RESEARCH

CONTINUING MEDICAL EDUCATION ARTICLE

Adipose-Derived Stem Cells in Aesthetic Surgery

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Abstract

Adipose-derived stem cells (ADSC) have come to be viewed as a ubiquitous solution for aesthetic and reconstructive problems involving loss of tissue volume and age or radiation-induced loss of tissue pliability and vascularity. As the theoretical potential of "stem cell therapy" has captured the public imagination, so the commercial potential of novel therapies is being exploited beyond scientifically sound, hypothesis-driven paradigms and in the absence of evidence establishing clinical efficacy and safety. Moreover, with variations in methods of isolation, manipulation and reintroduction described, it is unclear how the practitioner with an interest in ASDC can harness the clinical potential in reproducible and scientifically measurable ways. This Continuing Medical Education (CME) article presents a summary of our understanding of what ADSC are, their utility within the field of aesthetic surgery and the current and future directions for adipose stem cell research.

Learning Objectives

The reader is presumed to have a basic understanding of the therapeutic applications of adipose-derived stem cells (ADSC) in aesthetic surgery. After reading this article, the reader should be able to:

1) Define ADSC within the context of stem cell biology and etymology.
2) Identify the areas within the fields of aesthetic surgery and medicine where the therapeutic potential of ADSC has attracted interest.
3) Summarize the pre-clinical and clinical evidence (or lack thereof) for the use of ADSC in aesthetic surgery and medicine.
4) Address the limitations of ADSC therapeutics and current and future strategies designed to circumvent these limitations.

The American Society for Aesthetic Plastic Surgery (ASAPS) members and *Aesthetic Surgery Journal* (*ASJ*) subscribers can complete this Continuing Medical Education (CME) examination online by logging on to the CME portion of *ASJ*'s website (http://asjcme.oxfordjournals.org) and then searching for the examination by subject or publication date. Physicians may earn 1 *AMA PRA Category 1 Credit*^{TM} by successfully completing the examination based on the article.

The term "stem cells" has come to be defined by both clinicians and the lay public alike as a ubiquitous cell-based therapeutic solution capable of tissue repair, regeneration, enhancement and

replacement. The idea that these cells are ready made solutions waiting for problems to address is a captivating one but far from the truth. Indeed, the fact that there exists disagreement within the scientific community about the "stem-ness" of cells harvested from mature tissues serves as a reminder that the field is more complex than has been credited. For some clinicians "adipose derived stem cells" have become synonymous not only with the isolate of cells with stem-like potential derived from adipose tissue but the lipo-aspirate (a heterogeneous mix of fat globules, fibrous interstitia, cellular debris, oil and blood) or the stromal vascular fraction thereof. Attempts to market lipo-sculpture as "stem cell therapy" is, at best etymologically misconceived. At worst, it is the conscious intent to mislead.[1]

Adipose tissue (sometimes known as "white adipose tissue" to distinguish it from infantile, thermoregulatory "brown adipose tissue") is an energy store and an endocrine organ, secreting several adipokine hormones.[2] While mature adipocytes are sensitive to ischemic changes, their multipotent precursors (ADSC) are relatively resistant to ischemia, a feature exploited in adipose tissue grafting.[3] The utilization of these cells for therapeutic purposes has a special appeal because the substrate is: (1) technically straightforward and relatively painless to harvest with no anesthetic complications most of the time; (2) relatively abundant with a higher cell yield per unit of tissue substrate than bone marrow or other mesenchymal tissue; (3) relatively redundant, with no adverse functional or aesthetic implications of harvest; and (4) ethically incontrovertible.[4–6] In comparison with BM-MSC (bone marrow-derived mesenchymal stem cells), they are genetically stable[7] and relatively resistant to senescence in culture.[8] There exists common misconceptions about what ADSC are; how they can be used, the evidence for their efficacy and the strategies currently being developed to enhance the exciting field of stem cell therapeutics. This article serves to help aesthetic surgeons and physicians by removing the obfuscation surrounding stem cell therapeutics and providing a platform on which an evidence based practice might be built.

