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Review Article

Adipose derived stem cells and their importance in surgical treatment of cleft lip and palate

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Abstract

Aim: Facial fat grafting has undergone noteworthy changes over time. Many different techniques have been followed by trying to advance the quality of the lipoaspirate and the survival of the fat graft after implantation.
Methodology: The current review emphasizes the role of Adipose derived stem cells in autologous fat grafting in the maxillofacial region.
Results: Fat transfer techniques have demonstrated utility in the correction of a host of deformities through soft tissue augmentation. More recently, there is evidence that fat grafts harbor stem cells, Adipose derived stem cells and that these pluripotent cells produce factors beneficial for wound healing and regeneration. This autogenous tissue may also improve scar caliber and minimize scar burden. Many researches have recently revealed that infant-derived Adipose derived stem cells are more biologically robust than those obtained from adult tissue.
Conclusion: It may be inferred from the above study that fat grafting may appear to enhance the esthetic outcome in the local anatomical region of the cleft.
Keywords: Fat grafting, adipose tissue, cleft lip, stem cells.

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1. Introduction

Cleft lip with or without cleft palate is the most common congenital malformation of the head and the third-most common birth defect. Cleft lip is a physical split or separation of the 2 sides of the upper lip. It appears as a narrow opening or gap between the philtrum and the lateral upper lip (incomplete cleft), or it may extend beyond the base of the nose and include the bones of the upper jaw and/or upper gum (complete cleft). It originates from failures in the fusion of the maxillary process to the ipsilateral medial nasal process within the first 5 to 6 wk of gestation [1].

Clinical management of cleft lip is a unique ongoing challenge in maxillofacial plastic surgery. The repair of the lip is usually performed during the first year of life, as early as safe for the patient (mean age of 4 months at time of surgery), while cleft palate repair follows between 12 and 18 mo of age. The surgical goals of cleft repair are to achieve normal facial appearance, feeding, speech, and hearing without significantly affecting the ultimate facial and psychosocial development of the child. The multidisciplinary management team comprises of surgeons, speech pathologists, social workers, audiologists, and nutritionists. The outcomes of primary surgical repair are often less than ideal, requiring many revision operations due to loss of function and compromised aesthetic outcomes [2].

2. Scarring in cleft lip surgery

Hypertrophic scar formation is a frequent postoperative complication of cleft lip repair, which impairs soft tissue form, function, or movement and restricts facial growth. Hypertrophic scar generally occurs within 3 to 6 month following the initial surgery. Rate of hypertrophic scar formation following primary cleft lip repair are sparsely reported and vary widely, from 8% to 47%. Surgeons, patients, and caregivers are often dissatisfied with the surgical results, and multiple lip revision operations are required throughout childhood to optimize aesthetics and function [3].

3. Adipose derived stem cells and it's uses in cleft surgery

Fat transfer techniques have demonstrated utility in the correction of a host of deformities through soft tissue augmentation. More recently, there is evidence that fat grafts harbor stem cells, termed adipose-derived stem cells (ADSCs), and that these pluripotent cells produce factors beneficial for wound healing and regeneration. This autogenous tissue may also improve scar caliber and minimize scar burden. Research by Balkin *et al* has recently revealed that infant-derived ADSCs are more biologically robust than those obtained from adult tissue [4].

Adipose tissue-derived stem cells (ADSCs) are mesenchymal cells, which have a capacity for self-renewal and which can also be differentiated into adipocytes, chondrocytes, myocytes, osteoblasts and neurocytes among other cell lineages, which has resulted in them being used in clinical trials for the treatment of conditions such as diabetes mellitus, liver disease, corneal lesions, articular and cutaneous lesions, among others. In addition, stem cells and, in particular, adipose tissue-derived cells, play a key role in reconstructive or tissue engineering medicine as they can be used to develop new treatments [5-8].

Among the advantages of ADSCs, the greater ease of access and harvesting by means such as subcutaneous lipoaspiration, a much less painful procedure than harvesting bone marrow stem cells, and their use, is less associated with ethical controversies because they are harvested from autologous fat, unlike ES cells. Adipose tissue has been one of the most studied tissues in the last decade due to its endocrine activity which is manifested in the release of adipocytokines, cytokines, transcriptional and growth factors, which forms a secretome. [9,10] Adipose tissue is no longer only considered an energy reservoir, thermal insulator or mechanical buffer, but its participation in a complex network of interactions with the endocrine, nervous and cardiovascular systems has been highlighted. It is a tissue that originates in the mesoderm and is formed by adipocytes and a fraction of stromal cells that include vascular smooth muscle cells, endothelial cells, fibroblasts, monocytes, macrophages, pre-adipocyte lymphocytes and ADSCs. ADSCs may undergo differentiation to mesodermal or trans-mesodermal lineages and give rise to cells that are naturally of ectodermal origin [9,10].

