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Review

Adipocyte Transplantation and Stem Cells: Plastic Surgery Meets Regenerative Medicine

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The technologies for adipose tissue harvesting, processing, and transplantation have substantially evolved in the past two decades. Clinically driven advancements have paralleled a significant improvement in the understanding of cellular, molecular, and immunobiological events surrounding cell and tissue transplantation. These new mechanistic insights could be of assistance to better understand the mechanisms underlying some of the observed clinical improvements. In addition to plastic and reconstructive surgical applications, adipose tissue has become central to an increasing number of translational efforts involving adipose tissue-derived progenitor cells. The growing interest in this area of research has resulted in the exploration of many novel research and clinical applications that utilize adipose tissue grafting and/or progenitor/stem cell-derived cell products obtained from this tissue source. Progenitor, endothelial, and mesenchymal stem cells derived from adipose tissue could therefore not only be central to plastic and reconstructive surgery applications, but also become the focus of an array of therapeutic solutions for many disease conditions, such as those affecting bone, cartilage, muscle, liver, kidney, cardiac, neural, and the pancreas, expanding the possible indications and translational potential of tissue, cell-based, and regenerative medicine strategies.

Key words: Stem cell; Adipose; Fat; Graft; Tissue remodeling; Lipofilling

INTRODUCTION

Cellular therapies, stem cell and regenerative medicine applications are rapidly evolving with advancements in one area that can radically affect the understanding of the biological implications for another one. One of the most intriguing fields of recent interest has been that of adipose tissue-derived stem cells and their potential for banking as an alternative or complement to cord blood banking for several of the potential therapeutic applications in which mesenchymal stem cells (MSCs) could be used (6,11–13,31,32). While most research and academic work has focused on tissue remodeling and differentiation of MSCs into specialized somatic cell types to replace damaged organs and tissues (3,5,15,34,40,41,48,53,56–59), a relatively lower level of attention has been directed to emerging evidence from cosmetic, plastic, and reconstructive surgery. In fact, autologous fat grafts have been used successfully for structural fat grafting in facial, lip, and hand rejuvenation and body contour improvement (7,8,18,20,38). Initially, most investigators concentrated their attention on fat, as autologous tissue, because of its ideal characteristics as a soft tissue filler, which is also abundant, readily available, inexpensive, host compatible, and can easily and repeatedly be harvested. However, in recent years an increasing level of attention has been directed towards progenitor/stem cell components, such as MSCs, that are cotransplanted with the fat grafts.

FACTORS AFFECTING ADIPOSE TISSUE ENGRAFTMENT

Successful engraftment and long-term survival of transplanted adipose tissue has progressively increased the interest in structural fat grafting, as a safe, long-lasting, and natural-appearing method for soft tissue aug-

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mentation (9,10,21). However, one of the main concerns after fat grafting has been the potential high rate of tissue resorption over time at the graft site, which may represent up to 70% of the initially implanted tissue volume. In addition, failure of engraftment and long-term survival of such a significant proportion of implanted tissue is often not homogeneous, generating irregularities and tissue defects in the recipient (2,43). The most acceptable explanation for posttransplant tissue absorption has been based on the Peer's cell survival theory, which states that the number of viable adipocytes at the time of transplantation may correlate with ultimate fat graft survival volume (35), but today we definitely know that aspirated adipose tissue consists of two components: lipid inclusion containing adipocytes and stromal cells (47) containing a stem cell compartment, which under certain conditions can differentiate into mature fat cells (14).