Defining Stem Cells with a Focus on Adipose-Derived Stem Cells (ADSC)

ADSC Lineage

Stem cells are cells capable of self-renewal and have multi-lineage differentiation potential, though their differentiation capability becomes more restricted as development progresses. Totipotent stem cells are cells with the potential for generating both embryonic and extra embryonic tissues; the typical totipotent cell is the fertilized egg. Cells harvested from the fetal blastocyst also have

extensive developmental potential and are known as pluripotent stem cells as they can give rise to the three germ layers (ectoderm, mesoderm and endoderm) from which all cells in the body originate.[9] Adult "stem" cells can also give rise to more than one cell type, but usually within the same lineage, hence they are defined as multipotent. They are also capable of self-renewal, but, unlike embryonic pluripotent stem cells, adult "stem cells" might have a finite life-span; hence some authors prefer to call them progenitor cells to reflect their ability to renew over a long period of time while already committed to a certain lineage. Furthermore, adult stem cells from mesenchymal tissue (of mesodermal origin) are sometimes termed "stromal cells" owing to their derivation from connective tissues. Blood is also a mesenchymal tissue but, confusingly, mesenchymal stromal cells harvested from blood are typically called hematopoietic stem cells (HSC) instead. The mesenchymal stem cell lineage is shown in Figure 1. While "mesenchymal stem cells" (MSC) is the term used here, the nomenclature has not been standardized and this term is synonymous with mesenchymal stromal, progenitor or regenerative cell, non-hematopoietic stem, stromal, progenitor or regenerative cell and others. MSC exhibit the potential to differentiate into cells of mesenchymal origin namely cartilage, bone and fat (usually referred to as mesenchymal tri-lineage differentiation); the potential to differentiate along non-mesenchymal lineages, such as neural and epithelial, has also been reported. Non-hematopoietic multi-potency marks the feature of these cells that can be harvested from muscle, marrow, dermis, the umbilical cord and fat. Adipose-derived stem cells (ADSC) exhibit tri-lineage differentiation potential[10] (as shown in Figure 2) but differ from bone marrow-derived mesenchymal stem cells (BM-MSC) in the expression of some cell adhesion molecules indicating that ADSC exhibits features unique from BM-MSC.[11]

Do ADSC Have All the Properties of MSC?

The lack of a definitive marker set for the categorical identification of MSC from different tissues makes comparative *in-vitro* analyzes challenging. Fibroblast-like morphology on a plastic culture plate and tri-lineage differentiation potential have traditionally been used to confirm the presence of these cells. The International Society for Cellular Therapy (ISCT) attempted to clarify this issue by introducing minimum criteria for a MSC which included the requirement for a candidate cell population to express the MSC markers CD73, CD90 and CD105 and to lack expression (<2% positivity) of the pan hematopoietic marker CD45, the monocyte/macrophage markers CD11b and CD14, the B cell markers CD19 and CD79α and CD34, the marker for primitive hematopoietic progenitors and endothelial cells.[6,8,11–14] ADSC surface markers are summarized in Table 1. The

presence of equivocal markers probably reflects heterogeneity of cell population and passage as well as cell isolation and culture condition protocols. For example it has been reported that early passage ADSC express CD34 which is may be lost in later passages depending on the culture conditions.[15]

ADSC Acquisition and Isolation

Procedures for the acquisition of ADSC, their variations and the clinical implications of variations in practice have been reviewed by Gimble and colleagues.[16] All methods require the acquisition of adipose tissue, usually in the form of lipo-aspirate. A common method for the separation of the cellular component from the supporting stromal tissue is enzymatic digestion (eg, collagenase ± trypsin). The tissue suspension is incubated and agitated gently at 37°C and the resultant cell suspension passed through a strainer to discard the un-dissociated tissue. The filtered cell suspension is then centrifuged. The resultant cell pellet is known as the stromal vascular fraction (SVF). The pellet is re-suspended in cell culture medium and seeded onto a culture plate. Isolation of ADSC from the SVF employs *in-vitro* differential adhesion cell culture techniques. When maintained in a humidified atmosphere of 5% CO_2 at 37°C, the spindle-shaped cells adherent to the plate will be almost exclusively ADSC. This process is summarized in Figure 3. However, even small changes in protocols for ADSC isolation and growth, when their expansion is required, results in cell product heterogeneity[17] and this lack of methodological standardization needs to be further addressed.