The cleft lip nasolabial repair has undergone numerous revisions with preserved ambitions of accurately restoring structures to their proper anatomic position such that the functional and aesthetic outcomes are optimized and the burden of secondary deformities is minimized. While such efforts have lessened the frequency and severity of secondary deformities of the lip and nose, problems can persist. In nature, the eye is attracted to differences and asymmetries. Simple alignment of anatomic landmarks is necessary but not sufficient to achieve the most anatomically normal-appearing lip and nose. The nasolabial complex exhibits contours (concavities and convexities) that require construction and restitution. Inequalities within anatomic boundaries and sharp breaks between normally blended transitions may result from an improperly executed repair, complications in wound healing, alterations in growth and development of facial elements, and the formation of a wide or obvious scar. Residual deformities will appear conspicuous as cleft lip stigmata, and a host of techniques have been employed to revise secondary cleft lip abnormalities, such as scar manipulation, lip landmark re-approximation, rhinoplasty and tissue augmentation with dermal or fat grafting[11-13].

While scarring is an important and unavoidable aspect of wound healing, patients scar in different and often unpredictable manners. Thus, even a properly performed cleft lip repair may result in scars that are obvious, unsightly and/or disfiguring. Although great advances have been achieved in decreasing the burden of scar formation through both technical and adjunctive measures, scar management remains a difficult problem worthy of intense investigative inquiry. Insight into the general principles underwriting wound healing and scar formation has been elucidated through unexpected observations. It was long assumed that inflammation was a critical feature of the healing process, that phagocytic cells were required to clear wound debris and that the inflammatory cell-mediated growth factor response was necessary to orchestrate downstream cell and tissue movements required for wound healing. However, unique experimental observations illustrate otherwise [14-16].

Intriguingly, during a defined period of gestation the fetus heals wounds in a regenerative and scarless fashion, completely restoring the cutaneous anatomy as well as its strength. Research efforts have demonstrated that scarless fetal wound healing occurs during a period of gestation where wounds heal with a paucity of inflammatory cell infiltrate. Moreover, scarless wound healing ceases when inflammation becomes a natural component of the biological healing process. Thus, inflammation does not appear to impact wound healing but instead coincides with scar formation. Thus, a therapeutic strategy that targets inflammation at the site of wound healing might diminish scar burden without impairing the overall healing process. Critical to such a therapeutic approach would be the development of a sustainable source of anti-inflammatory mediators capable of interacting with the surrounding wound environment [11,16].

Growth factors are an essential element of the natural wound healing process. When compared to acute wounds, analysis of fluid collected from chronic wounds reveals depressed levels of growth factors as well as elevated proinflammatory cytokines. In theory, given the critical importance of growth factors and cytokines in orchestrating the molecular and cellular events required for proper wound healing and scar formation, the application of growth factors to sites of injury held great promise in clinically promoting the healing process. In practice, however, the benefits of such an approach were not fulfilled. Likely, the wound healing process requires a medley of growth factors applied in a strict and regulated spatiotemporal manner [11,13,17].

Adipose tissue is a plentiful source of mesenchymal stems cells that promote wound healing and minimize scar burden by both reducing the inflammatory response to injury and providing growth factors in a physiological manner. Experimental evidence suggests that ADSCs tune down inflammation by impairing lymphocytic proliferation and by suppressing a variety of T-, B- NK- and perhaps dendritic-cell functions. Intriguingly, ADSCs also secrete a complement of growth factors that govern the wound healing process and, when applied to sites of wounds *in vivo*, ADSCs remain viable and secrete growth factors in a continuous regulated manner. The benefits of ADSCs on wound healing and scar formation are not merely theoretical. In experimental models, ADSCs bolster wound healing by nurturing angiogenesis, promoting granulation and triggering reepithelization. Moreover, ADSCs appear to benefit overall wound cosmesis by decreasing scar size, enhancing color quality and improving scar pliability. Recent evidence from our group revealed that infant adipose tissue, as opposed to older cell donor tissue, contains robust ADSC populations with enhanced angiogenic and osteogenic capabilities. Similar to older donor cells, infant ADSCs also possess paracrine osteogenic activity. Our experimental evidence suggests that infant adipose tissue might represent an ideal substrate from which to extract ADSCs with regenerative capabilities [8,11-13,15,16]

