Anti-Aging Specialist Explains
Regenerative Cell Therapy

By Sharon McQuillan, M.D.

Through the use of adult stem cells, regenerative medicine is a revolutionary approach to treating many degenerative conditions that occur with age, including the appearance of aging. This field joins nearly all disciplines of science and holds the realistic promise of repairing damaged tissue by harnessing the body's ability to heal itself. While most every degenerative disease is being studied for potential treatment with stem cell therapy, regenerative medicine has also attracted attention for its newfound ability to improve aesthetic enhancements such as autologous fat grafting and skin rejuvenation.

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Use of adipose-derived mesenchymal stem cells (ADSCs) is a natural progression in aesthetic medicine. ADSCs can be found in abundant quantities within adipose tissue, which presents a clear advantage since subcutaneous fat is readily available in a majority of patients and can be easily isolated under local anesthesia. These cells have been reported as very similar to mesenchymal stem cells isolated from bone marrow (Bunnell, 2008); however, the amount of stem cells harvested from fat can be up to 500 times more than those collected from bone marrow (Meliga, 2007).

ADSCs offer proliferative potential, as well as the ability to differentiate into adipocytes, blood vessels, cartilage, bone and cardiac tissue. Several beneficial effects have been associated with ADSCs, including their ability to reduce inflammation and promote tissue healing through local secretion of cytokines and growth factors, which can recruit stem cells from other tissues to facilitate repair and healing of the affected tissue.

One of the newest and most successful applications of stem cells involves cell-assisted lipotransfer (CAL). Although fat grafting has been practiced for over 100 years, consensus on a standardized method of harvesting and fat graft survival has yet to emerge. This renewed interest in utilizing fat as a filler stems from the increased number of liposuction procedures performed, as well as recent trends in revolumization and a new appreciation for the regenerative potential of the stem cells contained in lipoaspirate. With CAL, autologous ADSCs are used in combination with autologous fat transfer.

Isolation of adipose-derived stem cells begins with harvested fat
Photo courtesy of Sharon McQuillan, M.D.

Once removed, half the volume of aspirated fat is processed for the isolation of ADSCs, while the other half is prepared for grafting. The stem cells and processed fat are combined and injected into various target areas such as the face, breasts and gluteal region. Adding stem cells to the fat used for autologous transfer enhances angiogenesis, improves fat graft liability and reduces post-operative atrophy. Significantly, ADSCs can be safely and effectively transplanted immediately after harvesting and isolation.

There are many processess involved in CAL. After fat transfer the ADSCs used in the repair process play a key role in adipogenesis and angiogenesis. With lipografting, the recipient tissue is injured and bleeding occurs. Cytokines, released by platelets, include platelet derived growth factor (PDGF), endothelial growth factor (EGF) and transforming growth factor beta (TGF-ß). Since the grafted adipose tissue is non-vascularized it experiences hypoxia and is temporarily fed by surrounding host tissue until direct capillary attachment is formed.

In response to tissue trauma, basic fibroblast growth factor is released from the extracellular matrix and dying cells. Surviving adipocytes die within a few months after transplantation and are replaced with next-generation cells. Theoretically, surviving fat grafts turn over within two to three months after transplantation. The number of ADSCs may affect the replacement process and post-operative atrophy of grafted fat, which commonly occurs during the first six months after grafting (Yoshimura K, *Regen Med* 2009). Research published by Yoshimura *et al.* suggests a strong correlation between the number of viable ADSCs contained in the fat graft and retention of the graft.

This advancement in autologous fat transfer makes the procedure more predictable and reliable, which is especially important in large volume grafts such as breast and gluteal augmentation. Loss of significant volume in a breast or gluteal fat graft may leave the patient with inadequate donor fat for a second treatment. Matsumoto *et al.* showed a 35% increase in survival at six months when stem cells were added to the graft. Additionally, the transplanted cells expressed vascular endothelial cell markers.

Yoshimura *et al.* performed and published results from transplantation of stem cell supplemented adipose tissue of the breasts in 15 patients. Overall,

clinical results were very satisfactory with no major abnormalities seen in magnetic resonance imaging or mammogram after 12 months. Post-operative atrophy of the injected fat was minimal and did not change substantially after two months. Surviving fat volume at 12 months was 155 ± 50 ml (right) and 143 ± 80 ml (left) following lipoinjection from an initial mean of 264 ml. These results suggest that CAL is a suitable methodology as an option for breast enhancement.

Other aesthetic applications for ADSCs include improving skin quality, skin repair, wound healing and wrinkle reduction. In addition to PDGF and TGF-ß, ADSCs produce cytokines including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF), all of which provide a regenerative effect on the skin. These growth factors activate and increase migration of dermal fibroblasts, which mediate collagen secretion. A study by Kim *et al.* showed that these activated dermal fibroblasts also have an anti-wrinkling effect. ADSCs and their soluble factors reduce UVB-induced apoptosis and stimulate collagen synthesis.

