STEM CELL HARVEST FROM ADIPOSE TISSUE

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Abstract

Background: Differentiation, self-renewal, immunomodulation, and angiogenesis are all capabilities of stem cells that can help improve tissue structure (Bacakova et al, 2018). In adipose tissue, there is a stromal vascular fraction (SVF) that contains a variety of cell types that can transdifferentiate between endothelial and adipogenic cell lineages. (Schreml et al., 2009). The procedure used to harvest adipose tissue, such as liposuction or excision, has an impact on the quality of SVF and ADSC recovered from it. Barzelay et al., 2015). **Literature review:** The two most common locations for stem cell harvest are bone marrow and fat tissue. Taking stem cells can be done in a variety of ways, depending on the source of the cells. Isolating stem cells from preimplantation embryos is how embryonic stem cell retrieval is done. Liposuction is the removal of subcutaneous tissue with the help of a suction pump and an aspiration cannula introduced through a skin incision. Suction-assisted lipectomy, suction lipoplasty, and liposculpture are other synonyms for liposuction. The advantage of liposuction over resection for obtaining stem cells is that it is less intrusive and can preserve neurovascular structures. In addition, liposuction preserves fluid balance and reduces patient discomfort. A high-speed cannula is used in liposuction to disrupt adipocytes. This procedure has the potential to cause cellular harm. Adipocyte viability and transplant survival will be improved by using less stressful adipose tissue extraction procedures. (Simonacci et al., 2017). Under general anaesthesia, the resected tissue was removed whole. In order to separate ADSC, fibrous tissue and visible blood vessels were removed from the retrieved adipose tissue. Resection of adipose tissue produced more SVF and ADSC cells than liposuction, according to Iyyanki et al and Faustini et al. This differs from the findings of a prior study by Schreml et al. **Conclusion:** Liposuction and resection both have advantages and downsides when it comes to extracting stem cells from fat tissue. There was no discernible difference in the removal of stem cells from adipose tissue by liposuction versus excision.

CHAPTER 1

INTRODUCTION

Differentiation, self-renewal, immunomodulation, and angiogenesis are all capabilities of stem cells that can help improve tissue structure. In tissues, stem cells can develop into a variety of cell types. Anti-apoptotic, anti-inflammatory, and pro-vascular properties are also found in stem cells. In vivo treatment of PFD with stem cells has been established in recent investigations (Cheng et al., 2020). Embryonic tissue, fetal tissue, particular places in adult organisms (fat tissue, bone marrow, skeletal muscle, skin, or blood), and differentiated somatic cells after genetic reprogramming (iPSC) are the most common sources of stem cells (Bacakova et al, 2018).

Because the retrieval method results in the demise of the embryo, embryonic stem cell harvesting has prompted ethical debate. Adult stem cells are primarily extracted from bone marrow and adipose tissue. 2015 (Dittrich, Beckmann, and Würfel). The extraction of stem cells from bone marrow is a difficult process. Under general anesthesia, 0.5-1.5 liters of blood are drawn from the iliac crest in order to extract stem cells from the bone marrow. Complications might include pain, bleeding at the puncture site, osteomyelitis, and soft tissue infection (Kara et al, 2016).

In adipose tissue, there is a stromal vascular fraction (SVF) that contains a variety of cell types that can transdifferentiate between endothelial and adipogenic cell lineages. In adipose tissue, there is a population of multipotent adipose tissue-derived adult stem cells (ADSC) that have the ability to differentiate into various cell lineages derived from the germ layer of mesoderm such as adipocytes, chondrocytes, and osteoblasts, hepatocytes, muscle cells, and endothelial cells. ADSC cells can differentiate into non-mesoderm lineages, such as neuron-like cells. These characteristics cause ADSC to be one of the most desirable cell populations for tissue engineering purposes (Schreml et al., 2009).

The procedure used to harvest adipose tissue, such as liposuction or excision, has an impact on the quality of SVF and ADSC recovered from it. Compared to resection treatments, liposuction is less intrusive, can preserve neurovascular systems, keep fluid balance, and reduce patient suffering. Outpatient liposuction can be done. For tissue engineering, the amount of ADSC cells recovered from liposuction aspirates is sufficient. Liposuction has been used since the 1970s and is a relatively simple surgery with few risks (Barzelay et al., 2015).