Recent studies reported that fatty tissue has the highest percentage of adult stem cells of any tissue in the body, with as many as 5000 adipose-derived stem cells per gram of fat compared with 100–1000 stem cells per millilitre of bone marrow. ADSCs are similar to bone marrow derived stem cells in that they are capable of differentiating into multiple mesodermal tissue types and show similar surface protein marker expression. Many studies demonstrated the ability of adipose-derived stem cells to undergo multilineage differentiation, not just into fat but also into bone, cartilage, skeletal muscle, cardiac muscle, blood vessels, nerves and skin. Furthermore, adult, lipid-filled adipocytes can dedifferentiate into stem cells and redifferentiate into other tissues such as bone [18-20]

ADSCs are different from bone marrow derived mesenchymal stem cells because they can be easily obtained using a standard wet liposuction procedure under local anaesthesia, without the need for expansion in culture. ADSCs are part of the stromal vascular fraction (SVF) of adipose tissue, together with a heterogeneous population of many other cell types, including preadipocytes, endothelial cells, pericytes, haematopoietic-lineage cells, and fibroblasts. The regenerative features of the SVF are attributable to its paracrine effects: SVF cells secrete vascular endothelial growth factor, hepatocyte growth factor, and transforming growth factor-b in the presence of stimuli such as hypoxia and other growth factors and strongly influence the differentiation of stem cells, promoting angiogenesis and wound healing, and potentially aiding new tissue growth and development [20-22].

In the mid-1990s, Coleman began to observe that the quality of the skin above the fat graft improved, not only as an effect of the filling but also with gradual improvement in the quality of the skin. There also appeared to be an improvement in the quality of the tissues into which fat is grafted, including softening of winkles, decreased size of pore and pigmentation improvement. He noted also that in the treatment of depressed scar, the fat grafted relieved the depression but also made the scar softer and sometimes it seemed to completely eliminate the scar, making it look like normal skin. Rigotti *et al* reported that the transplantation of lipoaspirates containing adult ADSCs is a highly effective therapeutic approach for the treatment of degenerative, chronic lesions induced as late effects of oncologic radiation treatments. Owing to the angiogenic factors released from ADSCs, lipofilling interrupted a vicious circle of vascular lesion, ischaemia, hyperpermeability, and fibrosis leading to increased ischaemia, and favoured the growth of a microvascular bed with the correct ratio of adipocytes to capillaries [12,23,24]

Because aspirated fat is relatively poor in adipose-derived stem cells, ADSC condensation seems important for obtaining better regeneration and retention. Supplementation of stromal vascular fraction or ADSCs can increase the ADSC/adipocyte ratio in the graft. Clinical results of ADSC supplementation remain controversial, but ASC condensation seems to lead to expanding applications of fat grafting into revitalization of stem cell-depleted tissue.[25,26]

Autologous fat grafting has an important role in facial rejuvenation. In fact, the unique regenerative potential of lipofilling leads to excellent results due to its filling properties and the role of ADSCs. For this reason, lipofilling has unique features, and plastic surgeons can use it not only to correct soft-tissue deficiencies but also to rejuvenate the skin of the face. The loss of facial volume, especially in the periorbital region, is an important component of aging and is due to the redistribution and atrophy of facial fat. Traditional approaches to facial rejuvenation have relied on subtractive surgical techniques, focusing on the excision of skin, muscle, and/or fat. Modern approaches concentrate instead on filling the "empty" facial compartments, mainly through fat grafting. Traditional fat grafting involves Coleman's harvesting technique with 2-mm side-port cannulas, followed by the distribution of a structural fat implant throughout the various dermal layers of the face, from deep to superficial. Disadvantages of traditional fat grafting include the risks of irregular fat accumulation, fat necrosis, and visible lumpiness. Because the eyelid skin is usually thin, the periocular area is most susceptible to contour problems, and thus, deep implantation of fat is recommended. In response to concerns such as those outlined above, many authors have recently focused on microfat grafting techniques [22,27-29]

