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Role of Nanofat Graft in Deep Dermal Acute Burn

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Abstract

A burn scar is defined as fibrous tissue that supplants normal skin subsequent to deep or full-thickness burns. These burn scars frequently result in considerable cosmetic disfigurement, contributing to substantial emotional distress, social difficulties, and behavioral disturbances. Across the historical continuum, burn scars have posed a significant therapeutic challenge, with most available treatment options proving inadequate in achieving satisfactory outcomes. The imperative for innovative techniques that offer more effective and enduring results continues to be a central obstacle in the management of burn scars Current therapeutic approaches for burn scars encompass intralesional administration of corticosteroids, 5-fluorouracil, or bleomycin, as well as the application of silicone sheets, laser therapies, and surgical interventions. These modalities demonstrate variable efficacy and are associated with notable limitations, including elevated recurrence rates, alterations in skin pigmentation, cutaneous atrophy, and significant pain. Fat harvested from the body contains a diverse array of cellular constituents. Adipocytes account for approximately 30% to 70% of the total cellular population within the harvested fat. Additional components include extracellular matrix elements, endothelial cells, mural cells, fibroblasts, adipose-derived stem cells, and various blood cells. Collectively, these cells contribute to the surrounding stromal environment, which supports adipose tissue regeneration and facilitates neovascularization. Aim: The aim of this review article was to assess the role of Nanofat graft in deep dermal acute burn.

Keywords: Nanofat; Graft; Deep Dermal Acute Burn

Introduction

Skin is the human body's largest organ, covering a surface area of about 2 sqm in an average adult. It consists of the epidermis and the dermis, deep within which are important skin appendage structures (including hair follicles, sweat glands and sebaceous glands). These deep structures are a source of proliferating epithelial cells (keratinocytes), which migrate into the clot and wound bed, playing an important role in the wound healing process. The loss of the physical barrier function of the skin opens the door to invasion by harmful microorganisms, which can lead to infection, and ultimately even to the development of sepsis. The repair process of burn injury, which begins as early as several hours after the traumatic event, may also be impaired by large fluid losses via the wound [(1)].

Burn injury

A burn injury results from skin contact with a heat source. The factors that can cause burn injuries include high temperature, electricity, friction, radiation and chemicals Burn injuries vary, and an increase in the body surface area affected by the burn injury affects wound morbidity and patient mortality Other important factors directly impacting on the severity of injury include the location of the burn, temperature and time of exposure to the heat source, with a synergistic effect between them [(2)]

Pathophysiology of burn injuries

- **Local effects of burn injuries**

Burn injuries cause coagulative necrosis of various layers of skin and underlying tissues. Because of its main function as a physiological barrier protecting underlying tissues, the skin usually limits the spread of damage

to deeper layers, but the extent of damage is determined by the temperature, the energy transmitted by the causative agent, and the duration of exposure In principle, the site of a cutaneous burn injury can be divided into three zones [3].

- **Systemic effects of burn injuries**

Burns involving more than 30% of total body surface area (TBSA) result in considerable hypovolemia coupled with the formation and release of inflammatory mediators, leading to a subsequent systemic effect, namely a characteristic cardiovascular dysfunction known as burn shock. It is a complex process of circulatory and microcirculatory impairment, generating oedema in both burned and unaffected tissues. Even with prompt intervention and adequate fluid support, this pathophysiological state remains completely irreversible [4]. Plasma extravasation is another feature of burn injury, resulting in increased systemic vascular resistance (SVR) and reduced peripheral blood flow. This results in hemodynamic changes, which include a reduction in cardiac output due to the diminished plasma volume, as well as a decrease in urinary excretion [3].

Another response of the body to a burn is oedema formation. Enema develops when the amount of fluid filtered out of microvessels is greater than the amount of fluid entering them. The process of oedema formation is biphasic. In the development of post-burn oedema, an important role is played by the rate of increase in tissue water content, which is clearly influenced by the type and amount of fluid resuscitation administered to the patient. The tissue water content reaches double the original volume within the first hour, with 90% of the increase observed in the first few minutes [5]. The body's hypermetabolic response has detrimental effects at the cellular and systemic level. At the systemic level, the structure and function of major organs (heart, liver, skeletal muscle, skin), the immune system and the transmembrane transport system are compromised. Wound healing is impaired, which increases the risk of infection, hampers rehabilitation and delays the reintegration of patients back into society [6].