As previously mentioned, evidence suggests that ADSCs participate in angiogenesis, as well as would healing. ADSCs isolated from human adipose tissue secrete VEGF. The levels of VEGF secretion increased five-fold when ADSCs were cultured in hypoxic conditions. These findings suggest that autologous delivery of ADSCs, which are regulated by hypoxia, enhance angiogenesis (Nambu *et al. Ann Plast Surg* 2009). Additionally, ADSCs express pericyte lineage markers, reside in a periendothelial location and stabilize endothelial networks (Traktuev Do *et al. Circ Res* 2008).

According to observations by Sydney Coleman, M.D., a plastic surgeon in New York, New York, U.S., fat grafts improve surrounding tissue. Dr. Coleman also noted improvement in the quality of aging skin and scars, as well as improvement in conditions such as radiation damage and chronic ulceration. Histologies of the tissue showed improvement in scarring by collagen deposition, local hypervascularity and dermal hyperplasia (Coleman SR, *Plast Reconstr Surg* 2006).

Mojallal *et al.* studied the improvement of skin quality after fat grafting via clinical observation and animal study. Clinical observation revealed a volumetric improvement, as well as skin improvements in the grafted area including skin texture, pore size, suppleness, skin color and scar quality. Histological analysis from preclinical studies showed the presence of mature adipocytes in large quantities. Additionally, thickening of the extracellular matrix that surrounds the fat tissue and the dermis can be seen after fat grafting. This thickening was attributed to an increase in type I collagen. We now know that these qualitative improvements are due to the presence of stem cells within the fat grafts providing growth factors and improved circulation.

Stem cells have also been used for facial skin rejuvenation by injection. A study by Park *et al.* injected ADSCs using a mesotherapy-like method for skin rejuvenation. A patient with photodamaged skin was injected intradermally with autologous, processed lipoaspirate containing 30% ADSCs, twice in a two week period. The patient showed improvement in skin texture and wrinkles two months post-procedure. Dermal thickness results were shown histologically via high frequency ultrasonography.

Studies are underway in our clinics for cell-assisted laser resurfacing. Cell-assisted laser resurfacing combines fractional ablative therapy with topical application of autologous ADSCs applied in a cell medium immediately after treatment. Histologic changes observed following fractional ablative resurfacing demonstrate neocollagenesis six weeks post-operatively via dermal fibroblast and growth factor activation. Furthermore, positive effects of ADSCs in wound healing have been observed. It is theorized that the combination of these two treatment modalities will result in a more pronounced diminishing of wrinkles and photodamage, with a shorter healing time.

In order to integrate stem cell medicine into a practice it is important to have the appropriate education / training, and a process that is fully validated, provides a consistent cellular product and is compliant with the current regulatory guidelines. Providing stem cell therapy at the point of care is also crucial as stem cell viability and potency diminish significantly over time.

The accepted method of ADSC isolation involves tumescent liposuction, cell washing to remove hematopoietic cells, enzymatic digestion, centrifugation to separate the stromal vascular fraction (SVF), filtration and preparation of the SVF in final suspension. ADSCs can be harvested and isolated easily, abundantly and with minimal patient discomfort in a 90 minute in-office procedure.

In autologous fat transfer, the method of harvesting affects the fat graft viability, as well as the stem cell yield. Stem cell yields are also affected by the location from which the fat is harvested and the time and

concentration of enzymatic digestion utilized. The abdomen is the preferred collection site for ADSCs in terms of cell yield and viability, although other sites can be used successfully (*Cell Tissue Res*, 2008).

There are many other factors that matter when harvesting adipose tissue, including the suction pressure, type and size of cannula used, the manner in which the fat is processed and the type of system employed. We have had the best success with a collection method utilizing less than 350 mm Hg suction pressure, cannulas no smaller than 3 mm in a closed system under sterile conditions, with decantation rather than centrifugation. This method results in a gentler treatment of the fat, leading to higher cell viability.

This established cell isolation technique provides a consistent, safe and compliant method for processing ADSCs. In our clinics we utilize materials that are produced according to cGMP regulations or regulated as clinical grade. We are collecting approximately 1 million cells per cc of fat extracted. This procedure consistently produces pre-determined acceptance criteria, making it easy to duplicate in a medical office setting and has been validated via reproducibility and robustness analyses.

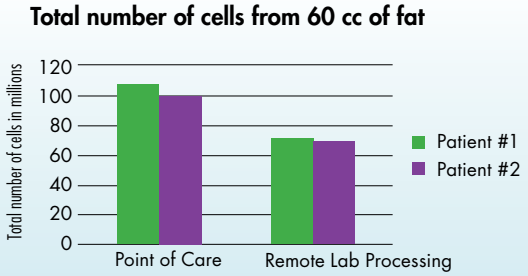
Offering stem cell therapies to patients not only requires procedural competence, but a thorough understanding of the regulatory considerations involved. Any technique employed to obtain stem cells must adhere to the current code of federal regulations for use in a physician's practice. The method described previously meets the federal code of regulations concerning autologous, homologous use of minimally manipulated cells at the point of care; therefore, it is not regulated by the FDA, but rather considered the practice of medicine.