The cell viability of ADSCs obtained by liposuction or excision was not found to differ in the current research. Although the rate of cell differentiation is higher than that acquired through resection, the quantity of ADSCs is higher than that obtained through liposuction treatments (Schreml et al., 2009). Resection of adipose tissue to extract stem cells is a more intrusive and unpleasant procedure for the patient (Faustini et al., 2010; Iyyanki et al., 2015). Except for upregulation of the aging initiator SERPINB2. from adipose tissue removed by resection, Barzelay et al. found no difference in the aging profile of active genes among stem cells from liposuction and resection in a study that compared the aging ratio of ADSC by assessing -galactosidase activity (Barzelay et al., 2015). Iyyanki et al. and Faustini et al. found that excision of adipose tissue produced more SVF and ADSC cells than liposuction (Faustini et al., 2010; Iyyanki et al., 2015).

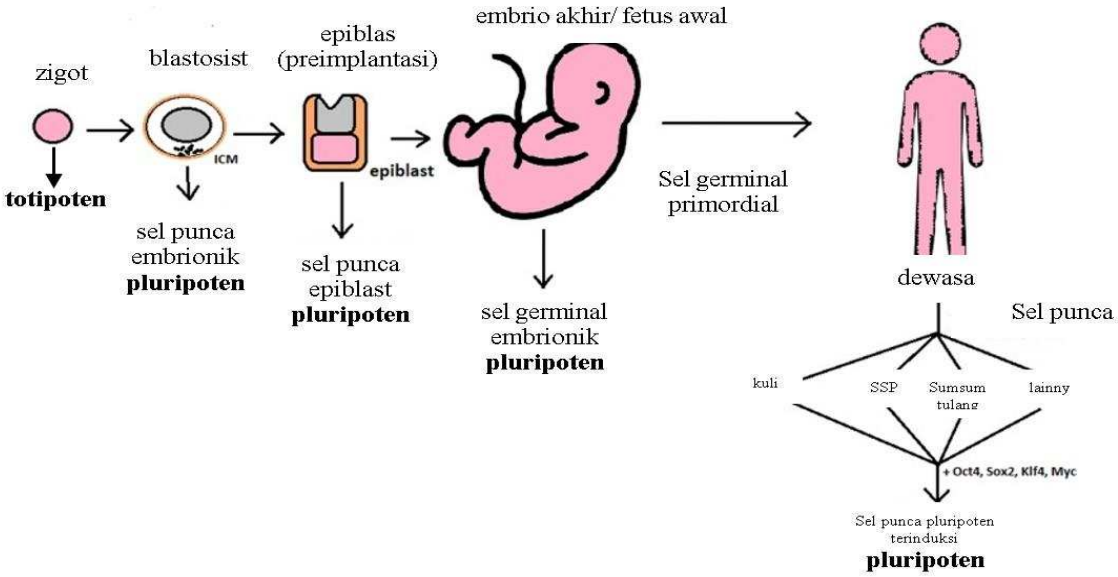
CHAPTER 2

LITERATURE REVIEW

2.1 Definition And Source Of Stem Cells

Stem cells are self-renewing cells that have the ability to differentiate into several types of cells. In both embryonic and adult cells, stem cells are found. Stem cells are divided into totipotent, pluripotent, multipotent, oligopotent, and unipotent stem cells depending on their ability to differentiate. Totipotent stem cells have the ability to divide and differentiate into any type of cell in the body. These cells have the greatest differentiation capability and can generate both embryonic and extraembryonic structures. A zygote, which is generated when a sperm and an ovum fertilize each other, is an example of a totipotent cell. The placenta is formed by these cells dividing into three germ layers. The blastocyst's inner cell mass develops pluripotency at 4 days. Pluripotent stem cells come from totipotent cells (Zakrzewski et al., 2019) .PSCs can generate cells in the germ layer but not extra-embryonic tissues like the placenta. Pluripotent stem cells, such as embryonic stem cells (ESC), are an example. The inner cell mass of a preimplantation embryo is used to make embryonic stem cells. Induced pluripotent stem cells (iPSCs), which come from the embryo's epiblast layer, are another type of pluripotent cell. Pluripotency is a spectrum that ranges from fully pluripotent cells like ESCs and iPSCs to less potent cells like fibroblasts (multi-, oligo-, and unipotent cells). The teratoma development assay is one method for determining the spectrum and activity of pluripotent cells (Zakrzewski et al., 2019).