The endocrine disruption that occurs after a burn alters metabolic pathways. Catecholamines drive hypermetabolism, while an increase in the secretions of cortisol, adrenaline and glucagon (which are catabolic hormones), together with an increase in pro-inflammatory cytokines, inhibits protein and fat synthesis The observed negative nitrogen balance in burn patients suggests that skeletal muscles are used as the main energy source [6].

Accelerated protein degradation leads to a significant loss of lean body mass (LBM) and muscle atrophy, resulting in reduced strength and compromised rehabilitation outcomes. Depending on the magnitude of LBM loss, certain dysfunctions occur. While alterations in the immune system, increased rates of infection and delayed wound healing are correlated with a 20% loss of LBM, patients with a 30% loss of LBM present inhibited cough reflexes, prolonged requirements for mechanical ventilation, as well as an increased risk of pneumonia and pressure sores [6].

An increase in gluconeogenesis activity associated with an increase in gluconeogenic substrates, which include glycerol (derived from the breakdown of triacylglycerols), alanine (derived from the breakdown of proteins) and lactate (a product of anaerobic glycolysis), leads to hyperglycaemia in patients with severe burns. Research has shown that serum glucose levels are persistently elevated in these patients, reaching of up to 180 mg/dL. This condition is further compounded by an attenuation of the suppressive effect of insulin on hepatic glucose release and enhanced hepatic glycogenolysis [7].

Thermal injury also triggers changes in the circulatory system. Cardiac function is subject to several modifications starting already at the time of injury. Before detecting any reduction in plasma volume, receptors on thermally damaged skin trigger a neurogenic response, initiating a rapid decrease in cardiac output. This is associated with an initial reduction, followed by a significant increase in the cardiac index starting on the third day post-burn [8].

Urinary dysfunction is a consequence of alterations in cardiovascular function and endocrine dysregulation (changes in angiotensin, vasopressin and aldosterone secretion). The development of hypovolemia, as well the diminished cardiac output following thermal injury brings down the glomerular filtration rate (GFR) as a result of reduced renal blood flow. These alterations usually manifest themselves in the form of oliguria, and

if not managed promptly and appropriately it can lead to acute tubular necrosis (ATN), renal failure and even death [9].

Thermal trauma also disrupts liver function. Research has shown that thermal injury alters hepatic expression and serum concentrations of acute phase proteins. Serum complement C3 and α2-macroglobulin concentrations in burn patients initially fall, and then gradually rise. The redirection of substrates to synthesise these proteins, the increased use of muscle proteins for energy production due to the hypermetabolic response and the impaired absorption of nutrients (including amino acids) in burn patients are the likely factors suppressing the synthesis of constitutive hepatic proteins [10].

Endocrine response is one of the systemic reactions observed in severely burned patients and is characterised by significant functional alterations in the hypothalamic-pituitary axis. During the early post-burn phase, there is a marked upsurge in so-called stress hormones, which include catecholamine, glucagon, and cortiso [11] .

Treatment of patients after thermal injury

While none of the established therapeutic approaches to date have been able to completely reverse the complex reactions induced by burns, there is a number of pharmacological and non-pharmacological strategies which are effective in modulating burn-associated metabolism [12].

- **Cooling of burned areas**

Research has shown that in the event of a burn, immediate removal of the cause and cooling of the injured area is beneficial to the burn victim. Reducing the elevated temperature of the burned tissue improves the physiological response. Importantly, it also provides palliative relief. The cooling agent should be applied as promptly as possible, but it must be at the right temperature. Extreme cold (e.g., ice) can cause further damage by reducing blood flow to the injured area (cold-induced vasoconstriction). Cooling of a large area of skin over a long period of time is likely to induce hypothermia. There is also a risk of frostbite on cooled surfaces. According to the available literature, the optimal temperature for cooling a burn injury is 10–20 °C [12].

- **Fluid resuscitation**

In the event of a severe burn, the first and most important therapeutic intervention is adequate resuscitation after a burn injury, fluid rapidly accumulates in damaged tissues and, to a lesser extent, in healthy tissues. Without resuscitation, burns greater than 15–20% TSBA can lead to hypovolemic shock, organ dysfunction and ultimately death of the victim. The 24-h fluid requirements of a burn victim are estimated using the Parkland formula for fluid resuscitation, which remains the most widely used protocol worldwide to date. Since its introduction by Baxter and Shires in 1968, it has become the gold standard for initial fluid resuscitation in burns [13].

