MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE TO TIGHTEN FACIAL SKIN

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ABSTRACT

Skin elasticity is an important aspects of facial care. This study aims to explore the potential of mesenchymal stem cells (MSCs) derived from adipose tissue (adipose-derived stem cells) in facial skin tightening and assess their advantages and limitations in clinical applications. The methods used include literature searches from various officially published online sources as well as analysis of research data regarding the process of isolation, culture, differentiation and clinical applications of ADSCs. The main findings show that adipose-derived stem cells (ADSCs) are able to form new collagen, increase skin elasticity, and repair tissue damage due to aging. The culture process involving isolation of cells from adipose tissue and differentiation of the cells into fibroblasts is essential to exploit the regenerative potential of these cells. Although this technology is promissing, this study also identified technical and regulatory challenges that need to be overcome, including patient health criteria, safe ADSc harvest procedures, isolation and culture processes that have a high chance of differentiation and post-application care of ADSc therapy. The significance of these findings is that a better understanding of the mechanism of action of ADSCs can make a positive contribution to the development of beauty therapies that are more effective and safer compared to conventional methods that are often unsatisfactory. Additionally, this approach is expected to provide a long-term solution for individuals who wish to maintain a youthful appearance without the risk of significant side effects. Thus, this research can be a step in integrating stem cell technology in the beauty industry, especially for anti-aging treatments.

Keywords : Adiposa Derived Stem Cell, anti aging, fibroblast, mesenchymal stem cells, tighten facial skin

INTRODUCTION

Facial skin care is a big concern for many individuals in this modern era. One of the commonly used methods to improve the firmness of facial skin is collagen injections and the use of skincare products such as anti-aging creams. Anti-aging can be translated as an attempt to prevent the aging process or stay younger looking (Pebrianti, 2023). Collagen injection methods or the use of products often do not provide satisfactory results, because they are designed for general use and do not consider the specific needs of each individual, thus making the treatment results less effective, and can trigger the risk of side effects such as skin irritation, allergic reactions, and even more serious

complications. Long-term use can also lead to dependence on certain products without significant improvement in skin condition (Atmaja, 2009). Many cosmetic and dermatological rejuvenation efforts such as lasers, vitamin C, topical antioxidants, topical antioxidants, topical growth factors, and retinoic acid, all of which are expected to induce collagen synthesis, thereby improving wrinkles and skin texture, but must be applied repeatedly for a long time. Anti-aging agents containing growth factors and anti-oxidants are needed to provide better results (Irianto, 2015).

Technological developments have opened up great opportunities in regeneration-based beauty treatments, one of which is stem cell technology. Stem Cells have the ability to renew themselves and differentiate into various other cell types, including skin cells (Suparno, 2022). Their ability to repair damaged tissue and stimulate new cell regeneration makes stem cells as a potential solution in the wolrd of beauty care. Unlike methods such as collagen injections that only provide temporary results, stem cells technology offers an approach that can provide long-term effects with less risk (Safitri, 2022). MSCs (Mesenchymal Stem Cells) are adult stem cells that have regenerative and immunomodulatory properties that can be used in tissue repair and wound healing. Their multipotent nature allows MSCs to differentiate into various cell types and can renew theselves (Santi, 2018). The basic characteristic of MSCs is that they are one of the most accessible primary cells and can be harvested easily from various tissues (Sisca, 2018).

Among the various sources of MSCs, adipose tissue-derived stem cells (ASCs) are the focus of attention due to their potential as a cell source for tissue engineering and regenerative medicine. Adipose tissue, or fat tissue, is proven to be one of the best sources for obtaining mesenchymal stem cells (adipose-derived stem cells or ADSCs) (Suparno, 2022). This tissue has advantages in terms of abundance, ease of extraction, and high regenerative potential. Recent studies have shown that ADSCs are able to stimulate the formation of new collagen, increase skin eladticity, and repair the structure of skin tissue that has been aging or damaged (Annita, 2024). These advantaged make stem cells from adipose tissue an attractive option for the development of beauty therapy, especially in facial skin tightening treatments (Tsuji, 2014).

