Nano Fat Therapy- A Boon to Regenerative Medicine

1.1 Background and Significance

Regenerative medicine has emerged as a revolutionary branch of medical science that focuses on harnessing the body's natural healing potential to restore or replace damaged tissues and organs. Over the years, many innovative therapies and techniques have been developed to enhance the regenerative process, and one such cutting-edge advancement is nano-fat therapy.

Adipose tissue is a well-organized compact tissue that contains a diverse cell population of progenitor cells, including mesenchymal stromal cells. Because of its availability and accessibility, adipose tissue is referred to as the "repository of stem cells". Anti-inflammatory, anti-fibrotic, anti-apoptotic and immunomodulating properties are found in adipose tissue products. (1) Autologous fat grafting is an aesthetic and reconstructive procedure that involves harvesting and injecting an individual's own fat into soft tissues to correct shape and other defects. (2)

The first "fat grafting" technique was performed in the late 19th century, in 1893, by Gustav Neuber (1850-1932), a German plastic surgeon, to treat orbital defects caused by osteomyelitis. Later Dr. Viktor Czerny (1842-1916) transplanted a lipoma into the breast to restore symmetry after a unilateral partial mastectomy in 1895. In the early twentieth century, preliminary reports of autologous fat grafting were available. It came into frequent use after Illouz's introduction of liposuction in the 1980s and Coleman's standardization of the fat grafting procedure in the 1990s. Adipose-derived stem cells were first identified in 2001. Since then, considerable research has been conducted on these multipotent mesenchymal-derived progenitor cells. (3)

Tonnard et al. in 2013 he discovered a new method of mechanically processing lipoaspirate to minimize the size of fat particles needed to create an injectable product known as nanofat. (4) Nanofat, a small bundle of stem cells with the ability to regenerate and rebuild tissues, offers translational and regenerative medicine applications. Applications of nanofat can be applied in various professions due to the wide range of applicability of its reconstructive and regenerative abilities. This chapter aims to provide an overview of nano-fat therapy, its applications in regenerative medicine and its potential benefits.

1.2 Definition and Composition of Nano Fat

There are two forms of adipose tissue: thermogenically active brown adipose tissue (BAT) and energy-storing white adipose tissue (WAT). (5) White adipose tissue consists of two main components: mature adipocytes and the stromal vascular fraction (SVF). The SVF is a diverse cell population consisting of endothelial cells, smooth muscle cells, pericytes, leukocytes, fibroblasts, mast cells, preadipocytes, and adipose-derived multipotent stem cells (ASCs). Adipose tissue has the largest percentage of adult stem cells of any tissue in the body, even surpassing bone marrow. There are up to 4,500 ASCs per milliliter of fat, but only 100 to 1,000 stem cells per milliliter of bone marrow. (6)

Subcutaneous adipose tissue has the highest tissue partial tension of oxygen (ptO2: 40-60 mm Hg) of all internal organs due to the high density of capillaries with parallel growth and low oxygen consumption. Mature adipocytes are extremely fragile and have a low tolerance to mechanical stress and ischemia. Conversely, preadipocytes, which have no metabolic activity and are 20 times smaller than mature adipocytes, are far more resistant to traumatic and ischemic shocks. (5) ASCs regenerate by paracrine secretion of cytokines and growth factors, with greater amounts secreted under hypoxic circumstances.

These factors include angiogenic cytokines such as VEGF, HGF, fibroblast growth factor 2, and basic fibroblast growth factor, as well as hematopoietic cytokines such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. Adipocyte regeneration is primarily mediated by ASCs, and these multipotent cells, together with a small fraction of adipose multilineage stress-resistant (Muse) cells, can differentiate not only into adipocytes and endothelial cells of the vascular system, but also into bone, cartilage, skeletal muscle, neurons, and skin. (6)

Nano fat is a unique form of adipose tissue that has been mechanically and enzymatically processed to create a powerful regenerative solution. Nano fat, which is typically obtained from the patient's own adipose tissue through a minimally invasive procedure such as liposuction, consists of a high concentration of mesenchymal stem cells (MSCs), pre-adipocytes, growth factors, and extracellular matrix (ECM) components. The extracted fat was mechanically emulsified and filtered until a liquid fat emulsion was formed, which is known as "NANOFAT". This concentrated cellular and molecular mixture makes nano fat an ideal candidate for regenerative therapy.