According to FDA CFR title 21 part 1271, which concerns the regulation of human cells and tissues, as well as cellular and tissue based products, physicians are not required to comply with the requirements of FDA regulation if the physician removes the cell products and implants them into the same individual during the same procedure. Additionally, the cells used must be minimally manipulated, meaning that the method of processing does not alter the relevant biological characteristics of the cells.

It is important to note that the increased focus on regenerative medicine has resulted in a variety of topical products containing stem cells to be introduced. Many of these skincare products are created using

Various stages of the cell isolation process
Photos courtesy of Sharon McQuillan, M.D.

Final product of the cell isolation process, black line at bottom of tube indicates the stromal vascular fraction where the stem cells are located
Photos courtesy of Sharon McQuillan, M.D.



Comparison of stem cell yields from point of care collection vs. off-site processing laboratory
Courtesy of Sharon McQuillan, M.D.

Test	1A	2A	2B	3A	3B
Cell Count	61.7e6	46.6e6	45.4e6	65.7e6	69.9e6
Viability	100%	100%	100%	99%	100%
CD34	72%	52.3%	68%	64.1%	51.2%
Sterility	No Growth	No Growth	No Growth	No Growth	No Growth
Endotoxin (EU/ml)	<0.5	<0.5	1.1	3.51	<3

Cell viability, amount of active stem cells, sterility and absence of toxin results from samples collected at the Ageless Regenerative Institute
Courtesy of Sharon McQuillan, M.D.

peptides or enzymes that help stimulate the skin's stem cells or protect the stem cells from damage or deterioration. Other products utilize plant based stem cells to promote healing and anti-aging effects. These plant based stem cells are derived from plants such as the Utuiller Spatlauber apple tree, Edelweiss plant and the date palm. However, currently, there is little to no clinical evidence to support the claims of these products and if any of these products actually altered the cell structure it would require FDA approval.

Until now, one barrier to the implementation of cell-assisted therapies into clinical practices has been the lack of an affordable, validated method for the isolation of ADSCs from a lipoaspirate. The automated methods undergoing FDA trials are not currently permitted for patient clinical use except under clinical trials or institutional review boards (IRB). Once FDA approved, the retail price of these devices is projected to be $70,000 to $90,000 with a consumable cost between $1,200 and $7,500.

In addition to the treatments currently provided in the U.S., there are several IRB approved protocols currently under investigation as part of our adipose program at Hospital Angeles (Tijuana, Mexico). These treatments are currently conducted using the accepted harvesting and isolation method previously described. The conditions being treated include chronic heart failure, chronic ischemia, acute myocardial infarction, critical limb ischemia, COPD, diabetes, advanced orthopedic indications, Parkinson's disease, glaucoma and multiple sclerosis. Preliminary data from these protocols is very encouraging in terms of successful patient outcomes. Oxygen dependent, end stage COPD patients are coming off oxygen, end stage CHF patients are able to walk the length of a football field and blind patients are achieving visual acuities of 20/50.

As the data suggests, stem cells possess enormous regenerative potential. Adipose stem cells can be harvested easily, abundantly and with minimal patient discomfort. The potential applications are virtually limitless. Clinical applications for patients can be performed in a medical office setting safely, legally and ethically using autologous ADSCs. The manual laboratory method yields approximately 1 million, 99% viable ADSCs per fat gram processed, and freshly processed cells are positive for cell markers CD34 and CD90, making them angiogenic and regenerative.

As aesthetic practitioners we owe it to our patients to provide only safe and compliant methods of these cutting edge treatments. We already understand the value of fat as a filler; the next step is to utilize fat for its regenerative value, both in the aesthetic arena and the traditional medical arena. We have only scratched the surface of ADSC's potential. The coming years will bring forth amazing scientific discovery. ■

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Dr. McQuillan is a board certified physician who specializes in aesthetic, anti-aging and regenerative medicine. She founded The Ageless Aesthetic Institute – a level 4 ACCME accredited aesthetic training program for medical professionals in order to standardize and elevate the practice of aesthetic medicine. Dr. McQuillan has educated thousands of medical professionals in the art and science of aesthetic and anti-aging treatments for over a decade. Dr. McQuillan lectures internationally on aesthetic and regenerative medicine for many organizations and is the medical director of the Aesthetic Fellowship, hosted by the *American Academy of Anti-Aging Medicine*. In 2009, Dr. McQuillan formed the Ageless Regenerative Institute in conjunction with a team of experts in stem cell therapies. This expert team has developed an approved method and protocol for the harvesting and isolation of adipose-derived stem cells for autologous transplantation.

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