However, this technology still faces various challenges, both in terms of technique and regulation. Therefore, it is important to understand how ADSCs work in improving skin structure, as well as evaluate their advantages, limitations, and application opportunities in the beauty industry, especially facial anti-aging treatments (Gonzales, 2018). This paper aims to assess the potential of adipose tissue-derived stem cells that differentiate into fibroblast cells to improve facial skin firmness to steering fat tissue stem cells from Adipose Derived Stem Cells (ADSC) to Mesenchymal Stem Cells (MSC) for therapeutic agents to tighten fibroblast tissue.

MATERIALS AND METHODS

This stage of writing is carried out by the literature search method, which is sourced from online searches, through Google Scholar, Pubmed, and officially published articles. The data processing stage starts from screening keywords that match the chosen topic, then proceeds to draw conclusions based on the data obtained and then narrowed down to get a conclusion.

The premise of this article is: The process of stem cell development is increasingly rapid, especially stem cells that are used in tissue regeneration, so this article examines the potential of Adipose stem cells as cells that can improve the aging process and as

anti-aging agents. Research was conducted with the keyword Adipose Derived Stem Cell (ADSc) from several websites that have been mentioned. Total of 22.146 literatures were filtered by adjusting the limitations of the keywords, such as ADSCs, anti-aging, fibroblast cell culture, and the application and regulation of the application of ADSCs in the field of beauty, especially facial skin tightening due to wrinkles. 40 literature sources were obtained to complement the data and journal literature.

RESULT AND DISCUSSION

In the collection of quality stem cells, screening is carried out first on the patient which aims to ensure that the patient does not have an infectious disease (Kot et al., 2019). In addition to infectious disease with other risks, the United Kingdom Advisory Committee on the Safety of Blood and Tissues regulations stipulate to carry out genetic checks to prevent the risk of cancer, in addition to checking family health histroy (Reinders et al., 2013). Patient consent through informed consent must also be considere, because it involves information before the cell collection process is carried out, and an understanding of how the procedure and risks will occur (Vaz et al., 2018). In terms of general health criteria, the patient should be in good physical and mental health with no infectious or contagious diseases that may affect the quality of the MSC (Kern et al., 2006). The suitable age for MSC patients is suggested to be around 18 to 60 years old, the youngest age is the most suitable age but the age limit may also vary depending on the regulation of the place. Patients should have a minimum body weight, usually around 55kg as it ensures they have enough tissue to harvest (Little et al.,2020).

In the health criteria in the physical examination, before the cells are taken, the msc patient must undergo a check-up which includes blood pressure, pulse rate, and body temperature (Herman et al.,2012). The level of haemoglobin also needs to be checked to ensure that it is within a healthy range. The healthy range is usually at least 12 g/dL for women and 12.5 g/dL for men (Pendleton et al.,2013).

Procedure for MSC Extraction

In the first stage, the patient consults a doctor (dermatologist or plastic surgeon) to find out the condition of the facial skin, medical history and expected results. The doctor will explain the procedure, benefits, risks and possible outcomes. The patient will be asked to fast for a few hours before the procedure as light anaesthesia is used. Patients are advised to avoid certain medications, such as anticoagulants, a few days before the procedure.

Patients must undergo health screening which is done through several processes. The next step is for the patient to understand informed consent. At this stage, the patient gives consent before the collection is done and understands the procedure and potential risks involved. Patients must undergo blood tests for HIV, HCV, HBS, HTLV-1, syphilis TV antibodies to ensure the absence of infectious diseases, next, cell harvesting is performed, where the source is from adipose tissue. Fat harvesting requires a 2 mm incision, the harvesting procedure is performed by experienced and supervised medical personnel, and follows safe and sterile protocols to prevent contamination. After harvesting, the next step is isolation and culture. Once MSCs have been harvested, they should be isolated and cultured under appropriate conditions to maintain cell viability and cell differentiation potential. Cultured MSCs are stored under sterile conditions to maintain cell quality. Usually, storage is done at low temperatures to keep the cells in an optimal state (Herman et al., 2012).