Mechanical emulsification and filtration break up adipocytes, leaving viable SVF intact. Although this term is a misnomer as there are no living fat cells after processing. The Tonnard approach uses mechanical disruption through small diameter luer lock connectors followed by filtration through a 500mm filter. The rapid technique allows the isolation of SVF as well as certain non-viable components of adipocytes. The active ingredient, SVF, stimulates the proliferation of the endothelium, the formation of collagen and the differentiation and formation of new cells. (3)

As a milestone, nanofat was described and published in 2013, and the methodology and definitions used in the mechanical isolation of stromal cells until May 2020 were systematically reviewed using PRISMA by Copcu HE and Oztan S (7). As a result of these investigations, the two most used acronyms, SVF and nanofat, are not able to accurately identify the mechanically obtained final product, a specific description of the mechanical extraction of stromal cells from adipose tissue was created for the first time. Instead of SVF or nanofat, we recommend using the abbreviation TOST, which stands for total stromal cell. Because although mechanical methods have several advantages over enzymatic methods, the most important is that no dissolving chemical such as an enzyme is used, the integrity and presence of stromal cells is maximized.

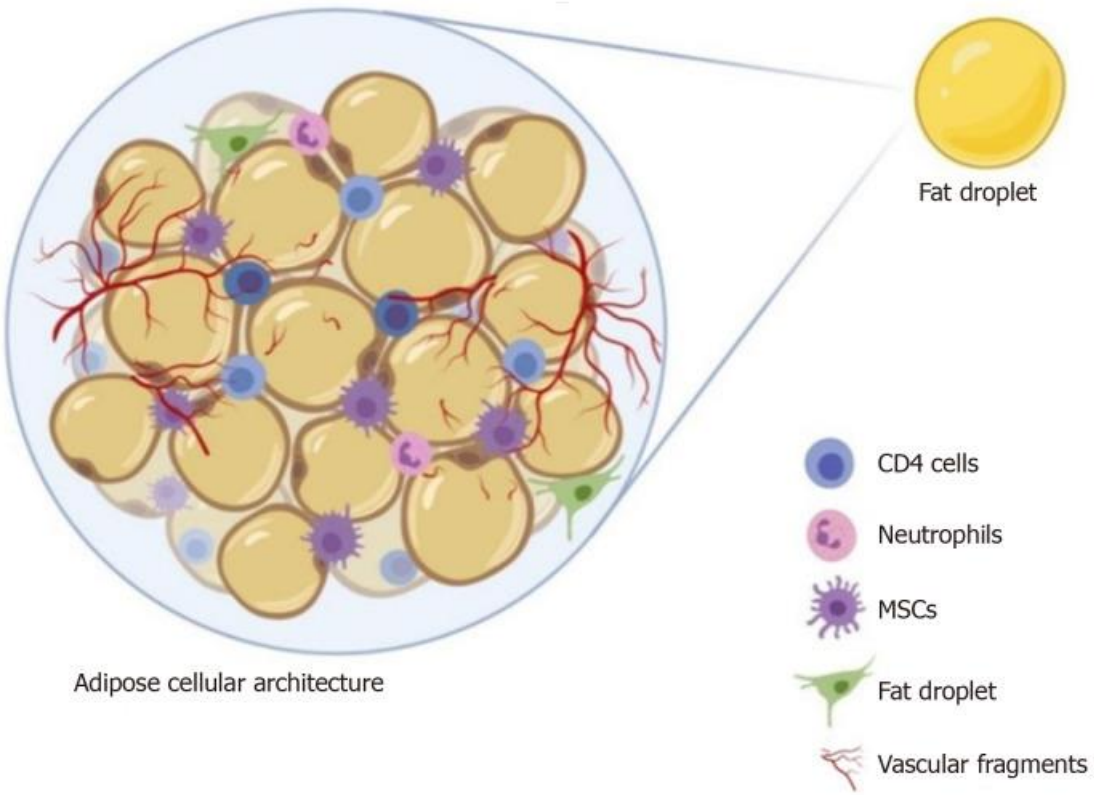

Figure 1 – Structure of Adipose Tissue (1)