Even Coleman, back in 2006, stated that tissue harvesting techniques may damage more easily mature adipocytes compared to immature progenitors such as preadipocytes (51). In addition, adipocytes could also be more fragile and less resistant to ischemia revascularization injury following transplantation, resulting in a more selective loss of mature cells in the posttransplant period (14). If the adult fat cells survive the trauma of tissue harvesting and transplantation, within 4–8 days after implantation they must became revascularized to access the proper supply of oxygen and nutrients required for long-term survival (42,50,52). In contrast, progenitor cells such as preadipocytes are more resistant to the trauma of both liposuction and postimplantation ischemia-revascularization injury, so they are more likely to selectively survive the overall harvest implantation procedure. This is in part due to the fact that immature progenitor cells have minimal metabolic requirements and can survive longer with minimal nutritional and oxygen requirements compared to mature adipocytes (55).

Because of the above-described competitive advantage of adipose-derived stem cells compared to the lipid filled adult adipocytes, some investigators believe that the major long-term effect of fat tissue transplantation may be related to the survival of the adipose-derived stem cells that are cotransplanted within the stromal cell fraction (37,51). Preadipocytes and adipose-derived stem cells might very well represent the only tissue surviving transplantation, and the variability in the frequency of progenitor cells between individuals may very well represent a critical factor responsible for the variability observed in the long-term survival of fat grafts (10). Very recent experimental works have stressed the relevance of donor tissue characteristics on the differentiation potential of grafted adipose tissue preparations (44,46). In fact, Stillaert suggested that while it was initially assumed that adipose tissue grafts played a "trigger" role in the induction of host-derived tissue remodeling and cellular morphogenesis, in the absence of a defined stromal and vascular fraction, fat grafts did not result in successful long-term engraftment and tissue remodeling.

These observations have progressively shifted the attention and interest of the plastic and reconstructive surgical community from the survival of the transplanted mature adipose tissue components to the stem cell/progenitor compartments and their proliferative, differentiation, and tissue remodeling potential. Observations on the migration and proliferative patterns in the microenvironment at the transplant site strongly suggest that there is more to be learned in tissue remodeling and organogenesis following adipose tissue transplantation and that the focus should increasingly shift from the survival of end-stage differentiated cells to an improved understanding of cell biology and tissue remodeling molecular events, as well as the fundamental embryological and developmental processes associated with adipogenesis (45).

EVOLUTION OF THE TECHNIQUES AND BIOLOGICAL IMPLICATIONS

Fat grafting transplantation techniques have essentially changed during the last two decades from simple free transfer of intact adipose tissue, which had a limited success in the consistent replacement of lost volume defects, to free composite fat cell transplantation strategies that, if properly executed, could have a high regenerative potential for both simple volume replacement and/or functional enhancement of recipient tissues.

After Coleman's fundamental work in the early 1980s (9,10,21), aspirated adipose tissue transplantation has gained reputation and consensus as the standard of care and is commonly performed in three stages: (a) harvesting of adipose tissue from a suitable donor site (consensus now exists on gentle manual 10-cc syringe aspiration) (36,54), with no significant differences observed when the adipose tissue was obtained from different subcutaneous donor regions (25,43); (b) purification of the lipoaspirate to eliminate the acellular oily supernatant and excess solution (by decanting, gentle centrifuging, or simple washing with saline or an appropriate physiologic solution); (c) reinjection of the purified fat tissue through a three-dimensional reimplantation technique, which creates multiple tunnels in the recipient tissue that could be more easily vascularized. This concept, in our opinion, was the most important innovation in the Coleman lipostructure technique (10).

Any variation of this original three-step technique could have biologic implications from cellular composition at tissue harvest to the ability and tissue remodeling

potential of the final cell product injected. Such modified techniques should be documented in the details and the proposed changes discussed in view of long-term outcome results obtained. It should be kept in mind that recent observations by Coleman (8) and several other surgeons (4,16,17,23–25,27–29,39) already suggest a role of stem cells and progenitors in the long-term tissue remodeling effects observed following adipose tissue grafting. In fact, it has been often observed that the quality of the skin above the adipose tissue graft improved, not only as a result of the filling effect of the grafted tissue, but also related to a gradual qualitative improvement in the quality of the skin itself, including softening of wrinkles, decreased size of the pores, and improvement in the pigmentation that could be observed over the first year of postautologous fat tissue transplantation.