Regulations related to MSC collection are contained in Permenkes No. 50 of 2012 which regulates stem cell laboratories for clinical applications and Permenkes No. 32 of 2018 in establishing stem cell service standards and related procedures. Health Law No. 17 of 2023 Provides a legal framework for health practices including the use of stem cells. International standards in the processing and storage of MSCs must follow international standards set by the Association of Blood Banks (AABB) and the Foundation for the Accreditation of Cellular Therapy-NetCord to ensure cell quality and cell safety are guaranteed (Little et al., 2020).

Only 0.05% of adipose tissue can be used as a source of MSCs (Kern et al., 2006). Adipose tissue produces more MSCs because it contains more progenitor cells, so it can be isolated more easily in large quantities (Pendleton et al, 2013). For clinical application of MSCs into the human body, 1 to 3 x 106 cells/kg body weight is required (Herman et al., 2012).

Adipose Stem Cell (ASC) Isolation

The mechanism of ASC isolation starts from the process of taking adipose tissue through a liposuction procedure (lipospiration), where fat tissue taken from the donor's body becomes the main material for isolation (Rodbell, 1966). The harvested tissue is then chopped into small pieces before being thoroughly washed using a wash buffer, such as phosphate-buffered saline (PBS), to remove contaminants that may affect the isolation results such as residual blood and carried tissue. Then the properly rinsed ASCs are further chopped under sterile conditions and washed again using PBS (Zhu, 2013). The next process is enzymatic digestion using collagenase enzyme which aims to break down collagen in the extracellular matrix which will then promote the release of stromal vascular fraction (SVF) which contains various types of cells, including mesenchymal stem cells (MSCs), immune cells, and endothelial cells (Kim WS, 2009). ASCs were added 0.075-0.5% collagenase enzyme and incubated for 30 minutes (Zhu, 2013). The volume of collagenase used is the same as the volume of ASC used. Besides collagenase, trypsin enzyme can be a cheaper alternative to digest adipose tissue (Markarian, 2014).

Collagenase enzyme activity can be neutralised by the addition of digested tissue samples with DMEM or a-MEM supplemented with 10% or 20% inactivated fetal bovine serum (FBS) (Zhu, 2013). This digestion process is performed at a specific time and temperature to ensure separation efficiency without damaging the cells of interest. The digestion results are then centrifuged at a certain speed to separate the SVF fraction from other components, lipids and residual enzymes. The SVF fraction obtained contains a heterogeneous population of cells that require further processing for purification. This fraction is then transferred to a culture medium that supports the growth and attachment of mesenchymal stem cells (Shah, 2013). Mesenchymal stem cells, which have adherent properties, will stick to the culture surface, while other cells that do not adhere will be eliminated through the washing process. These adherent cells are then propagated through repeated subcultures to increase the population and number of stem cells available (Revilla, 2019).

The next stage is the characterisation of ASCs to ensure that the isolated cells truly have characteristics typical of mesenchymal stem cells. The ability to form colonial peaks is an indicator of the potential and proliferation of ASCs Surface marker characterization is generally done by incubating subcultured cells using primary and secondary monoclonal antibodies labelled with dyes. Such as fluorescinate isothiocyante (FITC) or allophycocyanin (APC). Subsequently the cells incubated with the labelled dye-conjugated secondary antibodies

are washed (Lin, et al., 2008).

This isolation and characterisation process is designed to produce high-quality ASCs that can be used in various research and clinical applications. By following strict protocols, this process ensures that the ASCs produced meet safety and effectiveness standards, so they can be widely used in the development of medical technology (Wulandari., 2016).