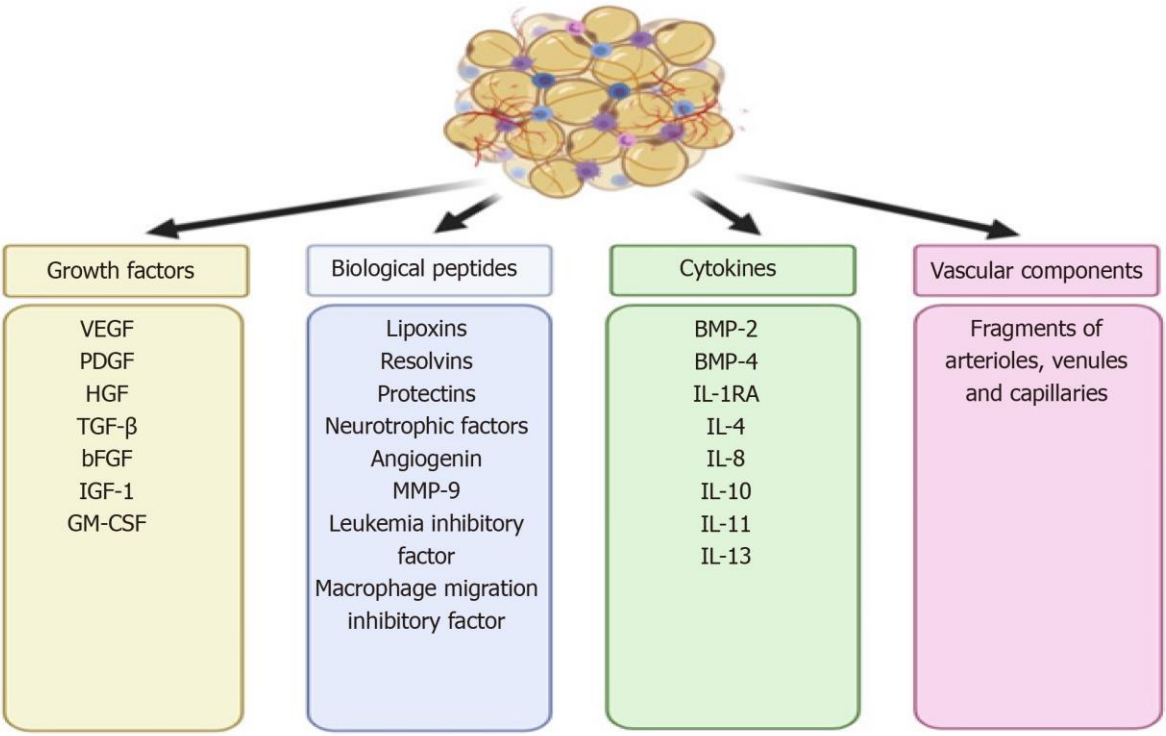

Figure 2 – Composition of Nanofat (1)

1.3 Processing of Nanofat

During fat processing, the obtained fat is cleaned of fluid, blood, cell fragments and oil. By removing these substances, the remaining fat becomes more suitable for injection and future fat graft collection. There are many techniques available for post-harvest fat processing, which are listed below. (8)

1. Gravitational separation (also known as decantation or sedimentation)

2. Centrifugation,

3. Rolling cotton gauze,

4. Washing and filtering systems

Tonnard described a preparatory protocol for nanofat harvesting (Figure 3) that involved infiltrating the lower abdomen with modified Klein's solution (lidocaine 800 mg/L and epinephrine 1:1000000) prior to adipose tissue harvesting with a multiport 3 mm cannula with 1 mm sharp side holes. (1)

The emulsified adipose tissue was once again filtered through a sterile nylon cloth and the effluent was collected in a sterile container labeled "nanofat". Compared to enzymatic digestion of adipose tissue, preparation of nanofats minimizes processing time, cost, and regulatory constraints.

Ferraro et al. found that centrifugation at more than 50 g caused structural damage to adipose tissue, increased necrosis and cell death, decreased adipogenic differentiation capacity, and decreased tubule formation. (9)

