regenerative Medicine.

The safety of MSCs by Intra-articular,[15] Intramuscular[16] and Intravenous[17] route is established widely. Up to 4 million MSCs/kg body weight are safe. There are no side effects as well. The successful results in our Clinical Trial in Osteoarthritis Knee patients can be attributed to two factors: (1) application of unprocessed SVF combined with PRP and (2) application of high dose of SVF, not reported so far in literature. Osteoarthritis anywhere else in the body has similar Pathogenesis and Morbid Anatomy with only minor variations. Thus, SVF with enrichment of Platelets may also be applied to a Vascular Necrosis of Femur, Frozen Shoulder; delayed healing at fracture sites, etc. Further research possibilities exist for application of SVF in Rheumatoid Joints as well. This is on account of not only its Regenerative Properties but also because of Immunoprotection exhibited by MSCs. It seems plausible that in Insulin Resistance Type II Diabetes patients, injection with 3-4 million SVF cells/kg of body weight may lower the Insulin requirement [8]. Chandra *et al* (2011) have demonstrated the hypoglycemic effect of stromal vascular fraction (Fig 7).

Further, with expansion of MSCs, it will be possible to get larger number of ICAs. This may be utilized to treat Type I Diabetes. Type I Diabetes patients are generally controlled with ~40 Units of Insulin a day. It is likely that there will be individual variations in dosage for Diabetic patients; yet, an entirely safe method can easily be repeated with periodic intervals to arrive at an optimal level without Hypoglycaemia as a side effect. The possibility of obtaining 95% purified MSCs not only opens up the possibility of its application to Retinal blindness[18] but may extend into arena of Neurorestoration elsewhere. Translation to Ectoderm unfolds a wide vista of applications for healing of surface wounds. SVF as well as simultaneous application of cultured MSCs have proved efficacious in cases of indolent Ulcers. A dominant etiological factor in Non-healing is autoimmune process at the local area of occurrence or at Systemic Autoimmune Disease. Immunomodulatory property of MSCs[19] may be pivotal in the healing of these Ulcers. The recovery from Peripheral Neuritis in a diabetic patient is explained on the basis of transgerminal neurotranslation. Combined application of SVF + MSCs/pure MSCs can be judiciously applied to several Degenerative disorders that merit research. A coincidental finding—turning gray hair to black is difficult to explain but would have been possible only with enhancing endogenous melanin production. It remains to be further established whether this could be due to a single translineage translation to Ectoderm (hair) or a Simultaneous Endogenous translation (melanin)[11].

Conclusion

The protocol to produce SVF, ICAs and purified ADMSCs is doable by medical doctors and Corporate Hospitals. It opens up vast possibilities for application in Clinical Research.

Acknowledgements

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Limitations of the study : Requires approvals from Ethics Committee, Institutional Committee for Stem Cell Research and DCGI.

Funding: None

Conflicts of interest : None declared.

Ethical approval : Approved by Institutional Ethics Committee of Total Potential Cells.

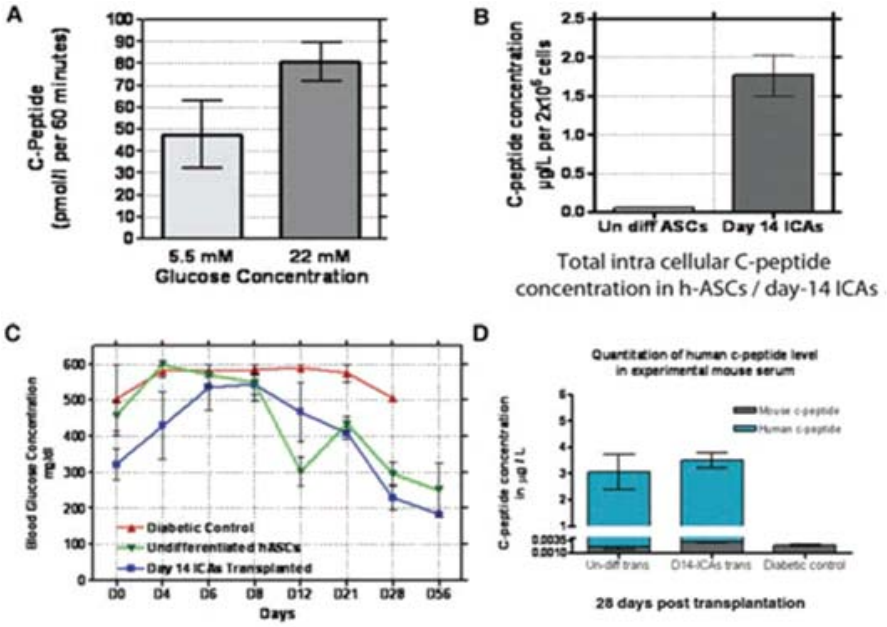

Fig 7 — Demonstration of hypoglycemic effect of SVF

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