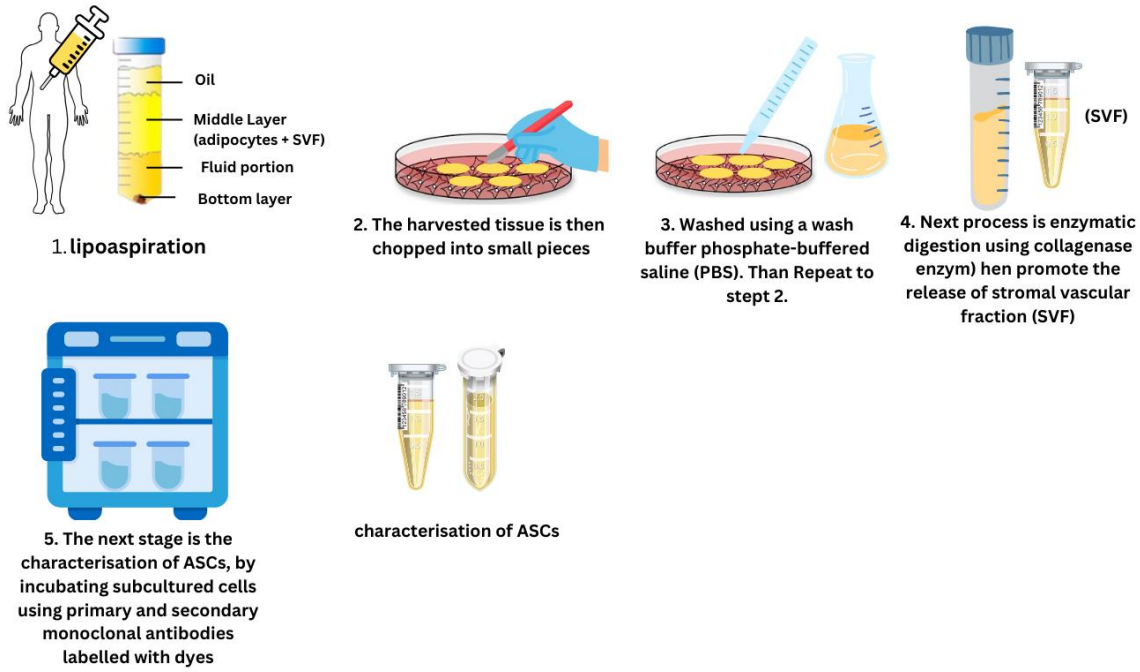

Figure 1. Adipose Stem Cell (ASC) Isolation

Adipose Stem Cell Culture

Cells are grown on ASC selection medium so that only ASCs can grow on the medium. The initial stage of cell growth is called primary cell culture. The grown cells can be sub-cultured at every one to two week period when confluence reaches 80%. Cells are sub-cultured and grown until the cells are finite. Cultured ASCs have an average finite time at the fifth passage. Cells are said to be finite when they can no longer increase in population and cells begin to show death which is characterized by the detachment of some cell populations from plastic culture plates. Finite in cells is an event that is not transformed and experiences telomere shortening due to the inactivity of the telomerase enzyme resulting in apoptosis which can affect the decrease in cell number. The following is the ASC culture procedure from the isolation results that will differentiate into fibroblast cells (Sari et al., 2016).

In the first stage, the MSC cell suspension obtained from the isolation results was dissolved into a culture tube containing complete medium, namely Dulbecco's Modified Eagle Medium (DMEM) + 1% non-essential amino acids + 1% penicillin/streptomycin solution supplemented with 10% fetal bovine serum (FBS) and incubated in a culture flask at 37°C with 5% CO2 under humid conditions, for 2 weeks. After 2 weeks of culture, the medium was discarded and cell colonies were fixed with absolute methanol and stained with 0.1%

crystal violet dye (Fatima, 2018).

The growth characteristics of MSCs were assessed by measuring plating efficiency, cumulative population doubling, and doubling time. The plating efficiency of MSCs was evaluated by culturing small amounts of cells for eventual colony growth. The number of colonies was counted after 2 weeks and this number was used to determine the plating efficiency, measured using the following formula:

PE: [Total number of colonies/number of cells initially grown] x 100

To determine population doubling, the initial cell count and the number of cells harvested at each passage were recorded. cells were counted with a haemocytometer and 10% of the cells were seeded into new culture tubes (Noviyantari et al., 2020).

