Phase Ib Open Clinical Trial to Assess the Safety of Autologous Mesenchymal Stem Cells for the Treatment of Nonrevascularizable Critical Lower Limb Ischemia

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Abstract

Background: Critical limb ischemia is a highly disabling disease, characterized by chronic pain at rest, ulceration and tissue tropism attributable to arterial occlusion. Despite significant advances in recent years in treating this disease, there are patients who, for technical reasons or because of the benefit/risk balance, have no therapeutic options other than amputation of the affected limb.

Objective: This study’s objective was to assess the feasibility and safety of autologous Adipose Tissue-Derived Mesenchymal Stem Cell (AT-MSC) implantation in patients with lower limb ischemia who are not candidates for surgical or endovascular revascularization.

Methods: This is a pragmatic, phase Ib, open, one-arm clinical trial, with a 1-year follow-up after cell implantation. The dose was 1 x 10^6 AT-MSCs/kg. AT-MSCs were diluted into a final volume of 25 mL of Ringer solution and injected as 25 aliquots of 1 mL into each injection site on the limb. Injection sites were selected below the knee at 25 different sites of the ischemic calf muscle along the tibial and peroneal arteries. Liposuction was done in the abdomen.

Results: A total of 7 patients underwent treatment for 21 months. Two patients showed no serious complications with the liposuction, only pain and mild infection. No serious cell implantation-related adverse event occurred during the follow-up, although 2 patients had to undergo amputation. The ankle-brachial index and clinical assessment of the limb improved during the follow-up.

Conclusion: In conclusion, AT-MSC treatment of critical limb ischemia is feasible, safe and has promising initial results for salvaging limbs in the short term.

Keywords: Mesenchymal stem cells; Peripheral arterial disease; Cell therapy; Lower limbs

Introduction

Critical limb ischemia is a sign of systemic atherosclerosis that expresses as a high cardiovascular risk and is defined by the TransAtlantic Inter-Society Consensus (TASC) [1] as a clinical condition characterized by chronic pain at rest, ulceration and tissue tropism attributable to proven arterial occlusive disease. With the increasing life expectancy of the population and the high prevalence of atherosclerosis, there are increasing numbers of cases of lower limb peripheral arterial disease. In recent years, the specialty of angiology and vascular surgery has greatly improved the quality of treatments and increased the number of surgical and endovascular techniques. More patients can now benefit from lower limb revascularization surgery, which has decreased the number of major amputations performed for this reason, resulting in better quality of life and financial outcomes [2]. Despite this improvement in treating patients with severe lower limb ischemia, there are patients who, for technical reasons or because of the benefit/risk balance, cannot undergo any of these procedures and end up requiring a major amputation, with the consequent deterioration in their quality of life and increased dependency [3].

Basic research into new therapies (genetics, cells, etc.) is continually advancing. There are numerous publications indicating the angiogenic ability of mesenchymal cells in experimental animal models [4, 5], as well as clinical trials in the early phases on patients with lower limb ischemia [6]. The study of Adipose Tissue-derived Mesenchymal Stem Cells (AT-MSC) is advancing due to its ease of use and performance, both at the experimental [7] and clinical [8] levels, with 17 clinical trials registered on ClinicalTrials.gov.

The objective of this study was to assess the feasibility and safety of a treatment using AT-MSCs in patients with critical lower limb ischemia who are not candidates for surgical or endovascular revascularization.

Methods

This clinical trial was designed as a pragmatic, phase Ib, open one-arm study to assess the feasibility and safety of mesenchymal stem cells in treating critical lower limb ischemia. All patients underwent a 1-year follow-up (Figure 1). The study was conducted in one hospital in the...
Community of Madrid (Spain). The recruitment started in April 2013 and finished in January 2015.

The study was approved by the Spanish Agency for Medicines and Health Products (AEMPS) and the Ethics Committee of La Paz University Hospital and registered at clinicaltrials.gov (NCT01824069).

**Study Population**

The study population consisted of patients diagnosed with critical lower limb ischemia without possibility of revascularization, either due to technical criteria (there is no available surgery to compensate for the lack of limb irrigation) or the benefit/risk criteria (intolerable surgical risk for the type of surgery required). These patients were therefore candidates for major amputation as the only surgical alternative for their clinical situation.