Interestingly, Coleman and others also observed an improvement in the quality of the tissues into which the adipose cell products were implanted (7–9,23,25,27, 37,49). With the increasing evidence suggesting a role of fat grafting for the treatment of an increasing number of clinical conditions, from radiation damage and breast capsular contracture to damaged vocal cords, chronic ulcerations, and tissue engineering of bones, it is now of critical and timely importance to carefully reexamine the potential and implications of autologous fat grafting, which appear to represent much more than the "filler" concept for which it was originally utilized. In fact, progenitor/stem cell proliferation and differentiation could significantly contribute to the observed long-term effects on tissue remodeling and may provide an explanation for the therapeutic and healing effects of fat grafting (9).

Coleman and others have recognized the relative lack of understanding of the molecular mechanisms underlying the observed clinical effects and positive long-term outcomes. Some of the studies recently reported suggest that the interaction between grafted adipose tissue and tissues surrounding the transplant site could result in triggering of direct or indirect repair mechanisms, which could involve angiogenesis and/or vasculogenesis (9). Additional studies have identified a possible role of the plasticity between preadipocytes and macrophages, so that at least a portion of the observed therapeutic effect could be attributed to an enhanced nonspecific immune response to the autologous tissue grafted, which might trigger the release of selected cytokines and growth factors that contribute to the recruitment of mononuclear cells. All of these events may contribute to a more efficient removal of damaged or dying cellular components, enabling a more efficient outcome and long-lasting tissue remodeling (9).

After more than 10 years of extensive fat grafting in several hundred aesthetic and reconstructive procedures, the considerations outlined above appear even more relevant (49) and justify further analysis of the possible relationships between recent improvement introduced in the technique of adipose tissue grafting (49) and the possible biologic implications in tissue remodeling.

Despite the overall good clinical results obtained using the classical Coleman technique, recent and ongoing efforts aim at further improving the qualitative and quantitative characteristics of the volume replacement techniques utilized to optimize the long-term "biological" effects. These technical modifications have been introduced sequentially and in a step-by-step fashion, often being developed in parallel between surgical teams in different parts of the world, but always with a very empirical approach, often basing the clinical relevance of the modifications introduced on outcome measures, but with very little understanding of the biologic reasons and mechanisms underlying the observed improved results.

A summary of empirical improvements derived from clinical experience in hundreds of cases over the past decade (49) is outlined below. An initial methodological consideration that was recently introduced consists in a transition from a single surgical session model to multiple sessions, which allowed the introduction of smaller volumes of transplanted adipose tissue that yielded the best results, both in terms of final volume gaining (23,49) and regenerative effects, which often appeared paradoxical. In fact, some of the more striking clinical results have been obtained in conditions in which it was possible to implant only minimal quantities of fat tissue, such as in patients with radiodermitic tissue, scars, and ulcers. The fact that smaller tissue volumes could result in improved clinical outcomes may be related to the improved survival of the transplanted cell clusters, avoiding excessive densities of loading at the transplant site. This could minimize posttransplant tissue necrosis and favor progenitor cell proliferation and migration in the absence of an excessive inflammatory microenvironment.

A second technical improvement has been related to the introduction of a smaller blunt cannula for adipose tissue harvesting, with a surface area penetration that is less than 50%, compared to the traditional cannula used by Coleman (2.0 vs. 2.8 mm diameter, respectively). This technical modification, which was originally introduced to adopt a more gentle fat tissue harvesting technique, could also allow for a much faster postsurgical recovery period. For example, a practical observation was that skin entrance holes of less than 2 mm diameter, as obtained with this improved technique, do not require any stitch for virtually scarless healing.