While simple liposuction does not appear to adversely influence the yield of ADSC[18] the use of ultrasound-assisted liposuction may impact negatively on cell yield and the proliferation capacity of viable cells (although this remains controversial).[19] High speed centrifugation (eg, 3000 rpm for 3 minutes) also results in a higher proportion of non-viable cells;[20] a problem that can be mitigated by using a lower speed (1300 rpm).[21] Moreover, contaminants such as blood, oil and fibrous interstitia may impair graft take through toxic effects on viable cells and the surrounding tissues and are prejudicial to appreciation of volumetric change.[22,23] In order to meet the dual demands of convenient fat processing and optimized graft viability a number of proprietary fat harvest and preparatory systems have been developed and marketed. Some of these systems are designed to improve the graftable quality of the lipo-aspirate while others use additional innovations to increase ADSC availability.

The Body-Jet® system of lipo-aspiration is used with the LipoCollector® or FillerCollector® systems (all Human med AG, Schwerin, Germany) to provide an end-to-end system of fat harvest, processing and delivery without the need for maceration or enzymatic digestion, washing or centrifugation. Also known as the BEAULI method, this approach is associated with excellent cell

viability[24] and is clinically safe.[25] Puregraft® (Puregraft LLC, Solana Beach, CA, USA) utilizes a closed membrane-filtration system whereby aspirated fat is introduced into the system and is washed and filtered in Ringer's lactate to remove contaminants. Revolve™ (Lifecell Corp., Bridgewater, NJ, USA) is a closed system that washes, filters and strains the lipo-aspirate and also agitates the tissue to improve the wash. This system has been demonstrated to be safe and cost effective (by decreasing operating time) when compared with non-proprietary fat aspiration and centrifugation for large volume lipo-grafting.[26] Three of the authors of the supporting paper were employees of the parent company and one was a paid consultant. Lipogems®, (Lipogems International S.p.A., Milan, Italy) utilizes a closed system whereby aspirated fat is macerated, washed, filtered and strained. Cell analysis revealed a higher component of ADSC in the preparation than standard lipo-aspirate alone which was attributed to the (non-enzymatic) maceration of fat in the system.[27] One of the authors of the supporting paper holds the patent for the Lipogems® system. Celution®, (Cytori therapeutics; San Diego, CA, USA) is a sophisticated closed system which first washes the lipo-aspirate then introduces a solution containing a proprietary enzymatic combination (Celase®) calculated by volume based on the mass of the lipo-aspirate. The mix is then agitated before the cell solution is centrifuged and washed.[28] In each of these closed systems, the processed graft is aspirated via a port. The Harvest® AdiPrep® system (Terumo BCT Inc., Lakewood, CO, USA) is a single use sterile pack for lipo-aspiration and centrifugation for use with the companies' proprietary centrifugation system. The Antria Cell Preparation Process® (Antria Inc., Indiana, PA, USA) utilizes Adipolyx™, the companies' proprietary enzymatic combination and buffer.[29] Tulip® GEMS (Tulip Medical Products, San Diego, CA, USA) are sterile, single use instruments for lipo-aspiration. Tulip® GEMS nanofat™ is an end-to-end proprietary system for refining aspirated fat for graft delivery by emulsifying the fat through repeated passage between two Luer-Lok syringes.[30] The Lipografter™ system (Marina Medical Inc., Sunrise, FL, USA) is a sterile, single use system designed to improve the economy of motion and hence quicken operating times for large volume lipoplasty. Unlike the other proprietary options, it neither washes, filters nor agitates the harvested fat but relies on gravity to separate the tissue for the tumescent mix.

Most of the evidence in support of the proprietary systems is based on pre-clinical work or small case series. While not a problem exclusive to proprietary systems, adequately controlled, independently funded, long-term volumetric data are lacking.

