Clinical Grade Human Adipose Tissue-Derived Mesenchymal Stem Cell Banking

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Abstract- In this study, our aim was to produce a generation of GMP-grade adipose tissue-derived mesenchymal stem cells for clinical applications. According to our results, we fulfill to establish consistent and also reproducible current good manufacturing practice (cGMP) compliant adipose tissue-derived mesenchymal stem cells from five female donors. The isolated cells were cultured in DMEM supplemented with 10% fetal bovine serum and characterized by standard methods. Moreover, karyotyping was performed to evaluate chromosomal stability. Mean of donors' age was 47.6 ± 8.29 year, mean of cell viability was 95.6 ± 1.51%, and cell count was between 9×106 and 14×106 per microliter with the mean of 12.2×106 ± 2863564.21 per microliter. The main aim of this project was demonstrating the feasibility of cGMP-compliant and clinical grade adipose tissue-derived mesenchymal stem cells preparation and banking for clinical cell transplantation trials.

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Introduction

Stem cells which can be derived from different sources demonstrate promising therapeutic evidences for cellular therapies (1). On the other hand, cellular therapy as an interesting area in biological sciences have grown dramatically (2,3). Among different types of stem cells, mesenchymal stem cells (MSCs) have increasingly used for cell therapy trials. MSCs are -multipotent cells with the ability to differentiate into mesoderm derived cells- can be isolated from almost all tissues for instance; bone marrow, umbilical cord tissue and blood, adipose tissue, dental pulp, synovial membrane and fluid, skin, etc. (4,5). Furthermore, they have some proven immunomodulatory, hematopoiesis, and regeneration capabilities that have led their utilization in clinical cell transplantation trials (1,6). Although bone marrow is the most common source for MSCs, the number of bone marrow-derived MSCs and their differentiation potential dramatically decrease with age (only 0.002% of total stromal cell population). In addition, the isolation of MSCs from bone marrow is an invasive and painful procedure. Therefore, scientists tried to find and develop an alternative source of MSCs. Human subcutaneous

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adipose tissue provides an easy and repeatable access to adipose tissue while the simple isolation procedures provide a clear advantage (1,7). Therefore, adipose tissue-derived stromal cells (ASCs) seem as a superior choice for cellular therapy because of several advantages such as a large number of isolated cells using a minimally invasive procedure (8). Furthermore, various basic, experimental, and clinical researches have revealed the enormous potential of ASCs in regenerative medicine (9-16). Significantly, increasing clinical transplantation of ASCs has shown the importance of several concerns regarding the clinical application of these cells and also has been accompanied by the regulations governing cellular therapies (2). In spite of the great hope and opportunity that have provided by stem cell therapy (using ASCs or other types of stem cells), there are some considerable risks (17). These risks can be divided into intrinsic and extrinsic factors. Intrinsic factors result from cell characteristics such as differentiation status, tumorigenic potential, proliferation capacity, life span, viability, etc. On the other hand, extrinsic factors as raw materials, manufacturing processes, storage and transportation condition, etc. are also important in all cellular therapy projects (17). Accordingly, it is recommended that the clinical grade preparation of stem cells (e.g., ASCs, bone marrow-MSCs) should be performed in accordance with current good manufacturing practice (cGMP) guidelines that are used for facility or manufacture, processing, packing controls (18,19) to overcome most of the extrinsic and some of the intrinsic risk factors, and ensure the safety, quality, characteristics, and identity of cell products (20-22). Additionally, cell manufacturing in accordance with GMP standards involves several issues similar to drugs manufacturing guidelines (6).

