Journal of Cancer Science & Treatment
JCST, 2(2): 111-114
www.scitcentral.com

ISSN: 2641-7472

Original Research Article: Open Access

A New Novel Technique for Isolation of Stromal Vascular Fraction from Adipose Tissue using Acoustic Waves for Clinical Applications

Abdulmajeed A Hammadi[1], Luay Rashan[2] and Rafie Hamidpour[3*]

[1]*Al-Yarmouk Hospital, Baghdad, Iraq*

[2]*Biodiversity Unit, Research Center, Dhofar University, Salalah, Oman*

[*3]*Department of Herbal Medicine, Pars Bioscience Research Center, Leawood, Kansas, USA*

Received August 22, 2019; Accepted September 30, 2019; Published May 21, 2020

ABSTRACT

The present work describes a new novel method for the extraction of Stromal Vascular Fraction (SVF) from adipose tissue and its significant clinical applications. The technique involves using a simple acoustic waves (acoustic bio modulation) to activate the separation of Stromal Vascular Fraction (SVF) from lipoaspirate solution. Further, it allows producing active viable cells in a short time and expenses. In addition, it is simple, not complicated, and non-invasive compared with others so far available. Up to our knowledge the technique used in this work can be described as a new. It has many advantages over other already described methods including the enzymatic and the mechanical methods.

Keywords: Stromal vascular fraction, Adipose tissue, Acoustic waves, Enzymatic methods, Mechanical methods

INTRODUCTION

The stromal vascular fluid (SVF) can be defined as a heterogeneous population of freshly isolated cells from adipose tissue. This cell population comprises many different cell types, such as mature endothelial cells, endothelial progenitors, pericytes, fibroblasts, mesenchymal stromal cells, macrophages and others, but excludes mature adipocytes. Conversely Adipose Derived Stem Cells (ADCSs) are cells found in adipose tissue and have shown to have properties of self-renewal and differentiation to several different cell types [1,2].

In the last 15 years we have seen a huge increase in the number of publications showing basic research and clinical studies using adipose-derived cells. There are a variety of methods available for the isolation of SVF cells, but overall they fall into two general categories: those which use proteolytic enzymes (collagenase) to dissociate lipoaspirate (enzymatic methods) and those which do not use proteolytic enzymes (mechanical methods).

Enzymatic methods yield significantly more nucleated cells from an equivalent weight of tissue than mechanical methods and tend to isolate a lower frequency of cells with hematopoietic origin and a higher frequency of stromal/stem cells [3,4]. Mechanical methods include techniques such as washing, shaking, incubation and centrifugation in order to separate stromal cell populations from lipoaspirate samples. However, mechanical methods offer the advantage of being less expensive and time consuming than enzymatic method.

The current work was undertaken to use some what a new technique that have some advantages over the enzymatic and the mechanical methods for the extraction of SVF from adipose tissue. Further, we wanted to see its clinical applications in four patients suffering from Osteoarthritis (OA) and Multiple Sclerosis (MS).

PATIENTS AND METHODS

Four patients of both sexes were enrolled in this study from October to December 2018. Two female patients aged 56 and 65 years old were suffering from grade 3 knee and hips OA. The other 2 patients were suffering from MS; 1 female

Corresponding author: Dr. Rafie Hamidpour, Department of Herbal Medicine, Pars Bioscience Research Center, Leawood, Kansas, USA, Tel: 913-432-0107; E-mail: rafie@parsbioscience.com

Citation: Hammadi AA, Rashan L & Hamidpour R. (2020) A New Novel Technique for Isolation of Stromal Vascular Fraction from Adipose Tissue using Acoustic Waves for Clinical Applications. J Cancer Sci Treatment, 2(2): 111-114.

aged 30 years old and the other is a male patient aged 45 years old. These patients were classified as secondary progressive MS disease (more than 4 years).

The two patients with knee and hip osteoarthritis were injected with 5 ml of the lipoaspirate fluid intra-particularly on 8 December 2018; whereas the other two MS patients were injected with 20 ml of the fluid aspirate intravenously on 16 October 2018.