The SVF is Not Synonymous With ADSC

The stromal vascular fraction is obtained by centrifugation of enzymatically-digested adipose tissue; a technique first described over 50 years ago.[31] The SVF contains, in addition to ADSC, other cell

populations that exhibit multipotent differentiation potential including pericytes, hematopoietic stem cell progenitors and adventitial cells.[32,33] SVF also contains pre-adipocytes, endothelial cells, fibroblasts, and immune cells.[6,14] Hematopoietic cells comprise up to 45% of the cellular content of the SVF, with endothelial cells contributing up to 20% and pericytes up to 5%.[13] Most of the remainder are differentiated cells of stromal origin (of which ADSC is one variety) comprising a small fraction of the total.[13] In the clinical literature, the SVF is sometimes erroneously used as a stem cell population synonymous with ADSC contained therein. Consequently, caution is required when interpreting results from clinical studies using SVF, especially when findings are interpreted as evidence of efficacy of ADSC in clinical practice. Importantly, the secretome is fundamentally dissimilar. ADSC express higher levels of the pro-inflammatory cytokines IL-12 and INF-γ, higher levels of the anti-inflammatory cytokines IL-10 and IL-13 and higher levels of the growth factors VEGF and IL-7.[34] Conversely, they express lower levels of the pro-inflammatory cytokines TNF-α and IL-1β and the chemokines IL-8, MIP-1α and MIP-1β.[34] The ADSC secretome is summarized in Table 2.

ADSC Heterogeneity

Not all MSC have an equal propensity towards tri-lineage differentiation.[8,35] And while ADSC exhibits excellent tri-lineage differentiation potential[8] both inter and intra-donor variability exists. Advancing donor age appears to adversely influence the differentiation (and possibly proliferative) capacity and angiogenic potential of ADSC.[36,37] ADSC from different harvest anatomic sites exhibit differences in tri-lineage differentiation, growth and proliferation, apoptotic potential and gene expression profiles.[36,38] For example, it has been shown that pre-Scarpa's ADSC exhibit enhanced proliferation and differentiation relative to sub-Scarpa's ADSC. This observation might be gender-dependent for, in another study, ADSC harvested from female subjects exhibited no such differences.[39] Abdominal adipose tissue has also been shown to be relatively resistant to apoptotic stimuli.[36] These differences are a function of gene (and, ultimately, growth factor) expression profiles.[38,40] The metabolic phenotype of the donor has also been shown to influence the behavior of ADSC. ADSC from obese donors have been shown to promote pro-inflammatory cell activation and adipocyte insulin resistance.[41] The immuno-modulatory properties of ADSC are also compromised in obese donors.[42] These observations have potential implications for graft take and post-surgical response in obese donors.

ADSC in Tissue Repair and Regeneration

The introduction of MSC to sites of tissue injury (eg, gut, kidney, skin) improves tissue homeostasis and repair. There is also evidence that signals at sites of tissue damage induce the recruitment of

intravenously delivered MSC (such as ADSC) to the area of need; a phenomenon known as homing.[43,44] ADSC promote angiogenesis and matrix remodeling through cytokine, chemokine and growth factor expression[2] and exhibit immuno-modulation through the expression of prostaglandin E2 and IL-10, suppressing the maturation of regulatory T cells.[2,45] More recently, it has become apparent that paracrine factors with immunomodulatory function are secreted by MSC in exosomes (30-150nm vesicles carrying proteins, nucleic acids and lipids) that are known to play a role in cell-cell communications both at short- and long-range.[46,47] The question of how ADSC promote tissue augmentation, repair and regeneration is important because understanding the process at a molecular level helps us to develop ADSC therapeutics.

Having summarized the properties of ADSC and introduced the mechanisms by which they influence tissue repair and regeneration, we will now turn our attention to the therapeutic applications in aesthetic and reconstructive surgery and medicine.