GMP-compliant cellular therapy

Bridging between stem cell basic science and clinical practice is one of the most complex organizational and regulatory areas (23). Consequently, advanced cellular therapy requires extensive validation, process control, and documentation and clearly speculates the critical importance of production methods, as well as the potential risks (2). Therefore, implementation of a quality management system in accordance with international standards such as GMP can greatly reduce these risks (24). The Food and Drug Administration (FDA) has defined human cells, tissues, cellular and tissue-based products (HCT/Ps), in two categories; the lower risk category or "minimally manipulated" products and the higher-risk category or "more than minimally manipulated" products. Accordingly, manufacturing the first ones must comply with good tissue practice (GTP) regulations as a level of control similar to that already practiced by most clinical laboratories and the second ones must be complied with both GTP and GMP (2,25). As we described previously we established a clean room facility as the most tangible aspect of cGMP, affiliated to brain and spinal injury research center to perform (stem) cell transplantation trials according to GMP standards (24,26). Our purpose in this study was demonstrating the feasibility of GMP-complaint and clinical grade ASCs manufactured for clinical cell therapy trials.

Materials and Methods

After obtaining informed consent, subcutaneous adipose tissues from five female donors were harvested under aseptic and sterile condition. Harvested tissues were placed in tissue container with wet ice and transferred to GMP facility.

Tissue digestion and cell isolation

All isolation and culture procedures were accomplished in the GMP facility (clean room) under laminar air flow cabinet and according to cGMP guidelines. Before processing, samples from tissue and transfer medium were sent to a reference laboratory to check probable microbiological contamination. Also during cell culture period, quality control and microbiological tests were repeated to recognize probable contamination during manipulation. Then, isolated adipose tissues were washed twice with phosphate-buffered saline (PBS; CliniMACS®, Miltenyi Biotec, Germany) to remove extra blood, small vessels, and connective tissues. Subsequently, cleaned adipose tissues were cut into very small pieces and were put into two or three 50 ml falcon tubes. Then 3 mg/ml collagenase-NB6 (GMP-grade, Serva Electrophoresis GmbH, Germany) was added to each falcon tube which was followed by incubation at 37 °C for 30 minutes. The digestion process was stopped by adding an equal volume of PBS and the samples were centrifuged at 600g for 15 minutes at room temperature. The Mature adipocytes, debris, and liquid portion were discarded, and the stromal vascular fraction (SVF) was precipitated. SVF resuspended in PBS was filtered through a nylon mesh. Next, the filtered suspension was centrifuged at 400g for 10 minutes at 20-C and then was transferred to culture flasks.

ASCs culture and subcultures

Approximately 50×103 mixed SVF cells per cm2 were transferred to each culture flask. Then DMEM supplemented with 10% fetal bovine serum (FBS; Pharma grade, Australian origin, and gamma irradiated, PAA, Austria) were added to each culture flask and placed into the incubator under the condition of 37°C, 5% CO2 and humidified air. After 48 hours incubation, the cells were washed to remove the culture medium, and non-adherent cells and the culture medium were renewed by fresh medium and every 72 hours the culture medium were renewed. At 85–90% confluence, the adipose-derived mesenchymal stem cells (ASCs) were harvested by enzymatic method and the cells were subcultured while cell count number, cell viability, and purity were evaluated by Trypan blue (Invitrogen, USA) and hemocytometer. ASCs at 3rd subculture were checked for chromosomal stability using the karyotyping method.

Characterization of ASCs

Flow cytometry

ASCs surface markers were analyzed using flow cytometry. Briefly, 1×10 5 cultured ASCs at 3rd subculture resuspended in FACS-buffer (3% BSA in PBS) and then appropriate fluorescein isothiocyanate (FITC) conjugated primary antibodies for 60 minutes at 4°C. CD44, 11b, 19, 34, 45, 105, 73, and HLA-DR; (all from Abcam, USA) were used and afterward, labeled ASCs were analyzed on a fluorescence-activated cell sorter (FACS; Partec, Denmark).

Differentiation potential

Adipogenic differentiation: Cultured ASCs incubated in adipogenic medium (DMEM supplemented with 50 µg/ml indomethacin, 10 -7 M dexamethasone, and 50 µg/ml ascorbate-sodium 2-phosphate (all from Sigma-Aldrich) for 25 days and each 72 hours culture medium was renewed. Then, ASCs were fixed by ethanol for 10 minutes and stained with oil red O.