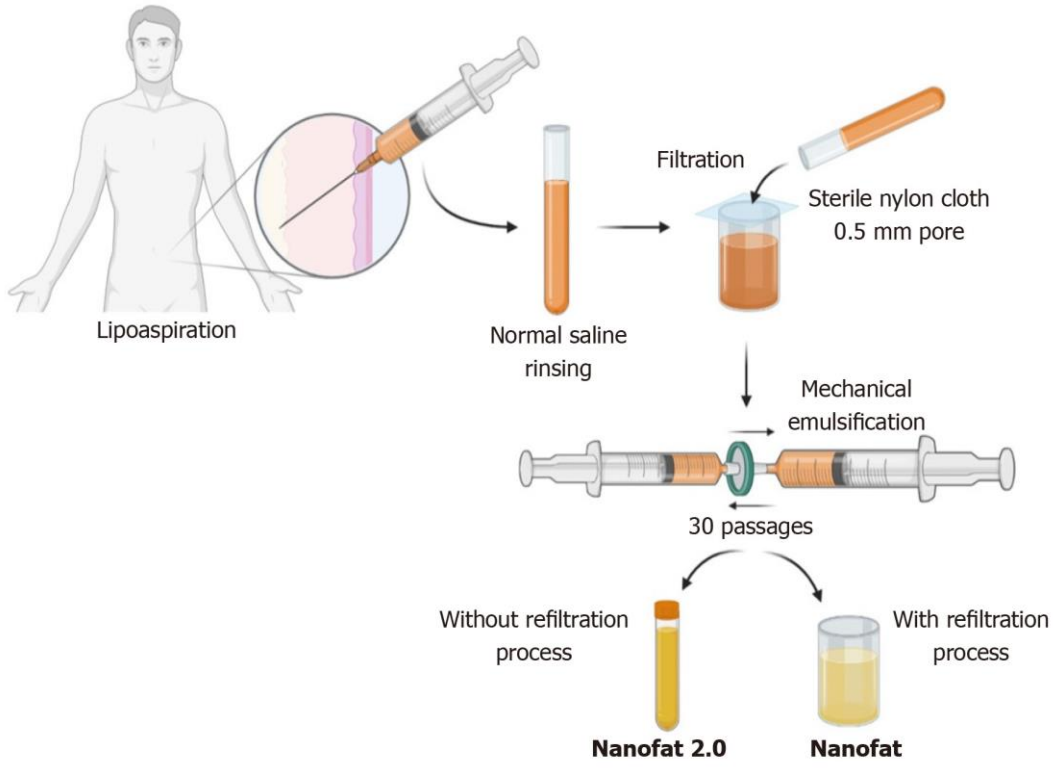

Figure 3 – Preparation of Nanofat (1)

1.4 Mechanisms of Action

Nano fat therapy works through three basic mechanisms: cellular replacement, paracrine signaling and ECM remodeling

1.4.1 Cell Replacement

Nano fat is rich in MSCs and preadipocytes, which have the ability to differentiate into various cell types, including adipocytes, fibroblasts, and endothelial cells. When these cells are injected into a target tissue or organ, they can replace damaged or lost cells, promoting tissue regeneration and functional restoration.

1.4.2 Paracrine signaling

Nanofat secretes a diverse array of growth factors, cytokines, and chemokines that have paracrine effects on neighboring cells. These factors stimulate cell proliferation, angiogenesis and immune modulation, creating a favorable microenvironment for tissue repair and regeneration.

1.4.3 ECM remodelling

ECM components present in nano fat, such as collagen and elastin, play a key role in providing structural support and promoting tissue remodelling. After injection, these components contribute to restoring the architecture and integrity of damaged tissues, facilitating proper function.

1.5 Applications of Nano Fat Therapy in Regenerative Medicine

Nano fat therapy has shown promising results in various areas of regenerative medicine. The following sections highlight its applications in various medical fields.

1.5.1 Cosmetology

Nano fat, when injected into facial tissues, provides specific benefits in terms of facial rejuvenation and skin regeneration. Growth factors and ECM components present in nano fat stimulate collagen production, improve skin elasticity and reduce the appearance of wrinkles and fine lines. In addition, the regenerative properties of nano fat promote angiogenesis, improve blood flow and tissue nutrition, thereby improving the overall quality and texture of the skin.

Nano fat grafting is also used to improve the appearance of dark circles under the eyes, malar bags, sunken eyes and blepharoplasty. (1) Because of its biocompatibility, lack of immunogenicity, and availability, fat grafting is considered the best soft tissue filler. (2)

1.5.2 Wound healing and scar reduction

Nano fat therapy has shown remarkable results in promoting wound healing and reducing scar formation. Regenerative components of nano fat facilitate tissue regeneration, speed up the healing process and minimize the formation of scar tissue. The paracrine signaling and remodeling effects of the ECM help modulate the inflammatory response, enhance neovascularization, and promote collagen remodeling, leading to improved wound closure, reduced scarring, and improved functional recovery.