The phenomenon of excessive fluid loading usually results from a combination of several factors, i.e., inaccuracies in calculating fluid requirements, unnecessary fluid infusions, increased use of sedation and analgesic infusions, and excessive administration of crystalloid solutions In order to improve the accuracy of fluid resuscitation, attempts are being made to introduce adjunctive measures in the form of modern minimally invasive procedures, such as the insertion of a pulmonary artery catheter or translung thermodilution, allowing for continuous monitoring of venous oxygen saturation, intrathoracic blood volume, total blood volume index and extravascular lung water index, but irrespective of the above urine output remains the main indicator of adequate fluid resuscitation. Isotonic crystalloid resuscitation fluids (lactate or acetate Ringer's solution) are recommended for fluid resuscitation. The simultaneous use of colloid and hypertonic lactated saline (HLS) is recommended as an option for fluid resuscitation [14].

- **Ventilation**

Airway management and ventilator support are often required in cases of severe burns, particularly in thermal lung injuries. Ventilation strategies for respiratory failure in critically ill patients, including those with severe burns, are still being developed. The introduction of a lung-protective ventilation strategy has reduced the incidence of ventilator-associated lung damage. Overall technological advances in the field of ventilation have shown measurable improvements in outcomes for patients with severe burns and inhalation injuries [15].

- **Surgical treatment**

Early excision and closure of the burn wound is sometimes described as the greatest advance in the treatment of patients with severe thermal injuries. In fact, this strategy is crucial for reducing the incidence of complications associated with severe burns The metabolic rate in patients undergoing total excision and wound coverage with an autograft and/or deceased donor skin within the first 72 h following severe thermal injury (50% TBSA) is 40% lower than the metabolic rate in patients with similar burn severity who did not undergo excision within a week. Immediate excision also offers additional advantages, which include reduced protein loss, lower risk of infection and sepsis, and less pain compared to patients with delayed reconstruction [(16)].

Eligibility for reconstructive burn surgery depends on several factors, including the extent and location of the burn, the patient's overall health, and the presence of other medical conditions. In general, patients with burns that affect functional or cosmetically significant areas of the body, such as the face, hands, and feet, may be good candidates for reconstructive surgery. The timing of the surgery is also important and is usually carried out after the burn wounds have healed [(17)].

Assessment of burn depth poses a major challenge even to experienced surgeons, as there are no precise methods to do so that can be used at an early stage (up to several days after the injury). Physicians can take guidance from a few important clues, such as the mechanism of the burn injury, redness or sensory preservation in the tissues, but such an assessment is subject to considerable error. That is why Laser Doppler Imaging (LDI), an accurate diagnostic tool with high sensitivity and specificity, has proven to be an important adjunct to clinical assessment. It is used to measure the degree of disruption of dermal microvascular blood flow and makes it possible to assess total depth with a high degree of accuracy. The use of LDI has resulted in shorter hospital stays, lower rates of surgical interventions, shorter decision-making times for grafting procedures, and overall cost efficiency [(18)].

Nanofat

Nanofat is an ultra-purified adipose tissue-derived product that is devoid of mature adipocytes but contains CD34+ rich ASCs, microvascular fragments [fragments of arterioles, venules, and capillaries as they are identified by immunohistochemical staining for CD31 and α-SMA], growth factors [vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor-beta (TGF-β), basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF)], biological peptides [lipoxins, resolvins, protectins, neurotrophic factors, angiogenin, matrix metalloproteinase 9, leukemia inhibitory factor, and macrophage migration inhibitory factor], and cytokines [BMP-2 and -4, IL-1RA, -4, -8, -10, -11, and -13] [(19)]. Nanofat behaves on the line of adipose tissue-derived mesenchymal stromal cells at the site of injury, these stromal cells initiate a site-specific reparative response comprised of remodeling of extracellular matrix (ECM), enhanced and sustained angiogenesis, immune system modulation, and cellular turnover [(20)].

- **Nanofat 2.0**

The unfiltered adipose tissue was initially called adult staminal cells by **Lombardo** *et al.,* [(21)] since they had higher proliferation capacity than the filtered cells. In 2017, **Lo Furno** *et al*., [(22)] modified the method. described by **Tonnard** *et al.,* [(23)] for nanofat, omitting the final filtration and squeezing the emulsified adipose sample through nylon cloth. **Lo Furno** *et al.,* [(22)] named this product "nanofat 2.0", which was highly rich in the stromal cell population and possessed an exponential proliferation capacity. They demonstrated faster epithelization of the wound gap within 8 d by placing nanofat 2.0.

