INTRODUCTION

Mesenchymal stem cells (MSCs) are adult non-hematopoietic multipotent stem cells. They have received considerable importance in cellular therapy in degenerative disorders,[1,2] traumatic injuries,[3-5] and autoimmune disorders.[6] They have also been tried in metabolic disorders such as diabetes[7,8] due to their multiple modes of action and their transgerminal translation potential. They hone to the sight of injury or lesion,[9] they are immunonaïve and in addition, they have immunomodulatory action.[10] In this study, we have gathered evidence of transgerminal potential of MSCs in in vitro condition in our GMP Class V, Department of Scientific and Industrial Research approved stem cell research laboratory. This will foster newer modalities of cellular treatment of various disorders.
MSCs are found in varying quantities in the stromal vascular factor (SVF) obtained from different tissues. Adipose tissue (AT) is the richest source for MSCs since SVF obtained therefrom contains approximately 30% of MSCs. This is likely to open up a vast panorama of various conditions that can be treated with cellular products derived from AT.

MATERIALS AND METHODS

Selection of subjects for lipoaspiration
AT was obtained from healthy subjects undergoing liposuction for cosmetic reasons at a planned procedure. Informed consent of the subjects willing to contribute the lipoaspirate for research was taken before procedure. All necessary approvals were taken with Institutional Ethics Committee and Institutional Committee for Stem Cell Research and Therapy.

Lipoaspiration
Twenty-five subjects who consented underwent lipoaspiration by the plastic surgeon. A patient was given local anaesthesia. 1–2 cm-long incision was made on abdomen ventrolaterally. Lipoaspirate was taken along with the injected ringer solution under vacuum pressure. Five hundred millilitres lipoaspirate was collected for research purpose from each subject. The lipoaspirate was uniform in consistency and was devoid of any clumps.

Isolation and characterisation of stem cells
Lipoaspirate was washed with phosphate-buffered saline (PBS). Lipoaspirate was then subjected to enzymatic digestion (collagenase type 1, 0.75%) for 2 h with manual shaking. Finally, the aspirate was centrifuged at 1000 g to pellet down the mononuclear cells (MNCs). The MNCs obtained are SVF. The pellet was then washed twice with PBS. 2000 cells/cm² were seeded in the flask for culturing with Dulbecco’s Modified Eagle Medium (DMEM) (HG) and 10% of foetal bovine serum. The cells, adherent to the surface of flask, were used for characterisation using immunocytochemistry (ICC) and flow cytometry. Flow cytometry results were confirmed at two laboratories. ICC imaging of CD44, CD90, CD105, CD34, CD45 and human leucocyte antigen – -antigen D related (HLA-DR) was done using MSC maker kit (Abcam, USA) following manufacturer’s instruction. Similarly, quantification of stem cell population was done with flow cytometry using CD 44, CD 90, CD34 and CD45 markers.

Transdifferentiation into trigeminal cell types
MSCs obtained were subjected to differentiation into neurogenic cells (ectoderm), chondrocyte (mesoderm) and islet-like cell aggregates ICAs (endoderm). Neural differentiation was done using commercially available differentiation media (Gibco, Thermo scientific, USA) according to manufacturer’s instruction. Similarly, chondrocyte differentiation was done using chondrogenesis media (Gibco, Thermo Scientific, USA). ICAs were generated according to Chandra, 2012 protocol, briefly the confirmed adipose-derived MSCs were plated to low attachment plates (HiMedia Laboratories, Mumbai) at density of 10⁶ cells/cm² in DMEM: F12 until 80% confluence is achieved. Thereafter, cells were put in serum-free media (SFM) according to the protocol. Differentiation was carried out in three stages; initially, cells were resuspended in SFM-A and were cultured in this media for 2 days. On the 3rd day, cells were shifted to SFM-B media containing taurine. The cell aggregates were cultured in this medium for another 2 days. Finally, cells were further shifted to SFM-C on the 5th day for 14 days. The cell aggregates were fed with fresh SFM-C medium every 2 days in this period.

RESULTS

Two millilitres of lipoaspirate yielded approximately 1 million SVF cells. SVF was found to contain ~30% MSCs [Figure 1], The figure also reveals cells positive for CD90(31.18%), CD34(12.58%) and CD105(1.37%). In

![Figure 1: Stromal vascular factor containing CD 90 cells – mesenchymal stem cells](image)
addition, SVF contained an endothelial precursor cell, T-regulatory cells, macrophages, smooth muscle cells, pericytes and pre-adipocytes.

Cultured and purified MSCs were confirmed with four positive markers CD29, CD44, CD90 and CD105 and were negative for three markers CD34, CD45, CD31 and HLA-DR12. Their translineage translation to mesoderm was confirmed, by evidence for chondrogenesis [Figure 2]. Endodermal translation was confirmed by obtaining insulin-secreting cells [Figure 3]. Ectodermal translation was established with a phenotypic translation to a neuronal-like cell [Figure 4].

**DISCUSSION**

The cost of finding a new molecular drug has been steadily increasing from $1.2 billion in 2001 to ~$3.0 billion in 2017 (Pfizer company report).

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 AT. This study reflects on a huge potential for plastic surgeons to utilise the skills to extend to a super speciality of regenerative medicine as an autologous source. The safety of MSCs by intra-articular, intramuscular and intravenous route is established. The dosages range from as low as 10 million MSCs to 5 million MSCs/kg body weight (BW). We have exhibited 3–4 million/kg BW SVF cells.

The human AT is an abundant source for stromal vascular fraction, containing a cocktail of mesenchymal as well as haemopoiesis, and other cells provide a base for cell therapy. Thus, it has established its place as a plausible source for therapeutic application (PRS 2015).

We have proven the therapeutic application of SVF with platelet-rich plasma for osteoarthritis of the knee. Osteoarthritis anywhere else in the body has similar pathogenesis and morbid anatomy with only minor variations; therefore, we believe that SVF will have therapeutic applications there also. SVF with enrichment of platelets can also be applied to avascular necrosis of femur and even to delayed healing at fracture sites. A further research possibility exists for application of SVF in rheumatoid joints as well.

Besides this, researchers have exhibited SVF to treat extensive burns, diabetic foot, breast augmentation, nerve regeneration and wound healing, diabetes and ischemic heart failure.
MSC translation to insulin-producing cells has a potential for application for type I and II diabetes.\cite{1,2} We have proven glucose-lowering activity of SVF in seven diabetic patients.\cite{26} MSCs have research potential for application to several neurological disorders that may range from children suffering from cerebral palsy\cite{27} to adults suffering from syringomyelia,\cite{28} Parkinson’s disease,\cite{29} Alzheimer’s disease\cite{30} etc.

CONCLUSION

The study adds the evidence for translineage translation of MSCs obtained from AT to existing literature. This opens up a wide panorama for research in therapeutic applications for unmatched challenges. The study also puts a product in the palm of the plastic surgeon available at the doorstep of the operating room.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

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