Grafting nanofat under and into the scar mass improves the quality, integrity, and texture of the scar, which improves tone, texture, thickness, elasticity, resilience, and color while reducing scar size. Autologous emulsified nanofat injection is a simple outpatient surgical method for scar repair with limited complications and high patient satisfaction. It reduces the symptoms and texture of all types of scars. For patients with significant facial burn scars where other therapeutic options are limited, nanofat grafting is a potential approach. (10) It can also be used to improve existing surgical treatment by having regenerative effects on the treated area and preventing contracture and adhesion during the postoperative period. (11)

1.5.3 Orthopaedic injuries and musculoskeletal conditions

Nano-fat therapy has shown promising results in the treatment of orthopedic injuries and musculoskeletal conditions such as osteoarthritis, tendinopathy, and cartilage defects. The regenerative properties of nano fat aid in tissue repair and regeneration by stimulating the proliferation and differentiation of MSCs into chondrocytes and osteoblasts. This promotes cartilage and bone formation, leading to reduced pain and functional improvement.

A study by Chen et al demonstrated that Nanofat is anti-osteoarthritis by alleviating joint pain symptoms and preventing cartilage degradation in OA rats through paracrine effects on anabolic, catabolic and hypertrophic chondrocyte molecules (12).

Given the multidifferentiation ability of adipose tissue, it could be extrapolated for use in avascular bone necrosis, mild to moderate osteoarthritis, tendinopathies, and fracture nonunion. (1)

1.6 Fate of Nanofat Graft

During adipose tissue transplantation, it is transferred, perivascular ASCs play an important role in the revascularization of the fat graft, which release angiogenic factors in response to ischemia. To achieve optimal graft revascularization, each droplet of fat graft (G) must engage with the capillary recipient site (R) in a 1:1 ratio to form a successful fat-recipient (GR)

complex. More fat droplets than capillary recipient sites will result in insufficient neoangiogenesis, which is thought to be the cause of fat resorption and necrosis. First described by Khouri and Khouri, it is known as the stoichiometric principle of fat grafting. (13)