A major effect of microfat injection is improvement in the viability of adipocytes via the disruption of fat lobules which is contrary to Coleman's thesis that preservation of the lobular structure is essential for fat survival. Moreover, Moscatello *et al* demonstrated that the greater surface area of the disrupted fat lobules on the recipient bed significantly improved fat survival after injection. More recently, various authors have proposed "ultra-micro" fat as a very superficial implant in the periocular and perioral areas. These newer techniques are based on fat harvesting with Coleman's traditional cannulas, followed by various modalities of fat processing to disrupt the large fat lobules harvested. Tonnard *et al* reported that manual fat emulsification provides a nanofat solution rich in the SVF and consequently ASCs, but devoid of viable adipocytes. Consequently, the indications of nanofat are reportedly limited to skin regeneration and do not include volume restoration. In fact, it may be questioned whether a nanofat transfer actually is a "fat grafting" procedure, as adipocytes did not survive the emulsification process [30,31].

The major effect of nanofat injection is probably a stem cell activity so nanofat injection might rather be considered as an in vivo tissue-engineering process. It might be logical to discard the dead adipocyte fraction from the nanofat and to inject the purified stromal vascular fraction only. Moreover, it is known that apoptotic cells release cytokines and attract macrophages that induce growth factors and play an important role in regeneration of the damaged tissue. Thus, coinjection of fragmented adipocytes might have a stimulating effect on stem cell differentiation and tissue regeneration. Aesthetically, the main surgical indications of lipofilling for facial rejuvenation are the correction of dark circles, as an adjuvant to blepharoplasty, or as an alternative treatment for hollow eyes and malar bags [32-35].

Fat reinjection is an important step in the overall success of the graft. Fat is injected in longitudinal tunnels that form a 3D mesh to promote revascularization and graft survival, as per Coleman's technique. In the upper eyelids, fat grafting is generally used to fill hollow eyelids. The injections are sometimes carried out in conjunction with blepharoplasty. The injection sites are located at the medial 2/3 of the upper eyelid, the inferomedial 1/3 of the eyebrow, and the lateral part of the eyebrow. In the lower eyelids, fat grafting helps restore volume, including that of the periorbital region. The injection points are located external to the zygomatic bone and in "the valley of tears". In these areas, injections should not be administered between the skin and muscle, as the skin here is thin and such injections may lead to palpable irregularities. A greater understanding of facial aging mechanisms, consisting of fat atrophy and ptosis of the different facial compartments, has allowed fat grafting to be considered as a possible technique for facial rejuvenation, particularly of the eyelids [31,32,34,35].

Patients who elect to undergo augmentation rhinoplasty often present with concerns of a low dorsum and a short nose. Both autologous grafts and synthetic implants can result in acceptable outcomes of rhinoplasty. In general, synthetic implants are associated with higher rates of complications, such as displacement and extrusion. Coleman emphasized that structural fat grafting to regions with thin skin, such as the periorbital area, must involve the delivery of minute fat parcels. The nasal dorsum is characterized by relatively thin skin and limited space so the implantation of large fat parcels is more likely to lead to dislodgement of the implant, nodulation, and skin irregularities. Therefore, often, autologous microfat transplantation is used to correct the profile of the nose [12,36,37].

ADSCs are an accessible and more flexible alternative for treating conditions that require tissue regeneration. Their therapeutic efficacy is founded on the applications based on these cells not only in experimental models in animals but also in an increasing number of human trials. Conditions that can be treated with ADSCs range from traumatic injuries, through to neurodegenerative and endocrine metabolic disorders, and postsurgical reconstructions. In relation to antineoplastic treatment, the paracrine and endocrine stimuli mediated by the secretomas of the ADSC may be beneficial, but may also promote tumour progression. The development of the use of ADSCs in the treatment of highly prevalent conditions involves not only cell transplantation, but intersects with genetic manipulation, epigenetic modulation and the effect of secretomas correcting pathophysiological alterations [5].

4. Conclusion

Since its introduction, lipofilling has become increasingly popular; however, its results are variable and unpredictable. Several modifications have been made to the procedures of fat harvesting, processing, and injecting. Surgical excision and low negative-pressure aspiration with large-bore cannulas minimize adipocyte damage during fat harvesting. Lipofilling can be used in various fields of plastic surgery due to its filler and regenerative effects, with minimal discomfort for the patient. However, one of the fundamental limitations is that the amount of the fat graft is strictly linked to the amount of adipose tissue in the patients. ADSCs are appealing for cell-based therapies involving tissue repair and regeneration.

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