Due to the availability of stromal cells and endothelial progenitor cells, nanofat 2.0 resulted in the healing of wounds and long-standing non-healing ulcers where a large volume of soft tissue augmentation was needed. Lo Furno *et al*l demonstrated that nanofat 2.0 possessed increased stromal cell and endothelial precursor density and higher proliferative capacity than nanofat. Since nanofat 2.0 is subjected to less mechanical stress in preparation, the viability of the cellular content of the product could be enhanced compared to nanofat the modified nanofats [(21)].

- **Vivo nanofat**

In 2018, **Bi et al**., [24] formulated the preparation of nanofat with a combination of enzymatic disintegration and mechanical emulsification of adipose tissue and named this technique "Vivo nanofat". The harvested lipoaspirate is rinsed with 1 mL of 0.2 mg/mL of collagenase I enzyme and the final volume is incubated at 37 °C for 15 min. The final concentrate is centrifuged at 300 G for 7 min and the supernatant fraction is filtered through a 0.6 mm sized cell strainer. The final effluent obtained is called Vivo nanofat. The cellular viability of adipocytes and stromal stem cells has been preserved to a great extent in Vivo nanofat although the authors claim that the concentration of collagenase used (0.075%) was less than the amount used for adipose stromal cell separation, the effects of their concentration in the final derivative need further exploration.

- **Delivery of nanofat**

The application and delivery of fat grafting to the recipient site are based on optimal vascularity for adipocyte survival. Nanofat can be delivered through micro-needling, intradermal, subcutaneous, and local infiltration depending on the need of the individual and the disease perse Delivering nanofat through small gauge cannulas reduces the recipient site morbidity, risk of bleeding, and poor graft uptake. In fat grafting, the revascularization starts from the peripheral zones; hence, the center of the graft experiences a longer ischemic time [25].

- **Applications of nanofat**

Stem cells are an important component of regenerative medicine with increased significance and use in clinical applications. The newer concept of "Regenerative Surgery" has a great scope in augmenting and managing soft tissue defects and reconstructive procedures of which adipose tissue-derived nanofat is gaining rapid attention [26]. Autologous fat grafting and the products of adipose tissue fragmentation have been used to restore the volume of soft tissue defects in the field of plastic surgery and soft tissue reconstruction. Considering the regenerative potential of adipose tissue, researchers are exploring to identify the key element responsible for its function. The adipose cells were considered the storehouse of progenitor cells and bioactive micromolecules. By concentrating the progenitor cells within the adipose tissue complex, the regenerative capacity of the adipose-based products is enhanced to aid in their applications [27].

1) Plastic surgery

Autologous fat transplantation or lipofillers remain the most suitable management modality available for breast reconstruction. Adipose tissue-derived nanofat can maintain natural breast shape and conceal the underlying prosthesis while augmenting breast size. In gluteal augmentation, fat grafting can replace implant-based gluteal augmentation if the patient has adequate and available fat stores [26]. Nanofat injections can reduce the atrophic scars due to the presence of adipose tissue-derived stromal cells and avoid the need for surgical procedures. The underlying mechanisms for scar retraction by nanofat are uncertain. Nanofat components can regenerate dermis and subcutaneous fatty tissues and enhance the dermo-epidermal junction. They regenerate by laying down adipose tissue-derived ECM, collagen deposition, and neoangiogenesis [28].

2) Dermatology and aesthetic surgery

The most common procedure for managing facial aesthetics is autologous fat transplantation. Though the transplanted adipose tissue gets absorbed easily, a few progenitor cells stimulate the process of regeneration. The cells present in nanofat in combination with platelet-rich fibrin (PRF) enhances the proliferation and adipogenic lineage differentiation [26]. Due to this combination treatment with nanofat and PRF, a trend towards the disappearance of wrinkles and improved facial contour and skin rejuvenation have been observed attributable to the autocrine and paracrine effects of stromal cells in nanofat and anti-aging properties of PRF this combination treatment enhances the long-term benefits and is being increasingly utilized in the restoration of facial contouring in the field of aesthetic and cosmetic medicine. The skin texture, elasticity, and moisture, and facial rejuvenation can be achieved with nanofat admixed with PRF [29].