The Aesthetic and Reconstructive Applications of ADSC

Lipo-Sculpture and Neo-Adipogenesis

Volume enhancement using fat has garnered exceptional interest within the last 10 to 15 years, with 12 clinical trials registered and 115 patents filed.[48,49] Breast augmentation using autologous fat, described over 30 years ago[50] offers the potential advantage of achieving an augment more natural in shape and feel without the use of prosthetic material and the associated risks of capsular contracture and anaplastic large cell lymphoma.[51] However, necrotic grafted fat is associated with pain, swelling and erythema, oil cysts, breast lumpiness and oncologically suspicious micro-calcifications on mammography. The challenge for proponents of autologous breast augmentation is to deliver long-term, predictable graft retention with acceptable rates of ecchymosis, striae, hematoma, infection and residual asymmetry without compromising oncological surveillance.[52] In a systematic review of 1453 cases of lipo-sculpture for breast augmentation the technique was found to be both efficacious (with retained volumes of between 55 and 82%) and reasonably safe with complications requiring hospitalization in 6%.[53] With a mean follow up of 16 months, doubts persist over long-term volume retention and the long-term reliability of oncological surveillance in lipo-sculptured breasts. In theory the survival of grafted fat might be enhanced using either the resident or an enriched population of ADSC as these cells exhibit enhanced survival and self-renewal and are mediators of angiogenesis and neo-adipogenesis via paracrine mediated cell recruitment and adipogenic differentiation.[54] The use of ADSC to enhance the survival of simultaneously injected autologous fat has been labelled cell-assisted lipo-transfer (CAL).[55] CAL is produced by the centrifugation of collagenase-treated lipo-aspirate, filtration of the re-suspended pellet to remove

the cellular and extracellular debris followed by a second centrifugation and the subsequent mixing of the resultant ADSC-enriched pellet with standard lipo-aspirate.[56] The therapeutic utility of CAL has been investigated with respect to autologous breast augmentation.[57] The authors reported reasonable long-term graft survival after the introduction of a mean of around 270 ml into each breast using chest circumference as a surrogate measure of volume retention. The evidence is not unequivocally supportive however, as another study, this time using MRI to quantitatively assess the influence of SVF-enriched lipo-aspirate for use in autologous breast augmentation found no difference compared with the lipo-aspirate-only control.[58] With such methodological variation it is difficult to draw concrete conclusions about the efficacy of ADSC and/or SVF-augmented lipo-sculpture. A recent small but high quality (triple blinded, placebo controlled) trial designed to investigate the benefit of enriching fat grafts with an expanded population of ADSC confirmed the therapeutic promise of this approach.[59] However, the cell population underwent *in-vitro* expansion prior to therapeutic re-implantation and hence there are significant translational hurdles to overcome before this research translates to a viable therapeutic option. The use of a vacuum device to pre-expand the recipient matrix has been shown to be an effective means of preparing the recipient matrix for receipt of the grafted fat[60] and has also been shown to enhance the retention of engrafted fat when compared with the results from other published studies using fat engraftment alone.[61]

The use of ADSC for post-oncological breast reconstruction has a controversial past. In 1987, the American Society of Plastic Surgeons (ASPS) published a position statement detailing their unequivocal objection to the practice of using autologous fat grafting for volume restoration on the grounds that micro-calcifications from necrotic fat would compromise oncological surveillance.[62] In the intervening years ADSC have been used for soft tissue augmentation following oncological breast surgery[63] and, to date, the theoretical concern that engrafted ADSC compromises oncological safety is unproven.[64,65] Of the two most recent systematic reviews of autologous fat grafting for post oncological breast reconstruction Groen et al identified 43 studies with a total of 6260 patients (of which 3020 patients contributed oncological data) revealing loco-regional and distant recurrence of 2.5% and 2.0% respectively, volume retention of between 44 and 82%.[66] Agha et al identified 35 studies with a total of 3624 patients (of which 2428 patients contributed oncological data) revealing a mean recurrence rate of 4.4% at 2 years and an overall complication rate of 7.3%. The most common complication was fat necrosis in 4.6% of cases. They performed a meta-analysis of the oncological data, revealing no statistically increased likelihood of oncological recurrence with fat grafting. This study did not examine volume retention.[67] A prospective, multi-center trial of CAL for 68 partial mastectomy defects reported good contour reconstruction in 54 of 65 breasts on blinded

MRI despite radiotherapy and no oncological recurrences at 12 months.[68] Nonetheless, the concern that engrafted fat obfuscates radiological breast surveillance continues to be debated in the literature.[69,70]