In addition to the scarless outcome, the previously observed absence of significant differences in adipose tissue characteristics between anatomical donor sites of

subcutaneous tissue allows for choosing donor sites for fat tissue harvest following the patient's preferences (e.g., cosmetic). Interestingly, also in this case a modification of a procedure dictated for convenience, cosmetic, or faster recovery period could also have profound biologic implications, as the aspiration of adipose tissue through a cannula that has less than 50% circular surface of aspiration generates an adipose aspirate with significant differences in the size distribution of the cellular clusters. This could, of course, have profound implications for exposure of progenitor/stem cells, survival, and posttransplant revascularization of the grafted tissue. To further obtain a size reduction in the final adipose tissue cell product to be used for clinical implantation, a gentle mechanical reduction of the aspirated fragments of adipose tissue has been recently obtained through a special device that allows for the use of much smaller injection blunt needles (21 gauge or less), therefore minimizing the resulting trauma at the receiving tissue site. As for the donor tissue harvest, this has allowed for a faster recovery period and has often allowed for the procedure to be performed with minimal local anesthesia.

The use of smaller injection needles was initially introduced for practical and cosmetic considerations. However, this technical improvement could also have significant biologic implications, as it has been observed in other fields of cellular transplantation. In islet transplantation for treatment of diabetes, for example, it is known that the size of the cell clusters and the density of loading of the transplanted tissue could deeply affect short- and long-term cell transplant outcomes, determining early inflammatory events, revascularization, and the overall fraction of tissue that eventually successfully engrafts. In fact, thinner strands of transplanted adipose tissue resulted in a reduced postimplantation swelling of the grafted sites, which has consistently improved the engraftment of the transplanted tissue (no failure of engraftment has been observed following over 200 procedures performed with this method) (49).

Because high efficiency in engraftment was observed, it became preferable not to overcorrect the volume defect. In fact, it is now recommended to eventually obtain the final desired filling effect in a second and final step procedure, which can be performed as early as 3 weeks following the initial procedure, as the stabilization of the grafted fat volume is generally observed in less than 2 weeks (49).

Interestingly, the improved results obtained by multiple small tract injections performed with smaller needles could also have an alternative explanation, in view of recent evidence indicating that localized oxidative stress is associated with triggering of cell migration and proliferation, including endothelial cells (19,30). This phenomenon could be responsible, in part, for the observed improved results following small needle injections of adipose tissue cell clusters, which contain endothelial and progenitor/stem cells. Similar to the effect observed in a cell-wounding assay in vitro (30), the needle tract could be responsible for molecular events, such as a localized increase in free radical generation, which has been shown to be associated with activation, proliferation, and migration of cells that could be associated with tissue remodeling and repair mechanisms (19,30). Therefore, a localized and transient inflammation could be important to trigger remodeling and tissue repair, unlike a chronic state of inflammation and/or generation of preinflammatory cytokines, which is associated with chronic degenerative disease conditions (22).

Another technically important consideration has been to obtain almost complete removal of the oily component from the final adipose tissue preparation, through multiple washing steps of the volume-reduced adipose tissue cell clusters, as the oily component could trigger undesired inflammatory events (49). It is thought that reduction of postoperative inflammation improves engraftment of the transplanted adipose tissue cell clusters (49). In addition, the use of sequential washing steps has been demonstrated superior to centrifugation, as used in the Coleman technique (10) for the presence of more preadipocytes, a better cellular proliferation, and, above all, overall better clinical results (26). The use of much thinner implantation blunt needles for fat transfer therefore appears to improve engraftment of the transplanted cell product as well as reduce the trauma and nonspecific inflammatory events at the recipient implantation site. Moreover, injection of smaller quantities of adipose tissue appeared to facilitate the formation of a three-dimensional network, which enhances the engraftment of the transplanted tissue. This led to the adoption of blunt fine needles, which were initially developed only for injection of eyelids, for adipose tissue infusion in other sites, thus allowing for achievement of significantly less trauma and pain, and overall improved clinical results.