Osteogenic differentiation: ASCs were incubated in osteogenic medium (DMEM with 10 mM ß–glycerol phosphate, 50µg/ml ascorbate sodium 2-phosphate, and 10-7 M dexamethasone, 1% antibiotic/antimycotic (all from Sigma-Aldrich) for 25 days. Afterward, cell fixation was done using ethanol for 10 minutes and staining using alizarin red.

Results

Demographic characteristics of adipose tissue donors are showed in Table 1. The mean of donors' age was 47.6 ± 8.29 year. Also, mean of cell viability was 95.6% ± 1.51 and about cell count (mean=12.2×106 ± 2863564.21 per microliter). Mean of tissue weight was 17.14 ± 1.99 gr. Cell processing time was ranged from 18-22 days (mean=20.2 ± 1.48).

Table 1. Adipose tissue donors' demographic characteristics, cell count, cell viability, cell processing time, and tissue weight

Age	tissue weight (gram)	Cell culture duration (day)	Cell count (/µl)	Cell viability (%)
60	13.5	18	16000000	95
40	15	20	9000000	98
44	17.4	20	12000000	96
52	16.8	21	10000000	95
42	23	22	14000000	94

The morphology of ASCs was similar to the morphology of bone marrow and umbilical cord blood-derived MSCs (Figure 1b).

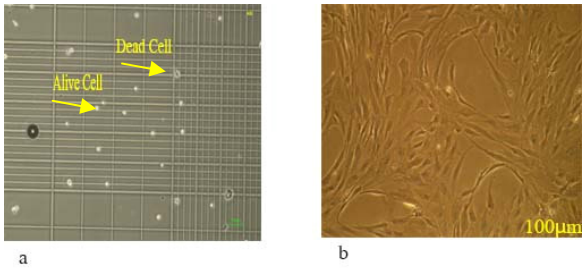

Figure 1. (a) Trypan blue staining on homocytometer (magnification: 40x). (b) ASCs morphology assessment by light inverted microscopy (magnification: 200x).

Characterization of ASCs

ASCs were negative for CD11b, 19, 34, 45, HLA-DR, and positive for CD105, 44, and CD73 (Figure 2).

Differentiation potential of ASCs

We evaluated the differentiation potential of ASC isolated by staining with Oil Red O (adipogenic), Alizarin Red (osteogenic) (Figure 3). Additionally, karyotyping which was performed at a 3th subculture, have depicted a normal diploid karyotype (Figure 4).

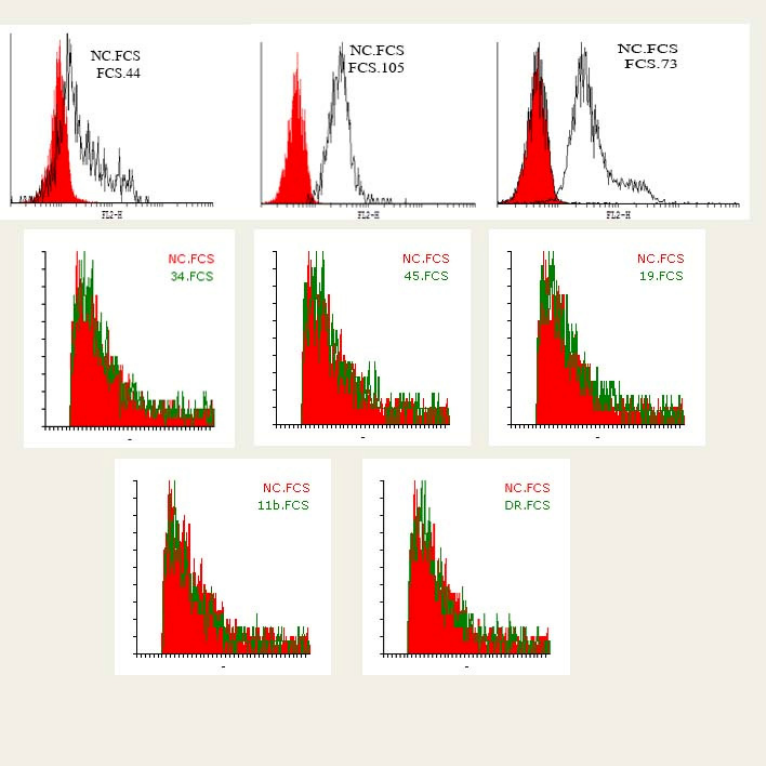

Figure 2. Cell surface markers of ASCs. Histogram analysis of cell surface markers. ASCs were positive for CD73, CD44, and CD105, and negative for CD11b, CD19, CD34, CD45, and HLA-DR.