The present method includes mini lipoaspiration to remove a homogenous population of cells from the fluid phase by local anesthesia to the skin. The new technique included:

1. Minilipoaspirate from the adult human abdominal subcutaneous tissue using local anesthesia (xylocaine 1%) after getting patient consent.
2. Infusion of a tumescent solution containing 250 ml of normal physiological saline 0.9% with 20 ml of xylocaine 1% with 10 ml of sodium bicarbonate (840 milliequivalent) and adrenaline 1/1000 1 ml, the whole solution is divided into 2 parts each to one abdominal side infused using special lipoaspiration cannula with small caliber opening to infuse the solution subcutaneously by reintroduction of the cannula frequently to get even homogenous solution distribution.
3. We wait for 20 min then we aspirate the solution using special lipoaspiration cannula with wide bore to get maximum amount of solubilized subcutaneous fat from both abdominal sides.
4. The mean volume of the lipoaspirate is 100 ml from both abdominal sides.
5. We injected the solution using wide bore needle in a 100 ml of sterile plastic bag for clinical uses.
6. We put the acoustic wave's probe of 10 cm diameter over the bag for 10 min, at the end we got a bilayer solution the upper one is the lipid layer and a pink lower solution.
7. We put the solution hanging over a stand for 5 min to allow gravity to separate the upper and lower layers.
8. We connected the solution bag to a 170 μm filter to pass the lower fluid layer through.
9. After collecting the lower layer of the solution we centrifuged it for 5 min (5000 rpm) with counter balance tube.
10. The stromal vascular fraction is extracted from the bottom of the tube using long spinal needle. We got a homogenous population of cells from the fluid phase and therefore, by using this new technique we did not capture of a specific population from heterogeneous mixture. Further, this fluid lipoaspirate allows the separation of two different tissue densities, because this method resulting into separation of 2 layers with resulting clear demarcation between adipose layer above and stromal vascular fraction below. The microscopic examination of the SVF fraction showed multiple types of cells including pericytes, periadiposite and mesenchymal T cells and other.

RESULTS

The results of the present preliminary study showed that the technique adopted in the current showed some advantages when compared with other already available techniques. Among these advantages is the amount of aspirate of SVF fraction obtained which reached up to 25 ml. This technique is rather simple, non-invasive and sterile. In addition, it produces active viable cells in a short time (10-15 min) and cheap to use. Further, it separates the different cell density fluids including the adipose and liquid ones.

Clinically, no evidence of treatment related adverse events was noted in any patient. Results show that the benefit reported so far is encouraging especially in two patients suffering from knee and hip OA using Womac scale (57% reduction). However, no response so far was reported from two patients suffering from MS.

DISCUSSION

Osteoarthritis is a chronic progressive degenerative disease associated with cartilage loss and degeneration. Current treatments are limited and advanced disease relies on total joint replacement. Total joint replacement may be associated with serious and life threatening complications including increased risk of infection, thromboembolism, myocardial infarction, stroke and even death post-surgery. In addition, the life span of the prosthesis is limited [5].

Recently, several methods are adopted to treat osteoarthritis using new techniques including injection of SVF and SVF combined with other techniques.

It has been reported that SVF combined with Platelet-Rich Plasma (PRP) has a great potential as a therapeutic agent in regenerative medicine especially in orthopedic conditions. The high numbers of Mesenchymal Stem Cells (MSCs) in SVF make it a suitable source for cellular medicine. Preliminary studies suggest that it is a safe and effective method for treating osteoarthritis. Both qualitative and quantitative measurements showed statistically significant improvements during the follow up period of 2 years. Additional studies with larger patient numbers and control subjects are needed to confirm the above results. Another limitation of this study is the combined effect of two modalities is unclear. Future studies with randomized groups considering each therapeutic agent separately and combined against a control are warranted. This clinical study of a combined intra-articular injection of SVF and PRP into the knee suggests a promising minimally invasive therapy for OA patients. In the United Kingdom, NICE published

guidelines in May 2014 ('platelet-rich plasma injections for osteoarthritis of the knee') [6]. These state that current evidence on PRP injections for osteoarthritis of the knee raises no major safety concerns. Nevertheless, the evidence on efficacy is inadequate in quality. NICE recommends that this procedure should only be used with special arrangements for clinical governance, consent and audit or research [6]. They also suggest that any further research into PRP injections for treating osteoarthritis of the knee should categorically describe patient selection and take the form of well-designed studies that compare the procedure against other methods of management. Outcomes should include knee function scores, patient-reported outcome measures and the timing of subsequent interventions [6].