These modifications of the technical procedure for fat tissue grafting have now been clinically tested for several years, during which performance of the three modified steps—gentle aspiration with a fine Mercedes cannula, the reduction of aspirated fat particles, and finally their washing with sterile saline before injecting the fat with a very fine blunt needle—has become the preferred option in the clinical setting at our institution (49) and elsewhere. The surgical procedure, although routinely performed under local anesthesia on an outpatient basis with no or very minimal sedation, is still a surgical procedure that must be performed in a sterile environment and proper surgical setting.

Finally, an unexpected finding observed in patients

undergoing cosmetic procedures was that, a few weeks after a "regenerative lipostructure" of the face and/or of the hands, the clinical results of treatments with Fraxel lasers seem to improve (Tremolada et. al, paper in preparation). In addition, the beneficial effect of hyaluronic acid fillers seems to be of much longer duration (Tremolada et al. paper in preparation). These findings are quite logical and are supported by recent experimental evidence (1) suggesting that the positive interaction between transplanted adipose tissue and hyaluronic acid fillers may open the possibility to improve long-lasting results, even in conditions of fat depletion, such as in some patients with HIV infections. In fact, in these subjects a lack of sufficient donor adipose tissue has represented a significant challenge in the clinical setting (Tremolada and collaborators; clinical trial in progress). Similar considerations exist for patients who need breast reconstruction but do not have enough donor fat tissue to use for these purposes (Tremolada and collaborators; clinical trial in progress).

ADIPOSE TISSUE GRAFTS MEET CELL BANKING AND CELLULAR THERAPIES

On the technical, cell processing side, a completely closed disposable system for collecting the aspirate, reduction of the adipose tissue particles, and effective washing of the final tissue product is now under development (Tremolada et al, manuscript in preparation) and could become of assistance for clinical transplantation, as well as for other research and tissue banking applications. In fact, the possibility of obtaining viable adipocytes in combination with metabolically active preadipocytes and stem cells, in the absence of any enzymatic digestion or growth factor additives to the final cell product, is very attractive and it is likely to become the focus of further studies in the field. The availability of a closed system, disposable kit for adipose tissue processing could allow for this procedure to be safely and economically performed in appropriate clinical settings by properly trained personnel.

A possible advantage of the proposed technologies under development for adipose tissue processing will be their application to the field of cell banking, as adipose tissue-derived stem cells maintain their proliferative and differentiation potential following cryopreservation (33). In fact, the well-described age-related reduction in the regenerative potential of progenitor cells, including those obtained from adipose tissue, has resulted in an increased level of interest in "earlier" banking of adipose tissue-derived cells during adult life, as younger adipose tissue contains progenitor and stem cells with a higher regenerative, tissue remodeling, and therapeutic potential, compared to the same cell types obtained from the same donor at a more advanced age, such as at a time in which the need for a therapeutic procedure may arise. In this direction, adipose tissue banking could become a useful complement and, for many applications, even an alternative to cord blood banking, especially for those subjects in which cord blood banking was either not available or did not represent an option.

SUMMARY AND CONCLUSIONS

In summary, the technologies for adipose tissue harvesting, processing, and transplantation have substantially evolved in the past two decades. Besides these clinically driven advancements, substantial improvements in the understanding of cellular, molecular, and immunobiological events surrounding cell and tissue transplantation have opened new options for the definition of novel therapeutic strategies. Adipose tissue has become central to translational research efforts aimed at identifying novel pathways and to better define the true potential of progenitor cellular components from this tissue source. This has resulted in an increasing interest in the exploration of many novel research and clinical applications. Progenitor, endothelial, and mesenchymal stem cells derived from adipose tissue could therefore not only become central to plastic and reconstructive surgery applications, but also represent the focus of an array of therapeutic solutions for several disease conditions, including those affecting bone, cartilage, muscle, liver, kidney, cardiac, neural, and the pancreas, therefore expanding the possible indications and translational potential of tissue, cell-based, and regenerative medicine strategies.

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