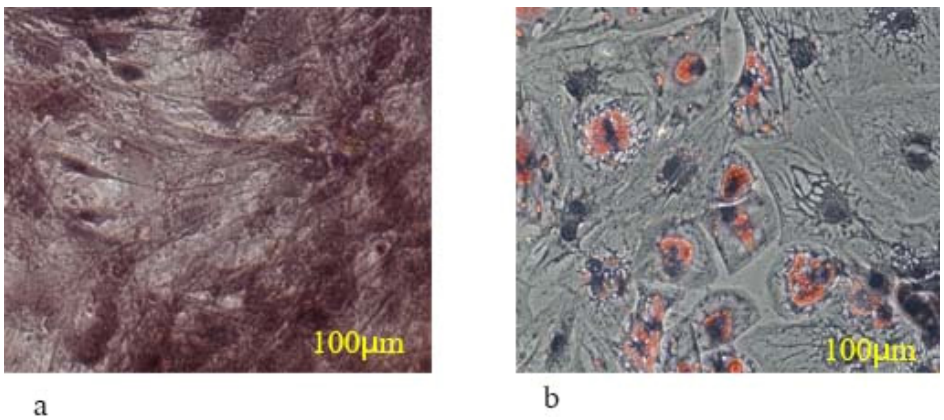

Figure 3. Light inverted microscopy photographs of ADSCs induced to differentiate into different lineages. (a) Osteogenic differentiation is demonstrated by Alizarin Red staining. (b) An adipogenic differentiation is demonstrated by Oil Red O staining (magnification: 200x).

Figure 4. Normal diploid (male) karyotype of adipose-derived mesenchymal stem cells at 3rd subculture.

Discussion

Regenerative medicine as an advanced therapy has been introduced for treatment of several diseases in recent years (6,27). Accordingly, various clinical cell transplantation trials are registered at different databases including www.clinicaltrials.gov, using MSCs as the most common used multipotent stem cells in cellular therapies (20). MSCs can be isolated from different tissues such as bone marrow, peripheral blood, adipose tissue, etc. Recently, advantages of ASCs in regenerative medicine are increasingly mentioned. For instance, in a published report in 2011, Lindroos *et al*., found 18 clinical trials that used human ASCs in regenerative medicine (18). This approach to stem cells and advanced cellular therapies seems to be accompanied by serious safety concerns (2). Accordingly, all steps of cell preparation for clinical transplantation must be performed based on appropriate standards and protocols such as GMP to achieve a reasonable safety and quality (3,23). In this study, we tried to demonstrate the feasibility of GMP-compliant and clinical grade ASCs preparation according to GMP standards. All procedures were performed in our GMP facility using relevant protocols and GMP-grade materials. ASC products have depicted all known characteristics of MSCs, which was reported by Dominici *et al*., (28). In accordance with the current state of cellular therapy regulations, all cell products should be manufactured adhering to cGMP requirements including facility, cGMP compatible or clinical grade reagents and materials if they are available. If some of them are not available, research grade reagents can be used for additional safety and quality tests. Accordingly, clinical and GMP-grade cell manufacturing cannot be achieved by transferr ing current basic cell processing methods into a cGMP facility (22). A reasonable quality assurance will be achieved in cellular therapy when the principles of cGMP are considered completely from tissue or cell isolation and collection to freezing, storage, and finally releasing for transplantation (1). Furthermore, all additional reagents must be justified and replaced with clinically compatible materials if possible (29,30). One of the most important reagents which should be considered as a potential risk of transferring immunogenic xenoproteins, infectious agents, and especially transmissible spongiform encephalopathy (TSE) is FBS. In accordance with the European Medicines Agency (EMEA) recommendations, its use should be avoided when manufacturers have a choice from "non-TSE relevant animal species" or non-animal origin (6,31). Considering these concerns we used FBS pharma grade as an Australian origin and gamma irradiated serum which is a high quality FBS that is compatible with current regulations and guidelines of biopharmaceutical products such as, EMEA guideline 1793/02 of the committee for proprietary medical products (CPMP); EMEA guideline 743/00 of the committee for veterinary medical products (CVMP); the Ph. Eur. (European Pharmacopoeia) current edition monograph of Bovine Serum (2262); the EU regulation 2005/567 for Advanced Therapies (AT); and US Code of Federal Regulation (9CFR). Additionally, 35 kGy effective single box gamma irradiation (for reduction of gamma sensitive viruses and mycoplasma) and a broad test panel (including mycoplasma, 10 different viruses, and Bovine Viral Diarrhea (BVD) antibodies) perform