Each patient signed the informed consent form prior to any procedure. To be included in the study, the patients had to meet all inclusion criteria and none of the exclusion criteria (Table 1).

**Material and Methods**

After the patients were included in the clinical trial, liposuction was planned. The procedure was performed some weeks after the inclusion by a plastic surgeon with the patient under local sedation. Some 150-200 cc of fatty tissue were obtained from each patient.

Once the adipose tissue has been obtained, it was sent to the manufacturing laboratory (Histocell SL, Bilbao, Spain), whose facilities include a clean room for the good manufacturing practice manufacture of the autologous stem cells used in this study (manufacturer’s authorization number, 4269E; manufacturing protocol PEI: 11-013; clinical research product, in accordance with Spanish and European legislation). The protocol of AT-MSC isolation was derived from that published by Zuk et al. in 2001 with modifications authorized for production under GMP conditions [9]. Cell cultivation and expansion continued in an authorized procedure until the required number of cells for implantation (dose) was obtained as per the protocol of another

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**Figure 1:** Flow diagram for the study procedures.
clinical trial by our group [10]. The AT-MSCs were characterized according to the guidelines of the European Medicines Agency, the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy [11]. Cells are plastic-adherent in standard culture condition; and express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79alpha, CD19 and HLA-DR surface molecules; and finally cells were differentiated to osteoblasts, adipocytes and chondroblasts in vitro.

For their administration, the cells were suspended in a sterile Ringer's lactate solution with 1% human albumin at 1 × 10⁶ cells/mL. Samples were taken before release to examine viability, DNA stability and pathogen controls (analysis performed by the manufacturer). For the implantation, 1 × 10⁶ AT-MSCs/kg of body weight were prepared for each patient (Table 2), AT-MSCs were diluted into a final volume of 25 mL of Ringer's solution and injected as 25 aliquots of 1 mL into each injection site on the leg using 21-gauge needles. Injection sites were selected below the knee at 25 different sites of the ischemic calf muscle along the tibial and peroneal arteries (Figure 2) [12].

The study patients continued with their previous medication and those prescribed specifically for the ischemia (heparin, analgesics and antibiotics), as well as the usual local treatments for patients with trophic lesions.

**Inclusion criteria**
1. The patient must have signed the informed consent.
2. Male or female over 18 years of age.
3. Rutherford grade IV-V chronic arterial ischemia affecting at least one limb.
4. Occlusion of direct arterial flow at the femoropopliteal or distal level.
5. No surgical or endovascular option for revascularization.
6. No acute infection.
7. Acute myocardial infarction or stroke in the past 3 months.
8. Medical or psychiatric illness that in the investigator’s opinion could be a reason for exclusion from the study.
9. Participants with congenital or acquired immunodeficiency.
10. History of active neoplasia or hematologic disease.

**Exclusion criteria**
1. History of active neoplasia or hematologic disease.
2. Uncontrolled hypertension (systolic blood pressure ≥ 180 and/or diastolic blood pressure ≥ 110).
3. Severe heart failure (New York Heart Association [NYHA] IV) or ejection fraction <30%.
4. Malignant ventricular arrhythmia.
5. Deep vein thrombosis in the past 3 months.
6. Active infectious disease.
7. Acute myocardial infarction or stroke in the past 3 months.
8. Medical or psychiatric illness that in the investigator’s opinion could be a reason for exclusion from the study.
9. Participants with congenital or acquired immunodeficiency.
10. Hepatitis B and/or C, tuberculosis or treponema diagnosed at the time of inclusion.
11. Major surgery or severe trauma in the previous 6 months.
12. Treatment with any investigational drug at the current time or in the 3 months prior to recruitment for this study.
13. Cardiopulmonary disease that in the investigator’s opinion could become unstable or sufficiently serious to rule out inclusion in the study.
14. Pregnant or breastfeeding women.