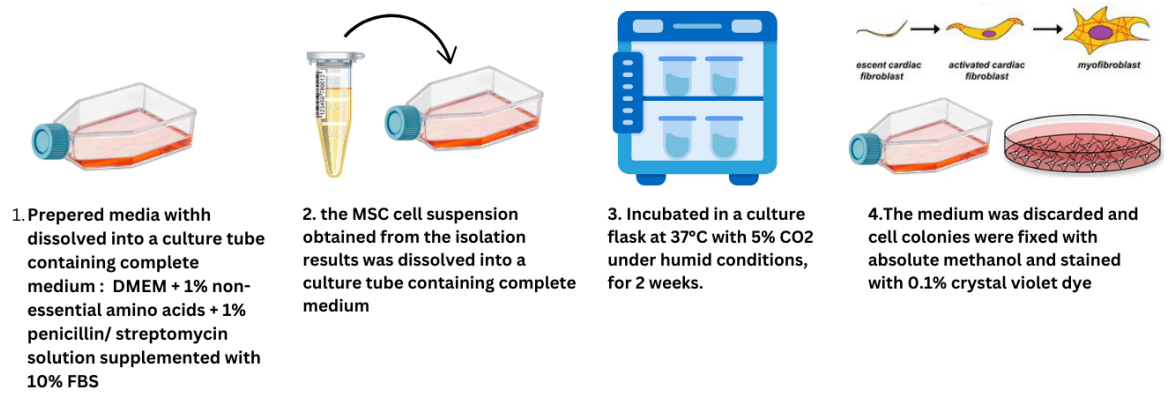

Figure 2. Adipose Stem Cell Culture

Differentiation of MSCs into fibroblast cells

For differentiation, MSCs were cultured in fibroblast induction medium for 2 weeks. Fibroblast stem cell differentiation media uses Dulbecco's Modified Eagle Medium (DMEM) with added growth factor Fibroblast Growth Factor (FGF) which helps to direct differentiation towards fibroblasts (Zhang et al., 2022).

Based on the research of Basil M. in 2019 with the title Differentiation of mesenchymal stem cells into fibroblasts, compositions comprising mesenchymal stem cell-derived fibroblasts, and methods of using the same' This study shows that DMEM enriched with FGF can increase the efficiency of mesenchymal stem cell differentiation into fibroblasts (Hasnah, 2019).

The differentiation medium was changed every three days. The fibroblast medium contained DMEM, 10% FBS, 1% penicillin/streptomycin solution, 5 ug/ml insulin and 1 ng/ml basic fibroblast growth factor. Differentiation of MSCs into fibroblasts was confirmed by morphological changes and through polymerase chain reaction (PCR) using marker genes for fibroblasts namely desmin, collagen 3, vimentin, and FGF7 (Sari et al., 2016).

Successful culture of adipose-derived stem cells (ADSCs) is evaluated based on parameters such as cell morphology, viability, and molecular characteristics. Successfully cultured cells showed spindle-shaped fibroblastic morphology with homogeneous distribution and a monolayer growth pattern on the culture surface (Han et al., 2019). Cell viability was assessed using trypan blue exclusion assay, with results >90%, indicating

optimal culture conditions (Wang et al., 2012). Immunophenotyping characterization was performed through flow cytometry analysis, showing positive expression of CD90, CD105, and CD73, and no expression of CD34, CD45, and CD14 (Han et al., 2019). In addition, successful culture is indicated by the ability to differentiate cells into three major lines-osteogenic, adipogenic, and chondrogenic-which can be confirmed through specific staining such as alizarin red, oil red O, and alcian blue (Hamra, 2016).