Multipotent stem cells have a smaller differentiation spectrum than pluripotent stem cells, but they can specialize to generate particular cell lineages. Hematopoietic stem cells, for example, can differentiate into a variety of blood cell types. Hematopoietic stem cells differentiate into oligopotent cells, which can only differentiate cells from the same lineage. Some multipotent cells, on the other hand, have been reported to convert to other cell types. Oligopotent stem cells have the ability to develop into a variety of cell types. The myeloid stem cell is an example of this type of stem cell, which can divide into white blood cells but not red blood cells. Unipotent stem cells have the smallest differentiation potential yet can divide indefinitely. These cells are a good candidate for regenerative therapy because of their capacity to divide into unipotent cells. A dermatocyte is an example of a unipotent cell because it can only create one type of cell (Zakrzewski et al., 2019).



Gambar 2.1. Perubahan potensi sel punca selama perkembangan manusia (Zakrzewski *et al.*, 2019)

Embryonic tissue, fetal tissue (fetus, placenta, amniotic fluid, and umbilical cord), adult tissue (fat, bone marrow, skeletal muscle, skin, or blood), and somatic cells are the most common sources of stem cells. After genetic reprogramming, they became distinct (iPSC). (Bacakova et al., 2018).

2.2 Extraction Of Stem Cells

Almost all types of tissues contain mature mesenchymal stem cells. The two most common locations for stem cell harvest are bone marrow and fat tissue. Taking stem cells can be done in a variety of ways, depending on the source of the cells. Isolating stem cells from preimplantation embryos is how embryonic stem cell retrieval is done.

2.3 Fat Tissue-Derived Stem Cells

Endothelial cells, blood cells, fibroblasts, pericytes, preadipocytes, macrophages, and various types of immune cells are all found in adipose tissue, in addition to adipocytes. The stromal vascular fraction refers to these non-adipocytes (SVF). Multipotent adipose-derived stem cells are a type of mesenchymal stem cell found in SVF (ADSC). ADSC was discovered for the first time in 2001. These cells resemble mesenchymal stem cells in appearance (MSCs). The immunomodulating, anti-inflammatory, and angiogenic properties of SVF and ADSC isolated from SVF are identical. SVF has two distinct advantages over ADSC. SVF, for starters, has diverse cell components, which means it can deliver superior therapeutic effects in animal experiments. Second, SVF is more accessible than ADSC. SVF was obtained without the need for cell separation and culture, as was the case with ADSC. Because SVF has little contact with chemicals, it is safer and meets fewer regulatory requirements. SVF can only be utilized for autologous therapy because the presence of multiple cell types can trigger immunological rejection. While ADSC can be utilized for both allogeneic and autologous therapy, it is most commonly employed for allogeneic therapy (Bora and Majumdar, 2017). Surface antigens (cluster of differentiation/CD) can be used to identify the cells in SVF (Bora and Majumdar, 2017).

2.4 Fat Tissue Stem Cell Removal

2.4.1 Liposuction extraction

Liposuction is the removal of subcutaneous tissue with the help of a suction pump and an aspiration cannula introduced through a skin incision. Suction-assisted lipectomy, suction lipoplasty, and liposculpture are other synonyms for liposuction. This treatment is usually performed for cosmetic reasons. In 1893, Neuber performed the first autologous fat transfer to cover depressed scars. Fisher then launched liposuction in 1974. Coleman devised a new approach in 1987 that lowers fatty tissue disintegration during the suctioning procedure. Dry liposuction is a type of liposuction that was first performed under general anesthesia without the use of any fluids. Next, apply a small amount of liquid to the fat, sometimes known as the wet technique. This approach causes the patient to lose more blood, necessitating blood transfusions. Dr. Jeffrey A. Klein invented the tumescent technique in 1985, which allows liposuction to be performed under local anesthetic with minimum bleeding. This treatment is the gold standard for liposuction procedures all around the world (Fransesco et al., 2019).