**Table 1: Inclusion and exclusion criteria.**

| Patients | Age, y | Sex | Weight, kg | Rutherford grade | TASC grade | Diabetes mellitus | Cells injected (millions) | No. injections* |
|----------|-------|-----|------------|------------------|------------|------------------|--------------------------|----------------|
| 1        | 56    | M   | 82.00      | 5                | D          | yes              | 80                       | 40             |
| 2        | 57    | F   | 73.00      | 5                | D          | yes              | 0                        | 0              |
| 3        | 71    | F   | 75.00      | 4                | D          | no               | 75                       | 35             |
| 4        | 89    | M   | 65.00      | 5                | D          | no               | 60                       | 35             |
| 5        | 79    | M   | 60.00      | 5                | D          | no               | 70                       | 35             |
| 6        | 41    | F   | 60.00      | 5                | D          | yes              | 60                       | 20             |
| 7        | 38    | M   | 70.00      | 5                | D          | no               | 60                       | 20             |
| 8        | 36    | M   | 63.00      | 5                | D          | no               | 60                       | 30             |

M, male; F, female

*Number of intramuscular punctures needed to complete implantation of the cellular dose used.

**Table 2: Patients’ demographic, clinical and ASC doses characteristics.**
Statistical analysis

The data were analyzed using SPSS v 0.20.0 software. Categorical variables were described as absolute and relative frequencies. Continuous variables were reported using the mean, standard deviation, median and range. A nonparametric Wilcoxon test was employed to compare the temporal progression and a before-after paired test of quantitative values. The significance value was set at <0.05.

Results

A total of 8 patients were included from April 2013 to January 2015. Their demographic and clinical characteristics are showed in Table 2. One patient died (ID, 2) 2 weeks after liposuction due to myocardial infarction. The death was assessed as unrelated to the procedure; this patient did not undergo the cell implantation. Ultimately, 7 patients underwent the treatment and were followed-up for 1 year. Liposuction and cell culture were performed in all cases. All patients had several arterial occlusions in the leg, which were rated as grade D using the TASC classification (Figure 3).

No serious cell implant-related adverse event occurred during the follow-up. Two patients showed no serious complications with the liposuction: 1 patient had pain in the injection area, and another patient had a mild infection, which was treated with antibiotics. Both patients had an excellent recovery in the first week post-treatment. There were 2 adverse events due to illness progression, which were not associated with the cell treatment, 1 patient underwent supracondylar amputation of the treated limb, and 1 patient underwent amputation of the treated limb 3 months after the cell injection.

The outcomes on the subjective symptoms and SF-12 are summarized in Table 3. There was a statistically significant (p<0.05, Wilcoxon test) improvement in health-related quality of life in the post-treatment period (p=0.046 in the 1st week; p=0.043 in the 4th week; p=0.028 in the 26th week and p=0.08 in the 52nd week).

Of the 7 patients, 2 had to undergo amputation, 1 of whom had to undergo a minor amputation. The number of patients was limited, but the efficacy results are very promising for some patients with such an advanced disease stage (Figure 4A and 4B), ankle-brachial index and clinical behavior of the limb, did not initially change, but improvement was observed during the follow-up. The ankle-brachial index increased from 0.33 ± 0.07 to 0.55 by the end of study, and the Rutherford category decreased from 5 to 3 by the end of the trial.

Discussion

Lower limb ischemia is a serious medical problem. Despite the clinical, surgical and endoscopic advances in treating lower limb ischemia, there are patients who eventually require limb amputation.

Since the start of stem cell use in adults (specifically mesenchymal cells), stem cell-based therapy has rapidly been introduced into many medical specialties. There are numerous studies registered on ClinicalTrials.gov that are studying the angiogenic capacity of stem cells.

Mesenchymal stem cells (MSCs) are able to cross the lineage boundaries and differentiate into various of cell types, including neurons, cardiomyocytes, hepatocytes and endothelial cells [13,14]. We chose adipose tissue as the source of mesenchymal stem cells because the tissue is available in large quantities, it can be harvested with minimal adverse effects for the patient, and the cells obtained have shown angiogenic capacity. Other research groups have used bone marrow-derived stem cells; however, in such cases, a cell-mobilization procedure is required, which can be dangerous for some patients. In our study, all liposuction procedures yielded a clinically useful number of cells with stem cell characteristics. The biological mechanisms of MSCs are not fully known; the MSCs synthesize bioactive factors and secrete them to modulate the action of other cells. It is well known the paracrine effects of the MSCs, through angiogenic growth factors, such as vascular endothelial growth factor, hepatocyte growth factor and insulin-like growth factor-1. Also, it has been determined in recent years that MSCs have the property of secreting a wide variety of extracellular vesicles containing exosomes and trophic factors, including growth factors and cytokines with angiogenic potential (VEGF and miR-126 in exosomes). It has been suggested that these vesicles can act as mediators of intercellular communication, through the transfer of proteins, mRNA
Figure 3: Arterial occlusion in the limb (type D TASC classification). Arterial calcification and occlusions highlighted with arrows.