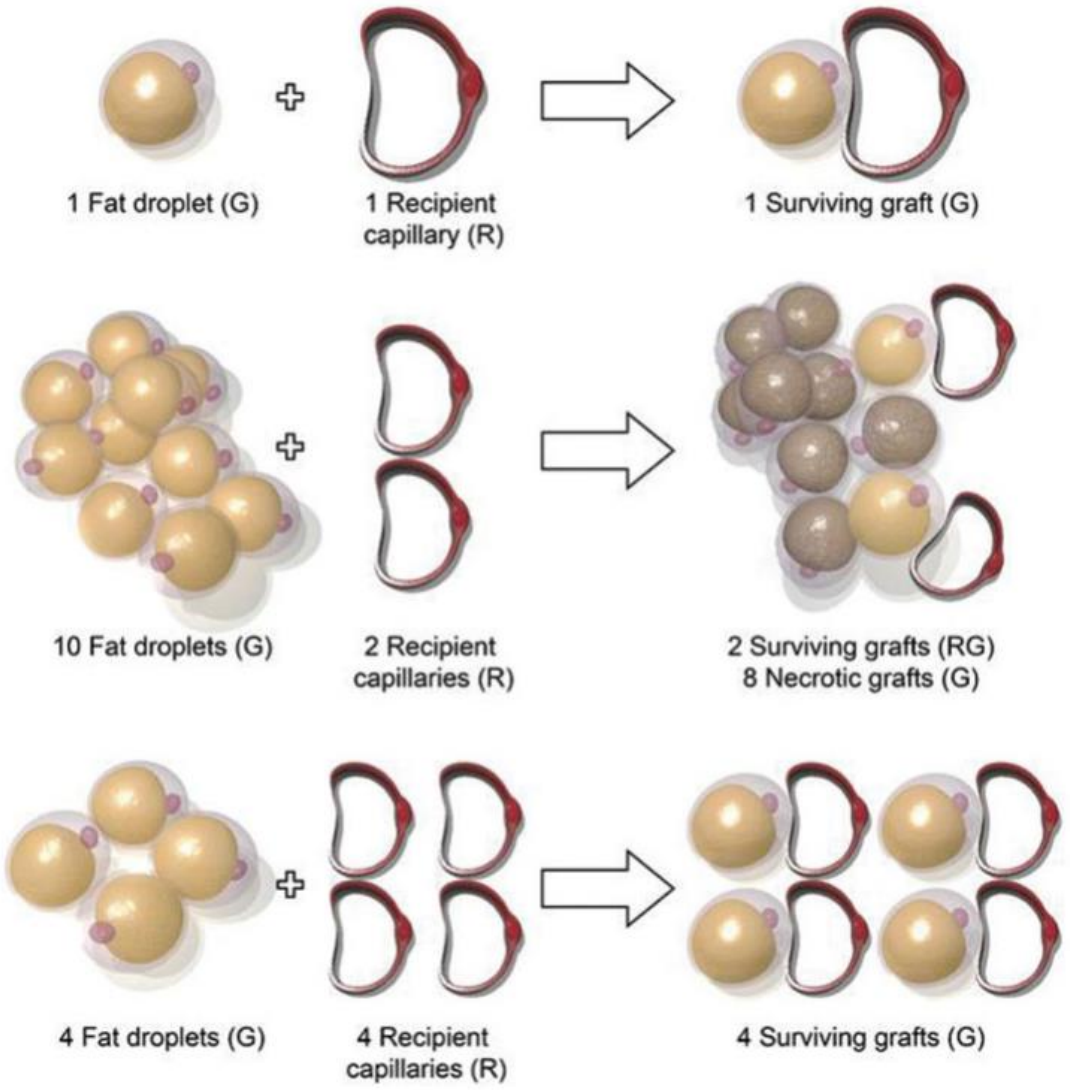

Fig 4- Stoichiometry principle of fat grafting (13)

1.7 Theories of Fat Graft Survival

Currently, many hypotheses have been proposed to explain how fat grafts survive after avascular surgical implantation. (14)

1.7.1 Theory of graft survival

According to this theory, first published by Peer et al., fat grafts survive after surgical transfer by diffusion of nutrition from the plasma until neovascularization develops from the recipient site. Thus, smaller volume grafts may be superior to larger volume grafts in terms of survival because smaller volumes are better adapted to ensure complete diffusion and perfusion.

1.7.2 Theory of graft replacement

According to this view, relatively few donor adipocytes survive the transplantation procedure; instead, grafted adipocytes are essentially replaced by donor ASCs that are transported concurrently in the graft.

1.7.3 Theory of host cell replacement

This idea states that no transplanted cells survive and that all cells are replaced by the recipient's cells. The grafted cells die and are replaced by connective tissue, new fat cells and the ingrowth of blood vessels from the recipient's tissue. Consequently, recipient integrity and environment are important factors in graft survival.

1.7.4 Three-Zone Survival Theory

For several days until revascularization, the transplanted non-vascularized adipose tissue is placed under ischemia (hypoxia) and nourished only by plasma diffusion from the surrounding host tissue. Many adipocytes die within 24 hours, and various factors of cell death and injury are released from the dying donor tissue and injured host tissue. Inflammatory cells such as macrophages and lymphocytes infiltrate and secrete inflammatory cytokines such as interleukins. Despite the death of adipocytes, ASCs are activated and try to heal the injured tissue in cooperation with infiltrating stem and progenitor cells from the bone marrow, which can be functional for up to 72 hours even in acute ischemia. (15)

According to the theory of Eto et al (16) 2012, when avascular fat is transported, it can be divided into three cellular zones. The survival zone, which is less than 300 m thick and contains adipocytes and ASCs that survive after transplantation, is the most peripheral zone.

A regeneration zone 600 to 1200 m thick is located directly below the survival zone. Adipocytes die and are resorbed in this environment, while ASCs survive and regenerate into new adipocytes. The central necrotic zone is the deepest zone where no cells survive due to hypoxia. There is no regeneration in this zone and the dead area is either resorbed or filled with fibrosis. (16)