Fat filler was originally only used for lipofilling. However, ADSCs have been discovered in adipose tissue, which allow for the regeneration of injured tissue via paracrine, immunomodulatory, chemotactic, and differentiation actions. As a result, fat transplantation has progressed over the last two decades. Fat extraction that is less stressful improves adipocyte viability and graft durability. Vacuum aspiration or syringe aspiration with or without tumescent fluid are the most common procedures for removing adipose tissue. The cells obtained using a syringe and those taken with pump-assisted liposuction had no changes in viability, activity, or adipogenic response (PAL). The tumescent approach produces hydrodissection and enlargement of the target fat layer, allowing for easier aspiration and a reduction in discomfort and ecchymosis. Meanwhile, the need of analgesics is increased due to the dry approach (Fransesco et al., 2019).

2.4.1.1 Liposuction procedure for obtaining stem cells

Subcutaneous infiltration with significant amounts of Klein's solution is the first step in the liposuction procedure. Low levels of lignocaine and epinephrine are present in this solution. Fat aspiration is also performed with the use of a pump and a small cannula, sometimes known as a micro cannula. Making a small puncture incision to introduce the needle is the first step in infiltration. Cannulas of various lengths are used to infiltrate the fluid into the subcutaneous adipose tissue to the desired depth. After that, the suction cannula is placed into a thick layer of fat. The vacuum is turned on, and the cannula is inserted in a radial pattern (Fransesco et al., 2019).

The non-dominant hand monitors the location and orientation of the liposuction cannula as it is placed into the incision area. To reduce the danger of perforation, a blunt tip cannula is employed, and the diameter of the cannula is reduced to reduce contour irregularities. The diameter of the cannula and suction tube is proportional to aspiration, while the length of the cannula and suction tube is inversely proportional. The negative pressure required for manual liposuction is created by withdrawing the syringe. PAL (power-assisted liposuction) is a liposuction technique that employs a machine to provide a cannula reciprocating motion. The usage of PAL decreases the energy consumed by the operator and speeds up the suctioning operation (Fransesco et al, 2019).

Sedimentation, filtration, washing, and centrifugation are all steps in the process of removing fat tissue. Lipoaspirates contain not only adipocytes, but also collagen fibers, blood, and detritus, necessitating fat processing. These substances have the potential to produce

inflammation in the recipient. Blood must be removed from adipose tissue because it causes the transplanted adipose tissue to degrade faster. When compared to decantation, centrifugation increased the quantity of ADSCs but decreased cell viability. Lemak aspirasi pada syringe diputar dengan kecepatan 3000 rpm untuk mengisolasi lemak selama 3 menit. After sentrifugasi, akan terbentuk 3 lapisan, yaitu lapisan pertama yang mengandung lipid, lapisan dua yang mengandung jaringan lemak, dan lapisan ketiga yang mengandung darah, cairan jaringan, dan lokal anestetik yang keluarkan dari dasar syringe. Normally, the second lapisan is used for jaringan lemak transplantation. The best pemrosesan method will increase the number of viable sel and decrease the transplantation rate (Fransesco et al., 2019).

2.4.1.2 Benefits of obtaining stem cells by liposuction

The advantage of liposuction over resection for obtaining stem cells is that it is less intrusive and can preserve neurovascular structures. In addition, liposuction preserves fluid balance and reduces patient discomfort. The ADSC collected from liposuction aspirate is sufficient for tissue engineering purposes. With an estimated ADSC frequency of 1-5 percent, or roughly 4,000-20,000 cells/mL, the amount of SVF collected from liposuction aspirates approaches 400,000 SVF cells/mL. Liposuction produced more ADSCs than resection, according to Schreml et al, however the difference was not significant (Schreml et al., 2009).