Mechanism of Stem Cell Application to Patients

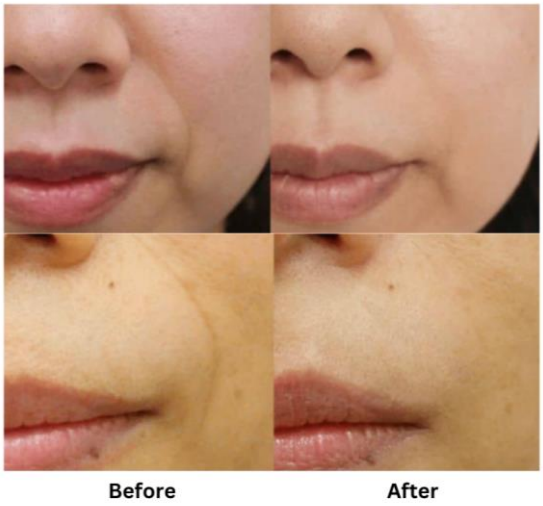

Figure 3. Differentiation of of MSC Therapy (Blu Clinic, 2022)

The use of stem cells from adipose tissue to tighten the face is known as facial rejuvenation, the application of cultured ASCs requires several steps performed by competent medical personnel. The processed stem cells are injected directly into the area of the face that needs improvement. Injections are performed using micro-techniques using fine needles, usually in the dermis or subcutaneous layer (Han et al., 2019). To improve results, some stem cells are mixed with platelet-rich plasma (PRP). The amount of ASC injected for facial skin tightening may vary depending on the procedure and doctor's recommendation. Generally, the dosage used ranges from 1 ml to 10 ml, depending on the area being treated. Stem cells that have differentiated into fibroblasts will then repair the skin from within by reducing signs of ageing such as wrinkles, sagging skin and hollows in the skin (Dirja et al., 2021).

MSC therapy increases skin cell proliferation and differentiation, resulting in more elastic, clear and hydrated skin. Stem cell therapy is carried out for 3 times of administration. After injection, MSCs start synthesising growth factors and cytokines such as VEGF (Vascular Endothelial Growth Factor), TGV-B (Transforming Growth Factor-beta), and IGF (Insulin-like Growth Factor). These molecules stimulate fibroblast cells in the skin to produce collagen and elastin, which are important for skin strength and elasticity (Bayu, 2021). Stem cells have a paracrine effect mechanism that can increase the proliferation and migration of dermal fibroblasts and epidermal keratinocytes and increase collagen synthesis in fibroblasts (Han et al., 2019).

Yin et al (2020) tested the efficacy of adipose stem cells for skin quality restoration in 50 women with an average age of 34.5 years. The test was divided into two behaviors, namely those who were only given stem cells (control) and those who were given stem cells with additional stromal vascular fraction (SVF) (inversion). The study was conducted for 6 months, with the results of the inversion group having a higher success rate (77.6%) than the control group (56.2%), judging by the improved wrinkles and texture.

Follow-up afterwards is post-procedure skin care. Patients will be given instructions on how to care for the skin to speed up recovery. Results will start to show within a few weeks to months after the procedure, which will see the skin become more elastic, wrinkles reduced, and the face

become firmer. To maintain these results, long-term follow-up is required (Zhum et al., 2013).

Patient care after adipose stem cell injection requires special attention to ensure optimal results and minimize the risk of side effects. After the injection procedure, the patient needs to perform health monitoring as monitoring for infection or other complications. This monitoring can use CT scan results to evaluate tissue regeneration that occurs after therapy (Ismi., et al., 2022). Patients are also advised to avoid strenuous physical activity for several weeks after performing the injection process to prevent stress on the injected area and support the healing process (Wulandari, et al., 2019). It is also important to keep the face and injection area clean by washing hands before handling the face. And protect the face from direct sunlight as the skin will be more sensitive after the injection procedure. Also, do regular check-ups according to the schedule set by the doctor to monitor the progress of recovery and treat any infections early (Zhum et al., 2013).

Patients who undergo the process of injecting stem cells from adipose tissue for facial tightening may experience some side effects. Generally, these side effects may include mild and temporary symptoms such as fatigue, headache, chills, nausea, and mild fever (Wulandari, et al., 2019). Some patients may also experience redness, swelling, pain, itching, and rashes, similar to the side effects experienced by patients who have used cosmetic fillers. There is also a risk of bacterial contamination during the culture and injection process. Therefore, it is important that this procedure is performed in a health facility that meets hygiene and safety standards (Ismi et al., 2022). There are also risks that can arise due to inflammation of the injected skin, namely granulomas, which are protrusions on the skin due to the inflammatory process. Therefore, it is important for patients to consult with their doctor before and after the procedure to ensure optimal results and minimize the risk of more serious side effects.