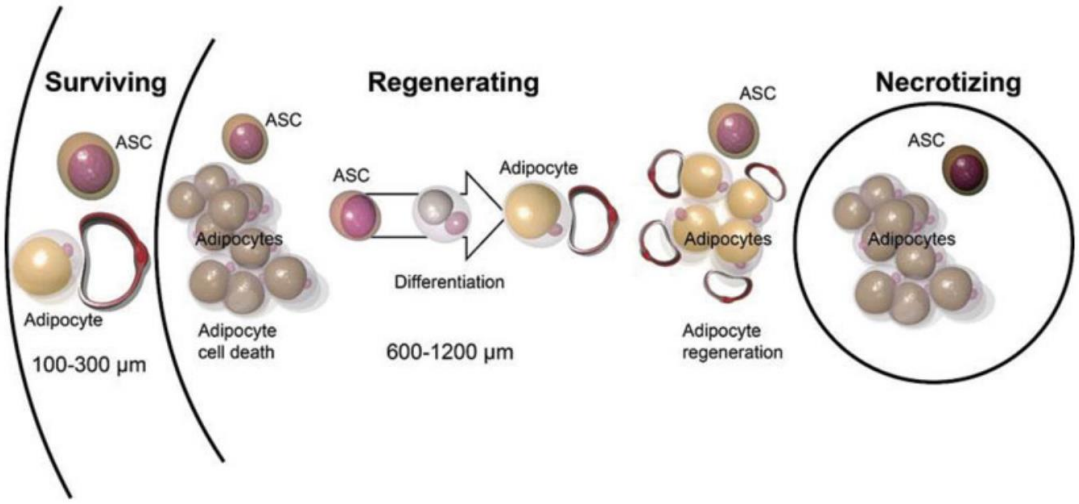

Fig 5- Three-Zone Survival Theory (13)

1.8 Complication of Nanofat Therapy

While nanofat injections for facial rejuvenation are generally considered safe, there are potential complications. These complications can vary depending on individual factors and the specific procedure performed. Some possible complications include:

1. Bruising and swelling: These are common side effects of any injection procedure and usually subside within a few days or weeks.

2. Infection: Whenever a needle or cannula is inserted into the skin, there is a risk of infection. Strict sterile techniques should be followed to minimize this risk.

3. Allergic reactions: Some individuals may have allergies to the materials used in the processing or injection of nanofats. Before the procedure, it is important to discuss any known allergies with the doctor.

4. Irregularities of contours: Improper injection technique can lead to uneven distribution of nano fat, which leads to irregularities of contours in the face. This can usually be corrected with additional injections or other cosmetic procedures.

5. Nerve Damage: There is a small risk of nerve damage during the injection process, which may lead to temporary or permanent numbness or loss of sensation in the face. Proper training and expertise in facial anatomy is important to minimize this risk.

6. Hematoma: In some cases, damage to blood vessels may occur during the injection

a process that leads to a collection of blood under the skin, known as a hematoma. Hematomas may require drainage if they are large or cause discomfort.

7. Fat embolism: In rare cases, the injected fat can enter the bloodstream and travel to other parts of the body, causing a fat embolism. This can be a serious complication that requires immediate medical attention.

8. Overcorrection or undercorrection: Achieving desired results with nanofat injections requires skill and experience. There is a risk of over- or under-correction, resulting in an unnatural appearance or unsatisfactory results.

9. Granulomas or cysts: Small, filled bumps or cysts may form under the skin as a result of injections. These are usually benign but may require further treatment or removal.

10. Asymmetry: In some cases, the results of nano fat injections can lead to facial asymmetry where one side of the face looks different than the other. This can usually be corrected with additional treatments or adjustments.

1.9 Advantages and Limitations of Nano Fat Therapy

Nano-fat therapy offers several advantages over traditional regenerative approaches. It is minimally invasive, uses the patient's own tissue for sampling, thereby reducing the risk of immune rejection or allergic reactions. Additionally, nano fat is readily available because adipose tissue is abundant in most individuals. Nanoscale processing of fat maximizes its regenerative potential by concentrating beneficial cellular and molecular components.

However, some limitations must be taken into account. Nano fat therapy is still a relatively new field and further research is needed to optimize processing techniques and standardize protocols. Long-term outcome studies are needed to determine the effectiveness and durability of therapy. In addition, regulatory approval and cost considerations may limit its availability in certain healthcare settings.

Conclusion

Nano fat therapy represents an exciting frontier in regenerative medicine and offers a promising solution for tissue repair and regeneration. Its unique composition, consisting of MSCs, preadipocytes, growth factors and ECM components, enables cell replacement, paracrine signaling and ECM remodeling. Applications of nano-fat therapy span many areas of medicine, including facial rejuvenation, orthopedic injuries, wound healing and scar reduction. While nanofat therapy has its advantages, further research is needed to fully understand its potential and optimize its use in clinical practice. However, the development of nanofat therapy holds great promise for advancing regenerative medicine and improving patient outcomes.

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