Table 3: Safety and quality of life results.

| Patients | Baseline visit | 1 week | 4 weeks | 26 weeks | 52 weeks | 1 week | 4 weeks | 26 weeks | 52 weeks |
|----------|----------------|--------|---------|----------|----------|--------|---------|----------|----------|
| 1        | 59.5           | 58.0   | ND      | 93.0     | 98.7     |        |         |          |          |
| 2        | 49.0           | ND     | ND      | ND       |          |        |         |          |          |
| 3        | 54.0           | 63.5   | 73.1    | 66.5     | 73.1     |        |         |          |          |
| 4        | 61.5           | 67.3   | 64.1    | 68.7     | ND       |        |         |          |          |
| 5        | 69.0           | 95.9   | 81.5    | 71.2     | 63.7     |        |         |          |          |
| 6        | 78.5           | 90.3   | 92.8    | 93.5     | 90.1     |        |         |          |          |
| 7        | 83.7           | ND     | ND      | ND       | ND       |        |         |          |          |
| 8        | 73.7           | 82.4   | 82.0    | 94.1     | 83.8     |        |         |          |          |

ND, Not determined

Figures 4: Patient progression before and after cell implantation.
and miRNA [15-17]. A novel approach that is currently generating a very promising new line of research, associated with growing evidence that epigenetic mechanisms are involved in determining MSCs' fate, is to analyze epigenetic modifiers of MSCs prior to the clinical treatment, recent observation indicated that inhibition of histone H3K9 methyltransferase G9a improves endothelial differentiation of adipose tissue-derived MSCs via upregulation of several endothelial markers, including VCAM-1, PECAM-1 as well as the factors that involved in blood vessel formation, such as von Willebrand factor and VEGFR-2 [18,19] and is also involved in the maintenance of DNA methylation at certain loci [20], it is possible to isolate the G9a-low MSCs based on the levels of its associated epigenetic modifications. Therefore, classification of the MSCs that intrinsically display the trend towards a given cell type might create a new direction for the clinical use of MSCs. MSCs may be considered a suitable therapeutic agent for the noninvasive treatment of lower limb ischemia because MSCs produce a downregulation of the inflammatory response, which could participate in the development of angiogenic processes [21].

The optimal MSC dose is not known but is likely based on the disease to be treated, its severity and the administration route. The small number of patients treated in the various studies performed to date complicates the extrapolation and interpretation of this data. It is tempting to associate better results with higher doses, but we must first verify that there is no toxicity or less viability of the massive cell implantation. We therefore decided to use a dose related to the patient’s weight, as has other published studies.

The best method for administering the cells is not known. There are studies that have administered the cells intravenously and even intraarterially, but both cases have shown some obstructive problems and a high percentage of cells retained in the lungs, liver and spleen during the first days following their administration. For these reasons, we believe that local administration, besides being well tolerated by the patients, ensures a greater number of cells at the point of interest for our treatment [22].

Our study found that liposuction appears to be a feasible but not harmless procedure (pain and infection) for this type of patient. The culture and expansion of the AT-MSCs represent a temporary delay of not less than 1 month from the decision to use the MSCs. In addition, MSCs from elderly patients [23], those with diabetes [24] and probably those patients with advanced stages of atherosclerosis have less regenerative capacity and could possibly secrete proangiogenic factors. The fact that a patient died during this waiting period indicates the type of patient who should be included in the study and the need to look for alternatives that do not involve a long treatment delay. Options could include allogeneic mesenchymal stem cell implantation, which takes advantage of its ability to not generate immunological rejection [25] due to the lack of major histocompatibility complex (MHC) class II and having a low MHC class I.