for more safety. For adipose tissue digestion, we used a GMP-grade collagenase, which is intended to use in clinical cell transplantation. It is compatible with the requirements of the EU-Guide to GMP. Furthermore, other clinical compatible reagents are commercially available. For instance, GMP-grade Ficoll-PaqueTM PREMIUM (GE Healthcare Life Sciences, USA), TrypLE™ Select (Invitrogen) and TrypZean (Sigma-Aldrich) as animal-origin free enzymes (22,32). Besides focusing on different reagents and materials that are used in clinical cell manufacturing, the manufacturing procedures must be designed based on cGMP standards to guarantee the safety and quality of cell-based product throughout the tissue or cell collection and isolation, cryopreservation, banking, releasing, and shipping process. Furthermore, cell manufacturing facility must be designed and installed in accordance with the relevant standards such as monitoring of air changes and its condition, temperature, humidity, airborne particles and probable contamination, etc. Likewise, all standard operating procedures (SOPs) must be documented (19).

Translating from the basic stem cell research into clinical cell transplantation is a complex process. During this translation, avoiding every probable risk and achieving a reasonable quality level should be considered. Therefore, (stem) cell preparation for the clinical application should be performed in accordance with cGMP standards. Although use of ASCs for treatment of various disorders is increasingly recommended by researchers, their clinical application is still in its infancy and needs more preclinical and clinical studies to demonstrate whether ASCs can meet expected scientific values and can be used as a successful cellular therapy.

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References

1. Thirumala S, Goebel WS, Woods EJ. Clinical grade adult stem cell banking. Organogenesis 2009;5(3):143-54.
2. Burger S. Current regulatory issues in cell and tissue therapy. Cytotherapy 2003;5(4):289-98.
3. Prince HM, Wall DP, Stokes KH, et al. Cell Processing for Clinical Trials and Commercial Manufacture. Cell Gene Ther 2004;1(1):15-21.
4. Tarte K, Gaillard J, Lataillade JJ, et al. Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. Blood 2010;115(8):1549-53.
5. Vishnubalaji R, Al-Nbaheen M, Kadalmani B, et al. Comparative investigation of the differentiation capability of bone-marrow-and adipose-derived mesenchymal stem cells by qualitative and quantitative analysis. Cell Tissue Res 2012;347(2):419-27.
6. Bieback K, Schallmoser K, Klüter H, et al. Clinical protocols for the isolation and expansion of mesenchymal stromal cells. Transfus Med Hemother 2008;35(4):286-94.
7. Schäffler A, Büchler C. Concise Review: Adipose Tissue-Derived Stromal Cells--Basic and Clinical Implications for Novel Cell-Based Therapies. Stem Cells 2007;25(4):818-27.
8. Lin G, Banie L, Ning H, et al. Potential of Adipose Derived Stem Cells for Treatment of Erectile Dysfunction. J Sex Med 2009;6(Suppl 3):320-7.
9. Reichenberger MA, Heimer S, Schaefer A, et al. Adipose Derived Stem Cells Protect Skin Flaps Against Ischemia-Reperfusion Injury. Stem Cell Rev 2012;8(3):854-62.
10. MacIsaac ZM, Shang H, Agrawal H, et al. Long-term in-vivo tumorigenic assessment of human culture-expanded adipose stromal/stem cells. Exp Cell Res 2012;318(4):416-23.
11. Mizuno H, Tobita M, Uysal AC. Concise Review: Adipose Derived Stem Cells as a Novel Tool for Future Regenerative Medicine. Stem Cells 2012;30(5):804-10.
12. Yarak S, Okamoto OK. Human adipose-derived stem cells: current challenges and clinical perspectives. An Bras Dermatol 2010;85(5):647-56.
13. Harris LJ, Zhang P, Abdollahi H, et al. Availability of adipose-derived stem cells in patients undergoing vascular surgical procedures. J Surg Res 2010;163(2):e105-12.
14. Yamamoto T, Gotoh M, Hattori R, et al. Periurethral injection of autologous adipose-derived stem cells for the treatment of stress urinary incontinence in patients undergoing radical prostatectomy: report of two initial cases. Int J Urol 2010;17(1):75-82.
15. Brown SG, Harman RJ, Black LL. Adipose-derived stem cell therapy for severe muscle tears in working German shepherds: Two case reports. Stem Cell Discovery 2012;2(2):41-4.
16. Wang Y, Lian F, Li J, et al. Adipose derived mesenchymal stem cells transplantation via portal vein improves microcirculation and ameliorates liver fibrosis induced by CCl4 in rats. J Transl Med 2012;10(1):133.