CAL has also been used to demonstrate superior volume retention over standard lipo-injection in facial filling for craniofacial microsomia[71] Parry-Romberg[72] and lupus erythematosus[73] and/or long-term maintenance of volume with one session of lipo-filling commensurate with between one and three sessions for standard lipo-filling.[74] In this study the facial soft tissue defect was varied and included, in the addition to the above, Barraquer-Simons Syndrome and trauma. Increased cell survival is thought to be due to angiogenesis induced by growth factors expressed by cells within the SVF[73] but also by immuno-regulatory mechanisms.[75] In a murine model of systemic lupus erythematosus (SLE), the injection of allogeneic ADSC reduced systemic expression of IL-17 and IL-6. It was observed that a consequent reduction in inflammatory cell infiltration of the renal interstitium reduced renal damage.[76] ADSC have been shown to downregulate pro-inflammatory, IL-17-expressing Th17 lymphocytes *in-vitro*,[77] cells that play a critical role in the pathogenesis of SLE. ADSC and BM-MSC have also been shown to inhibit T cell induction indirectly in the presence of antigen-presenting cells, with upregulated expression of anti-inflammatory cytokines being one of the proposed mechanisms.[78,79] Furthermore, there is some evidence to suggest that MSC from different sources inhibit T cell proliferation by different mechanisms (T cell apoptosis or cell cycle arrest) which varies on the secretome.[80] MSC also inhibit maturation and activation of B cells[81] and natural killer (NK) cells.[82]

The addition of platelet-rich plasma (PRP) to ADSC or SVF has also been advocated as a means of improving cell survival following lipo-injection. When a specimen of fat-infiltrated tissue is cross-sectioned and examined at a cellular level, the specimen may be reasonably divided into three concentric zones; the peripheral zone, the regenerating zone and the central zone. The peripheral zone acquires its nutritional requirements from adjacent tissue. The central zone is unable to do so and undergoes necrosis. The regenerating zone undergoes necrosis of mature adipocytes which are then regenerated by ADSC, which exhibit enhanced survival capacity in such conditions. PRP is hypothesized to enhance localized neo-angiogenesis and stimulate ADSC proliferation and differentiation in the regenerative zone. However, with evidence for[83] and against, in both the preclinical[84] and clinical[85] settings, it remains unclear whether there is any clinical role for PRP in the context of ADSC tissue engineering. Regardless of the evidence, the use of PRP in aesthetic practice continues unabated.

Skin Rejuvenation and Wound Healing

A number of studies have demonstrated that the subcutaneous injection of ADSC promoted collagen synthesis and angiogenesis in mice.[86,87] In a murine model of UVB-induced rhytids, subcutaneous injection of ADSC improved the appearance of the rhytids.[88] They further demonstrated that the conditioned medium of ADSC (containing the growth factors PDGF, VEGF, FGF, KGF, HGF and TGF-β1), when incubated with dermal fibroblasts, promoted fibroblastic migration, reversed UVB-induced fibroblast apoptosis, enhanced fibroblast synthesis of types 1 and 3 collagen and fibronectin and suppressed fibroblast synthesis of the collagenase, matrix metalloproteinase 1 (MMP1). Park and colleagues demonstrated the therapeutic utility of this approach by improving rhytids in a clinical pilot.[89] However, once more attempts to explain the influence of ADSC on skin quality have yielded evidence of multiple mechanistic effects. Kim et al demonstrated that ADSC-conditioned medium (ADSC-CM) inhibited melanogenesis in B16 melanoma cells, suggesting that ADSC might also play a role in preventing sun-induced melanosis.[88] Moreover, ADSC have been demonstrated to protect dermal fibroblasts against oxidative stress. ADSC-CM has been found to contain antioxidants including IL-6, Superoxide dismutasae (SOD2) and insulin-like growth factor-binding proteins. In an *in-vitro* model, this conditioned medium was protective against peroxide-induced apoptosis of cultured fibroblasts.[90] Derby and colleagues demonstrated that ADSC, when injected subcutaneously, migrated into the dermis and expressed the endothelial stem cell marker p63. They suggested that the effect of ADSC on skin was not only the result of growth factor expression, angiogenesis, paracrine cell recruitment or free radial scavenging, but also to trans-differentiation of ADSC to endothelial stem cells.[91]