The main conclusion of our phase I study is that treatment using AT-MSCs appears to be safe and feasible and is well tolerated by patients. However, we propose the use of allogeneic cells from young and healthy donors for future studies, as suggested in other lines of research. This would enable us to treat our patients sooner and possibly more effectively. Facilitating the application of this treatment could also open the way for using AT-MSC therapy in not so limited patients, in previous disease phases, such as intermittent claudication, and as an adjunctive treatment for patients revascularized by angioplasty or bypass.

Another important conclusion is the objective improvement in our patients' quality of life, according to the results of the SF-12 test. This result would initially suggest a placebo effect [26,27], but the long follow-up indicated that the effect disappeared, and the result is still significant.

Although there are few cases from which to draw conclusions, the efficacy results encourage us to continue this line of research by assessing the increase in cell doses, the number of cells per dose and especially the use of allogeneic cells from young donors with high proliferation capacity and secretion of proangiogenic factors.

A significant limitation of this study is the limited sample size. Although we extended the planned recruitment time by 2 years, we encountered numerous difficulties in finding nonrevascularizable patients due to advances in the current treatment, especially endovascularization that enables some type of procedure to be performed for almost all patients.

Conclusion

AT-MSC treatment of critical limb ischemia is feasible, safe and has promising initial results with respect to salvaging the limb in the short term. It would be interesting to be able to assess this therapy in patients with lower degrees of lower limb ischemia and use allogeneic MSCs that obviate the cell limitations of patients with advanced cardiovascular disease and thereby shorten the preparation time necessary to perform the treatment.

Author Contributions

L. Riera del Moral designed and supervised the drafting of the entire article, selected the patients, performed clinical evaluations of the patients, conducted the statistical analysis, interpreted the data analysis and wrote and gave final approval for the manuscript.

A. Salazar Álvarez helped select the patients, and performed clinical evaluations of the patients. Performed the statistical analysis, interpreted data analysis, wrote and final approval of the manuscript.

Stefanov Kiuri S: Selected the patients and performed clinical evaluations of the patients. Interpreted data analysis, wrote and final approval of the manuscript.

Tong H: Performed the statistical analysis, interpreted data analysis, wrote and final approval of the manuscript.

Riera de Cubas L: Selected the patients and performed clinical evaluations of the patients. Interpreted data analysis, wrote and final approval of the manuscript.

García Olmo D: Designed and supervised the entire article. Interpreted data analysis, wrote and final approval of the manuscript.

García Arranz M: Designed and supervised the entire article. Interpreted data analysis, wrote and final approval of the manuscript.

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Disclosure of Potential Conflicts of Interest

Prof. García-Olmo D. and Dr. García-Arranz M. have applied for 2 patents related to adipose derived mesenchymal stem cells titled “Identification and isolation of multipotent cells from non-osteochondral mesenchymal tissue” (WO 2005/057649) and “Use of adipose tissue-derived stromal stem cells in treating fistula” (WO 2006/136244). The remaining authors have no other financial or competing interests to declare.

References

1. Dormandy JA, Rutherford RB (2000) Management of peripheral arterial disease (PAD). TASC Working group TransAtlantic Inter-Society Consensus (TASC) J Vasc Surg 31: S1-S296.
2. Varu VN, Hogg ME, Kibbe MR (2010) Critical limb ischemia. J Vasc Surg 51: 230-241. [PubMed]
3. Bertelé V, Roncaglioni MC, Pangrazzi J, Terzian E, Tognoni EG (1999) Clinical outcome and its predictors in 1560 patients with critical leg ischaemia. Chronic
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10.4172/2157-7633.1000391

Critical Leg Ischaemia Group. Eur J Vasc Endovasc Surg 18: 401-410. [PubMed]

4. Rehnman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, et al. (2004) Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 109: 1292-1298. [PubMed]

5. Kim Y, Kim H, Bae Y, Bae Y, Suh K, et al. (2007) Direct comparison of human mesenchymal stem cells derived from adipose tissue and bone marrow in mediating neovascularization in response to vascular ischemia. Cell Physiol Biochem 20: 867-876. [PubMed]

6. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, et al. (2002) Therapeutics angiogenesis for patients with limb ischemia by autologous transplantation of bone-marrow cells: a pilot study and randomised controlled trial. Lancet 360: 427-435. [PubMed]