17. Herberts CA, Kwa M, Hermsen HP. Risk factors in the development of stem cell therapy. J Transl Med 2011;9(1):29.
18. Gir P, Oni G, Brown SA, et al. Human adipose stem cells: current clinical applications. Plast Reconstr Surg 2012;129(6):1277-90.
19. Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. Stem Cell Res Ther 2010;1(2):19.
20. Fekete N, Rojewski MT, Fürst D, et al. GMP-Compliant Isolation and Large-Scale Expansion of Bone Marrow-Derived MSC. PloS One 2012;7(8):e43255.
21. Maekawa T, Kimura S, Kasai Y. Development of Novel Advanced Cell and Gene Therapy and GMP-Controlled Cell Processing. JMAJ 2005;48(2):81-4.
22. Arjmand B, Aghayan HR. Cell manufacturing for clinical applications. Stem Cells 2014;32(9):2557-8.
23. Cuende N, Izeta A. Clinical translation of stem cell therapies: a bridgeable gap. Cell Stem Cell 2010;6(6):508-12.
24. Larijani B, Arjmand B, Amoli MM, et al. Establishing a cGMP pancreatic islet processing facility: the first experience in Iran. Cell Tissue Bank 2012;13(4):569-75.
25. Burger SR. Design and operation of a current good manufacturing practices cell-engineering laboratory. Cytotherapy 2000;2(2):111-22.
26. Arjmand B, Emami-Razavi SH, Larijani B, et al. The implementation of tissue banking experiences for setting up a cGMP cell manufacturing facility. Cell Tissue Bank 2012;13(4):587-96.
27. Warnke PH, Humpe A, Strunk D, et al. A clinically-feasible protocol for using human platelet lysate and mesenchymal stem cells in regenerative therapies. J Craniomaxillofac Surg 2012;41(2):153-61.
28. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315-7.
29. Romagnoli L, Giuntini I, Galgano M, et al. Cell-based medicinal products and the development of GMP-compliant processes and manufacturing. BMC Proc 2011:5(Suppl 8):O3.
30. Hartmann I, Hollweck T, Haffner S, et al. Umbilical cord tissue-derived mesenchymal stem cells grow best under GMP-compliant culture conditions and maintain their phenotypic and functional properties. J Immunol Methods 2010;363(1):80-9.
31. Brooke G, Rossetti T, Pelekanos R, et al. Manufacturing of human placenta derived mesenchymal stem cells for clinical trials. Br J Hematol 2009;144(4):571-9.
32. Ilic N, Brooke G, Murray P, et al. Manufacture of clinical grade human placenta-derived multipotent mesenchymal stromal cells. Methods Mol Biol 2011;698:89-106.