3) Application in facial skin rejuvenation

Since nanofat has no filling ability, nanofat grafting achieves the purpose of facial skin rejuvenation by injecting regenerative cells and extracellular elements. 6 months after nanofat transfer, the clinical effect of facial

skin rejuvenation in the surgical area is significant, without complications or other adverse reactions [23]. It was used nanofat combined with platelet-rich fibrin injection to treat facial skin aging, and the results showed that 103 patients who received nanofat combined with platelet-rich fibrin injection had more significant improvement in facial skin texture and satisfaction than 128 patients who received hyaluronic acid injection, without causing any complications (eg, infection, allergic reactions, or paresthesia). In vitro experiments have shown that ADSCs can whiten the skin by inhibiting melanin synthesis by downregulating the expression of tyrosinase and tyrosinase-associated protein 1 [25].

4) Application in scar repair

Because the scar tissue contains fibrotic tissue and has a hard texture, the injection of fat into the scar requires that the injection needle be thin enough. Nanofat particles have a small diameter, smoothly pass through a 27-G needle, and have a relatively large contact area with the scar after injection, so nanofat has unique advantages in treating scars repair. **Bhooshan et al.,** [30] applied nanofat for local injection therapy in the scar, and the results demonstrated that nanofat scar injection could effectively improve scar characteristics and symptoms.

- **Complications of nanofat grafting**

The lesser the fat graft is manipulated and the sooner it is injected, the higher the chances of its survival in the target site minor complications related to the harvesting are due to the liposuction technique. The possible complications range from bruising, hematoma formation, donor-site pain, infection, contour irregularities, and damage to the underlying structures when the aspiration cannula enters peritoneal or muscular territories [31]. Breast augmentation with lipofilling was associated with complications such as fat necrosis, oil cyst formation, and calcifications when performed in large volumes into poorly vascularized areas. Cellulitis at the donor site, transient digital numbness infections at both the recipient and harvest sites , and cyst formation in 10% of hand rejuvenation patients, along with the common complications of fat grafting such as temporary dysaesthesia , fat necrosis and reabsorption of the grafted fat were also reported [32].

Facial rejuvenation by lipofilling involves complications related to the fat graft injections in "dangerous" areas of the face such as the glabella and nasolabial folds. Accidental intra-arterial injections may result in cerebral or ocular artery thrombosis resulting from the reflux of fat into the ophthalmic artery and the internal carotid artery. To prevent such devastating complications, confirmation of the absence of blood reflux into the syringe before injecting the graft is a necessary routine, along with a slow pace of injection at low pressure, and the use of a blunttip cannula [33].

Mbiine et al., [34] performed a systematic review to evaluate the existing clinical research involving the use of autologous adipose-derived stromal vascular fraction in the treatment of scars. They demonstrated that, Lipoaspirate processing to obtain the SVF was only performed in 11 studies while the rest of the studies used nano-fat graft which didn't require this step. Stromal vascular fraction (SVF) and ADSC lipoaspirate processing: Among the 10 studies that processed lipo-aspirate to obtain the SVF, 8 described the lipo-aspirate techniques to obtain the stromal vascular fraction (SVF) while 2 didn't have a description. Of the 8 studies that described the process, 7 reported enzymatic digestion with collagenase as the method for fat break down while one study by Gentile described using the Celution system for mechanical breakdown of the fat. Five of the 10 SVF studies described a mean cell concentration harvested from the lipoaspirate. Four of the five studies reported mean cell concentrations ranging between 1-6×106 cells/ml and 1×108 cells/ml of SVF suspension.

ElSherbeny et al., [35] showed that, when comparing nano fat group, (group A) with control group (group B) as regard the quality of healing using Vancouver scar scale, group A showed significant change in vascularity than group B with a 90% pink in group A vs 95% red in group B. As regard pliability, group A showed more significant pliability with a (90%) yielding versus (85%) firm. There was no statistical significance between group A and B as regard height and pigmentation. The mean of overall total Vancouver score was 3.75±1.86 in group A and 5.40±1.5 in group B which is statistically significant.

Conclusion

Nanofat grafting has emerged as an innovative therapeutic adjunct in the treatment of deep dermal acute burns, providing regenerative and reparative benefits that surpass traditional interventions. Ultimately, nanofat has the potential to enhance scar quality, promote angiogenesis, modulate inflammation, and accelerate wound

closure by directly delivering adipose-derived stem cells, paracrine factors, and extracellular matrix components to injured tissue. Nano fat therapy is an additional treatment modality that has the potential to provide the unfortunate burn victims with a statistically significant improvement in the aesthetic outcome, as well as a better outcome in terms of greater wound healing and a reduced need for skin grafts. It is an effective treatment option for treating post-burn scarring and improving the quality of life of patients due to these benefits.

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