7. Moon MH, Kim SY, Kim YJ (2006) Human adipose tissue derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. Cell Physiol Biochem 17: 279-290. [PubMed]

8. Lee HC, An SG, Lee HW, Park JS, Cha KS, et al. (2012) Safety and effect of adipose tissue derived stem cell implantation in patients with critical limb ischemia: a pilot study. Circ J 76: 1750-1760. [PubMed]

9. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell WJ, et al. (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7: 211-226. [PubMed]

10. García-Olmo D, García-Arranz M, Herreros D (2005) A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum 48: 1416-1423. [PubMed]

11. Bourin P, bunnett BA, Castella L, Dominici M, Katz AJ, et al. (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 15: 77-85. [PubMed]

12. Yang S-S, Kim NR, Park K-B, Do YS, Roh K, et al. (2013) A Phase I Study of Human Cord Blood-Derived Mesenchymal Stem Cell Therapy in Patients with Peripheral Arterial Occlusive Disease. Int J Stem Cells 6: 37-44. [PubMed]

13. Huang B, Li G, Jiang XH (2015) Fate determination in mesenchymal stem cells: a perspective from histone-modifying enzymes. Stem Cell Res Ther 6:35. [PubMed]

14. Papp B, Plath K (2013) Epigenetics of reprogramming to induce pluripotency. Cell 152: 1324-1343. [PubMed]

15. Marks H, Stunnenberg HG (2014) Transcription regulation and chromatin structure in the pluripotent ground state. Biochem Biophys Acta 1839: 129-137. [PubMed]

16. Du W, Zhang K, Zhang S, Wang R, Nie Y, et al. (2017) Enhanced proangiogenic potential of mesenchymal stem cell-derived exosomes stimulated by a nitric oxide releasing polymer. Biomaterials 133: 70-81. [PubMed]

17. Vlasiou AV, Magdaleno S, Settenquist R, Conrad R (2012) Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochem Biophys Acta 1820: 940-948. [PubMed]

18. Hu GW, Li Q, Niu X, Hu B, Liu J, et al. (2015) Exosomes secreted by human-induced pluripotent stem-derived mesenchymal stem cells attenuate limb ischemia by promoting angiogenesis in mice. Stem Cell Res Ther 6: 10. [PubMed]

19. Culmes M, Eckstein HH, Burgkart R, Nüssler AK, Guenther M, et al. (2013) Endothelial differentiation of adipose-derived mesenchymal stem cells is improved by epigenetic modifying drug BIX-01294. Eur J Cell Biol 92: 70-79. [PubMed]

20. Shinkai Y, Tachibana M (2011) Since G9a functions as the main writer for H3K9me1/2. Genes Dev 25: 781-788.

21. Zhang T, Termanis A, Özkan B, Bao XX, Culey J, et al. (2016) G9a/GLP Complex Maintains Imprinted DNA Methylation in Embryonic Stem Cells. Cell Rep 15: 77-85. [PubMed]

22. Nakagami H, Morishita R, Maeda K, Kikuchi Y, Ogihara T, et al. (2006) Adipose tissue-derived stromal cells as a novel option for regenerative cell therapy. Atheroscler Thromb 13: 77-81. [PubMed]

23. Dimarino AM, Kaplan AI, Bonfield TL (2013) Mesenchymal stem cells in tissue repair. Front Immunol 4: 201. [PubMed]

24. Wu W, Niklason L, Steinbacher DM (2013) The effect of age on human adipose-derived stem cells. PRSG 131: 27-37.

25. Minteer DM, Young MT, Lin YC, Over PJ, Rubin JP, et al. Analysis of type II diabetes mellitus adipose-derived stem cells for tissue engineering applications. J. Tissue Eng. 2015; 6:2041731415579215. [PubMed]

26. García-Gómez I, Elvira G, Zapata AG, Lamana ML, Ramírez M, et al. (2010) Mesenchymal stem cells: biological properties and clinical applications. Expert Opin Biol Ther 10: 1453-1466. [PubMed]

27. Whalley B, Hyland ME, Kirsch I (2008) Consistency of the placebo effect. J Psychosom Res. 64: 537-41. [PubMed]