On the other hand, MS is a chronic inflammatory demyelinating disease of the CNS which involves the loss of myelin-forming oligodendrocytes that can be followed by a spontaneous and an efficient regenerative process called remyelination [7]. It affects the people of almost all the ages in many parts of the world, mostly young people, especially more prevalent in women and among those in northern latitudes. Relapsing-Remitting Multiple Sclerosis (RRMS) is the most common form of MS.

In 2009, the first trial of Stromal Vascular Fraction (SVF) isolated from adipose tissue was conducted with 3 progressive male MS patients. The SVF contains Endothelial Progenitor Cell (EPC), MSCs, T regulatory cells, endothelial precursor cells, pre-adipocytes, as well as anti-inflammatory M2 macrophages. The SVF cells were infused by IV and intrathecal. Infusions were very well tolerated without any patient complaints or side effects. A couple of months after stem cell infusion their clinical conditions were improving and their brain MRI had no other new lesions [8].

Adipose-derived Mesenchymal Stem Cells (ADMSCs) are another source of MSC [9] with the advantage that the samples for stem cell production can be taken with a minimally invasive lipectomy procedure. With the exception of a report of 3 patients treated with stromal vascular fraction (cells from unexpanded adipose samples) [10] and another two small studies [11,12] the potential of adipose tissue as a source of stem cells has not been explored. In conclusion, the present study demonstrates that infusion of ADMSCs is a safe and feasible procedure in patients with SPMS. Although the study was not powered to determine the efficacy, some hint of efficacy was observed by the use of MRI and evoked potentials. Larger studies would be needed to investigate the potential therapeutic benefit of the technique.

The most pronounced changes following SVF treatment were the high levels of interleukin-10 in the peripheral blood, lymphoid and CNS tissues along with the induction of regulatory T cells in the lymph nodes which indicate potent immunomodulatory effects. The data indicate a SVF cell effectively ameliorated the EAE immune pathogenesis and supports the potential use of SVF for treating MS. This is the first evidence, to date, to elucidate a mechanism of action of SVF treatment in an inflammatory, autoimmune disease. Our data supports key immunomodulatory signaling between cell therapies and T cells in this T cell-mediated disease. Together, treatment with SVF mediated immunomodulatory effects that diminished effector cell activities, promoted regulatory T cells and reduced neuroinflammation [13].

The results of the current study showed some advantages using the new technique. This technique resulted in the isolation of adult stem cells in addition to other already known cells that can be isolated from the SVF fraction rather than other sources of obtaining adult stem cells such as using bone marrow, dental progenitor and endometrial tissue. Second, this method resulted into separation of two layers with no clumps and clots. Furthermore, it allowed separation of viable cells within a short time and with no contamination. Furthermore, using this technique provides more energy to the fluid and this may lead to yield or produce active viable cells within a short time and expenses. Clinically, we used the cells harvested by this technique on four patients. Despite the short observation time, the results of the present study suggest that using SVF therapy for both OA and MS patients are safe and well tolerated and without any complications or side effects. Second, it is premature to discuss the outcome of the potential effect of using it in the patients under treatment. Our preliminary results showed that intra articular injection of freshly isolated SVH cells obtained from adipose tissue in the first two patients suffering knee and hip osteoarthritis, are encouraging and there is a good response so far obtained [14].