The therapeutic utility of using ADSC for skin rejuvenation of the face has been investigated in a number of clinical studies. The use of SVF, with or without the addition of platelet-rich plasma (an additional source of growth factors and cytokines) promoted angiogenesis and inflammation in facial skin when injected subcutaneously.[92] "Nanofat" grafting (using an emulsified fat suspension delivered through a 27G needle) has been shown to improve the appearance of rhytids in the absence of viable adipocytes.[30] Here, the clinical efficacy was attributed to viable cells of the SVF. The topical application of ADSC-CM following CO_2 laser resurfacing improved blinded objective and subjective parameters when compared with laser resurfacing alone in a trial of atrophic acne scars.[93]

As collagen synthesis and angiogenesis are key features of ADSC-mediated dermal rejuvenation, some of the experimental evidence presented in the preceding section might just as readily be applied to wound healing. However, while they are paradigmatically similar, the inducing stimulus is different as is the aesthetic result. Mediated by toll-like receptor signaling, MSC appear to express either pro- or anti-inflammatory phenotypes which, in turn, influence wound healing by the resultant scar formation.[94] The balance of these opposing phenotypes is determined by the ligands

present in the extracellular milieu which is different (and perhaps less predictable) in the context of cellular destruction, bacterial ingress and localized ischemia associated with trauma and/or burns. In this context the potential for hypertrophic scarring is clear.[95,96]

An exhaustive list of the extensive preclinical experimental evidence for the use of ADSC in wound healing and scar management is beyond the scope of this paper. In a small study comparing CAL with traditional fat grafting for the management of facial scarring, contour restoration was maintained at 12 months in two-thirds of the CAL cohort but in just over one third of the lipo-aspirate cohort.[97] Similarly, Gentile et al observed scar regeneration and aesthetic improvement following "nanofat" injection (lipo-injection using a 27G or smaller cannula).[98] Of practical interest is recent work on the development of reliable delivery systems for ADSC to enhance wound healing. Promising experimental results have been obtained using stem cell-loaded hydrogels as dermal substitutes[99] with further refinements offered by manipulation of the material properties of the hydrogel.[100–102] Optimal biocompatibility and mechanical properties of the delivery system to be used are crucial and need to be selected according to the type of tissue to be bioengineered.

ADSC for Alopecia

In 2011, Festa et al reported evidence for the paracrine regulation of follicular stem cells by resident intradermal precursor cells of adipogenic lineage.[103] A number of pre-clinical studies subsequently provided evidence for the up-regulation of follicle cells by growth factors expressed by ADSC.[104,105] Thus, the use of ADSC or SVF has garnered interest in the management of alopecia areata with several small clinical studies claiming efficacy when ADSC-conditioned media (ADSC-CM) was applied to the scalp or when cellular isolates were injected into the scalp. In a study of 27 healthy females with mild (Ludwig type 1) alopecia, the application of ADSC-CM after micro-needling resulted in significant increases in both hair density and thickness.[106] In a study (retrospectively registered) of 20 healthy subjects with mild alopecia, injection of autologous SVF into the scalp improved hair follicle density, hair thickness (stand diameter) and reduced hair pullout.[107] A study of 9 patients (of which 5 patients were injected with a mix of purified adipose tissue with ADSC and completed adequate follow up) reported increased hair density and thickness after injection. Changes to the anagen/telogen balance was also sought but the numbers were too low to draw meaningful conclusions. Moreover, one of the authors is the chief medical officer of the parent company of Puregraft® and Kerastem® used in the study.[108] There are additional limitations to each of these studies and higher quality clinical evidence is needed to build on the promising pre-clinical evidence.