The advantage of stem cell therapy is that the procedure is non-invasive, using material from the patient's own body, so the risk of stem cells being rejected or turning into cancer is very small. The results of stem cell therapy provide natural-looking results.

In a case study, ADMCs were injected intradermally into wrinkled skin. After 2 months, it was found that skin texture and thickness improved. This occurred because ADMSCs have paracrine effects of the secretome which plays an important role in various regulation of physiological processes including cell growth, replication, differentiation, signalling, apoptosis, adhesion, and angiogenesis. However, the administration of the therapy must be done autogenously to avoid tissue rejection reaction, fat tissue harvesting cannot be done to the elderly, thin people and is not mass-produced. The secretome is derived from stem cells that show secreted molecules can cause tissue repair. The secretome can be found in ADMSCs cultures. This medium is called conditioned medium (CM). CM was injected using microneedles into 30 volunteers' facial skin and observed for 3 months. CM injection showed significant improvement in skin brightness, skin smoothness, skin elasticity, and decreased melanin index (Qian et al., 2023)

In a study (Jeong et al., 2014) compared adipose tissue-derived stem cells (ADSCs) with fibroblasts in the repair of skin wrinkles caused by photoaging, and found that ADSCs were as effective as fibroblasts in increasing collagen protein production. In the study (Lv et al., 2021) stated that these ADSCs have anti-aging effects on aging cells and on animal models of premature aging. These ADSCs can also accelerate mitophagy, eliminate intracellular ROS, and will ultimately increase the number of mitochondria. Fragmented micro adipose

tissue containing ADSC cells in combination with hyaluronic acid scaffolds will crosslink and can repair soft tissue defects such as deep wrinkles (Svolacchia et al., 2022).

In the study (Park et al., 2008) there are many clinical applications for mesenchymal stem cell treatments, including ADSCs in restitution of skin defects and wound healing. In previous studies, these ADSCs and conditioned ADSC media (ADSC-CM), would stimulate collagen synthesis and fibroblast migration during wound healing. This can also be attributed to various other factors produced by ADSCs (proven in the journal of dermatological sciences). Treatments for skin aging such as lasers and topical regimens induce new collagen synthesis through the activation of skin fibroblasts or growth factors (Pustovalova et al., 2016).

To confirm this, (Park, B.S., et al2008) conducted animal studies in vitro and in vivo, including pilot studies using volunteers and autologous ADSCs (Jo et al.,2021). For the in vitro case study, adipose tissue samples were obtained from elective liposuction with the client's consent, while for the in vivo case study of adipose tissue-derived fat stem cells (ADSC) in the skin, studies were conducted in mice and human patients. In mice, it was found that intradermal injection of ADSC and ADSC-CM led to a small increase in skin thickness and increased collagen expression (Ou et al., 2022).

In human patients, two injections containing the processed ADSC were performed on pre-photographed skin. As a result, the patient showed a general improvement in skin texture and wrinkles after two months (Pittenger et al., 2019). This was observed through comparison of medical photographs and skin thickness measurements using 20 MHz high frequency ultrasound. These findings indicate that the use of ADSC can benefit human skin regeneration and repair (Rahmadewi et al., 2020).

In Jason's 2014 MSC application case titled Stem Cells for Skin Rejuvenation, there was a woman who had painfully developed small bone fragments around her eyes after receiving a facelift.

SUMMARY

The use of adipose stem cells as an anti-ageing therapy shows promising results in improving skin quality and correcting signs of ageing. ADSCs are able to stimulate collagen and elastin production, and have anti-inflammatory effects that may help reduce oxidative stress. However, despite this great potential, further research is needed to overcome challenges in clinical applications, including understanding the mechanisms of differentiation and immune response. Strict regulatory and ethical considerations must also be adhered to in order to ensure the safety and effectiveness of these therapies. Going forward, the development of better strategies to utilise ADSCs in beauty care is expected to provide wider and sustainable benefits.

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