There were no significant differences in adhesion, growth rate, or differentiation potential between ADSC and MSC from bone marrow when they were compared. CD13, CD29, CD44, CD71, CD90, CD105/SH2, SH3, and STRO-1 were all present in both stem cell groups. ADSCs have been discovered to be multipotent stem cells with the ability to differentiate into adipogenic, chondrogenic, osteogenic, and neurogenic tissues. (2009) (Schreml et al.) By measuring -galactosidase activity, Barzelay et al were able to compare the aging ratio of ADSC. Except for the overexpression of the aging initiator SERPINB2 from adipose tissue removed by resection, no significant variations in the aging profile of active genes were detected between the two groups. However, there was no significant variation in -galactosidase activity, division rate, or ADSC differentiation capacity as a result of this discrepancy. (Barzelay et al, 2015).

2.4.1.3 Disadvantages of stem cell extraction by liposuction

A high-speed cannula is used in liposuction to disrupt adipocytes. This procedure has the potential to cause cellular harm. In the current investigations, however, there is no difference in cell survival between ADSCs obtained via liposuction and those obtained by

resection. According to Schreml et al, ADSCs acquired via liposuction have a lesser degree of differentiation than those produced through resection.

In the liposuction procedure, ADSCs were able to differentiate into two or more lineages 33.33 percent of the time, but in the resection method, they were able to differentiate into two or more lineages 79.17 percent of the time. On liposuction and resection procedures, the rates of differentiation of adipogeny, osteogenesis, combined adipogenesis and osteogenesis, and combination chondrogenesis and osteogenesis were 53.33 percent vs 79.17 percent, 26.67 percent vs 58.33 percent, and 12.50 percent vs 13.33 percent, respectively. Differences in the osteoblast induction media employed could account for the poor differentiation into osteocytes (Schreml et al., 2009).

2.4.2 Fat tissue excision is used to remove the excess fat

Adipocyte viability and transplant survival will be improved by using less stressful adipose tissue extraction procedures. Excision versus aspiration has been shown to be superior in several experimental and clinical investigations. The shape and viability of the excised adipose tissue can be preserved with resection while the adipocytes are not harmed. Adipocyte function was found to be significantly decreased in aspirates from traditional liposuction in another investigation. Up to 90% of adipocytes can be damaged by high vacuum pressure. Excision and liposuction with a large cannula can assist maintain the tissue's original structure and limit the risk of cellular rupture. The diameter of the device used to harvest fat has an inverse association with the amount of cellular damage (Simonacci et al., 2017).

2.4.2.1 Fat tissue excision procedure for extracting stem cells

Under general anaesthesia, the resected tissue was removed whole. In order to separate ADSC, fibrous tissue and visible blood vessels were removed from the retrieved adipose tissue. The tissue was then sliced into smaller sizes as a result of this (diameter 1-4 mm). Collagenase NB 6 GMP was used for enzymatic digestion and occasional mixing with a plasma-thawing apparatus for 30-60 minutes at 37oC. After incubation, 300 mL of phosphate buffered saline (PBS) was added to the tissue to wash away any leftover collagenase (Schreml et al., 2009).

After that, a blood set was used to transmit the sediment. The network storage bin was centrifuged at 400g for 10 minutes. After the centrifugation procedure, the supernatant was discarded. Then 10 mL of 0.9 percent sodium chloride and 3 mL of citrate phosphate dextrose (CPD) solution were given. A syringe was used to extract the cell pellets (Schreml et al., 2009).

2.4.2.2 Benefits and drawbacks of obtaining stem cells by resection

Resection of adipose tissue produced more SVF and ADSC cells than liposuction, according to Iyyanki et al and Faustini et al. This differs from the findings of a prior study by Schreml et al. Fat tissue collected using different procedures exhibited varying densities, according to Iyyanki et al. Liposuction aspirates are less viscous than resection aspirates. The resection method has the disadvantage of being a more invasive and unpleasant procedure for the patient (Faustini et al., 2010; Iyyanki et al., 2015).

CHAPTER 3

CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusion

Liposuction and resection both have advantages and downsides when it comes to extracting stem cells from fat tissue. There was no discernible difference in the removal of stem cells from adipose tissue by liposuction versus excision.

3.2 Recommendations

It is suggested that more research on the extraction of stem cells from fat tissue be carried out.

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