These patients were injected with fluid lipoaspirate only on 8 December 2018 and they showed good response after two months following the injection. However, they are under careful follow up and we hope to do full scans including magnetic resonance imaging after 4 and 6 months and other scans to see the outcome. It seems that our preliminary results are consistent with results of other investigators who reported the benefit at 6 months. Yet, a long-term follow up data is required.

On the other hand, it is not surprising not to observe any response in the two MS patients simply because these two patients were injected with the fluid lipoaspirate only two months ago, i.e., 8 December 2018. In order to investigate the outcome and the potential therapeutic benefits on MS patients a couple of months (6-18) are needed.

Finally, we would like to indicate that current therapy in our laboratories are in progress using SVF in combination with a new potentially active anti-inflammatory and immunomodulatory agent and we hope to disclose these data later [15].

CONCLUSION

Our reports indicate that our preliminary results are consistent with results of other source and investigators who reported the benefit at 6 months. Yet, a long-term follow up data is required.

GUARANTOR

The corresponding author is a guarantor of submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Murphy MB, Moncivais K, Caplan AL (2013) Mesenchymal stem cells: Environmentally responsive therapeutics for regeneration medicine. Exp Mol Med 45: e54.
2. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, et al. (2013) Stromal cells from the adipose tissue-derived stromal cells/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ICST). Ctrotherapy 15: 641-648.
3. Markarian CF, Frey GZ, Silveira MD, Chem EM, Milani AR, et al. (2014) Isolation of adipose-derived stem cells: A comparison among different methods. Biotechnol Lett 36: 693-702.
4. Baptisa LS, do Amaral RJ, Carias RB, Aniceto M, Claudio-da-Silva C, et al. (2009) An alternative method for the isolation of mesenchymal stromal cells derived from lipoaspirate samples. Cytotherapy 11: 706-715.
5. Schrama JC, Espehaug B, Hallan G, Engesaeter E (2010) Risk of revision for infection in primary total hip and knee arthroplasty in patients with rheumatoid arthritis compared with osteoarthritis: A prospective, population-based study on 108,786 hip and knees joint arthroplasties from the Norwegian Arthoplasty Register. Arthritis Care Res 62: 473-479.
6. (2017) NICE Platelet-rich plasma injections for osteoarthritis of the knee NICE interventional procedure guidance IPG491. Available at: https://www.nice.org.uk/guidance/ipg491
7. Minagar A, Jy W, Jimenez JJ, Alexander JS (2006) Multiple sclerosis as a vascular disease. Neurol Res 28: 230-235.
8. Riordan NH, Ichim TE, Min WP, Wang H, Solano F, et al. (2009) Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. J Transl Med 7: 29.
9. Gimble JM, Katz AJ, Bunnell BA (2007) Adipose-derived stem cells for regenerative medicine. Circ Res 100: 1249-1260.
10. Riordan NH, Ichim TE, Min WP, Wang H, Solano F, et al. (2009) Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. J Transl Med 7: 29.
11. Ra JC, Kang SK, Shin IS, Park HG, Joo SA, et al. (2011) Stem cell treatment for patients with autoimmune disease by systemic infusion of culture-expanded autologous adipose tissue derived mesenchymal stem cells. J Transl Med 9: 181.
12. Stepien A, Dabrowska NL, Maciagowska M, Macoch RP, Zolocinska A, et al. (2016) Clinical application of autologous adipose stem cells in patients with multiple sclerosis: Preliminary results. Mediators Inflamm 2016: 1-5.
13. Shahbeigi S, Sepehry AA, Oger J (2014) The use of mesenchymal stems cells in the treatment of MS: An overview of open labels and on-going studies. J Neurol Neurophysiol 5: 4.
14. Bansal H, Comella K, Leon J, Verma P, Agrawal D, et al. (2017) Intra-articular injection in the knee of adipose derived stem cells (stromal vascular fraction) and platelet rich plasma for osteoarthritis. J Transl Med 15: 141.
15. Damia E, Chicharro D, Lopez S, Cuervo B, Rubio M, et al. (2018) adipose derived mesenchymal stem cells: Are they a good therapeutic strategy for osteoarthritis? Review open access article. Int J Mol Sci 19: 1926.