ADSC on Biological Scaffolds for Repair of Bony Defects

The conditions for *in-vivo* bone formation include osteo-progenitor cells, an osteo-conductive scaffold, osteogenic growth factors, angiogenesis and non-immunogenicity. Hence, the combination of ADSC on an osteo-conductive scaffold carrier with or without osteogenic growth factors is particularly promising as a means of repairing and regenerating critical size bone defects. Hyaluronic acid (HA),[109] atelocollagen,[110] Poly-L-glycolic acid (PLGA), b-tricalcium granules,[111] fibrin,[112] carboxymethylcellulose hydrogel[113] and even human bone allograft[114] have all been used as a biological scaffolds for ADSC and pre-adipocytes. The possible advantages of seeding ADSC onto osteo-conductive scaffolds for the repair of critical sized bone defects has been studied extensively; in some cases adeno- or retro-viral vectors have been used to induce expression of osteogenic or angiogenic growth factors in ADSC, but a review of this pre-clinical work is beyond the scope of this article. The utilization of human ADSC was examined using critical sized defects in immuno-incompetent animal models[112,115] and then clinical studies.[111,114,116] Critical size traumatic and surgical bone defects have been regenerated using autologous ADSC seeded on fibrin glue [116] and b-tricalcium with[117] and without[111,118] the addition of recombinant bone morphogenetic proteins (BMPs). Human bone allograft has also been used with ADSC and recombinant BMP-2 to reconstruct orbitozygomatic defects in Treacher-Collins syndrome.[114] A summary of the research for the use of ADSC on scaffold carriers for the management of critical sized craniofacial bony defects is provided by Griffin et al.[119] Exosomes are membrane vesicles of endocystic origin that engage in cell signaling through RNA, protein and lipid transfer [120]. Recently, exosomes have emerged as an alternative to whole cells as a means of paracrine signaling.[47,120] ADSC-derived exosomes, seeded on a PLGA scaffold have been shown to accelerate healing of a critical size murine calvarial defect.[121] The therapeutic promise of this approach is based on the fact that, as a tissue free system, it offers obvious commercial potential. Enhancing our knowledge of the mechanistic aspects of cell based therapy and the translational hurdles of commercializing the approach for the regeneration of bone defects has been reviewed by Grayson et al.[122]

ADSC on Biological Scaffolds for Repair of Cartilaginous Defects

There is a paucity of readily available, biomechanically suitable, autologous cartilage graft material for use in aesthetic and reconstructive surgery. An example of the need for such material is in secondary or reconstructive rhinoplasty where the dearth of septal cartilage necessitates the use of dissimilar autologous or synthetic materials.[123] While chondrogenic differentiation of MSC still presents several challenges (*in-vitro* chondrogenic differentiation is notoriously capricious) the potential of human ADSC for cartilage engineering was first demonstrated over 15 years ago. In this series of experiments ADSC were chondrogenically pre-differentiated *in-vitro* then suspended within

an alginate gel. The construct formed histologically mature cartilage in a subcutaneous pocket of an athymic mouse.[124] A number of additional pre-clinical studies using chondrogenically pre-differentiated ADSC, a variety of scaffold carriers and both articular (knee) and elastic (ear) cartilaginous defect models have added further confirmatory evidence to this concept.[125] While ADSC have been shown to differentiate into functional chondrocytes they may not do so as readily as BM-MSC *in vivo*.[126] With variable degrees of success reported in the literature, a clear picture of the potential of ADSC as a cellular resource in chondrogenic engineering remains unclear.

ADSC on Biological Scaffolds for the Repair of Neural Defects

ADSC seeded in fibrin nerve conduits have also been shown to exhibit the potential to induce nerve repair and regeneration. This effect has been attributed to the synthesis of neurotropic growth factors.[127] The clinical potential of this application is clear, offering the prospect of nerve repair without the donor site morbidity of a nerve graft and circumventing the need for *ex-vivo* Schwann cell expansion.[128] Studies investigating the use of ADSC with natural and synthetic conduits for peripheral nerve repair have been summarized elsewhere.[129]

The Limitations of the ADSC Therapeutics

Age-Related Functional Decline

The demand for aesthetic interventions is fueled largely by the ageing process and the interpretive effects of the ageing process on self-image and esteem. Like all cells, ADSC are subject to ageing. Stem cell renewal ability appears to decrease with ageing and is associated with down-regulation of SIRT-1 (silent information regulator 1) by micro-RNAs, with miR-486-5p proposed to play a role in ADSC replicative senescence.[44,130] Given a plethora of studies examining the effects of ageing on MSC, it is useful to briefly summarize the effect of ageing on cell differentiation, proliferation migration.

It has been demonstrated that ageing is associated with the downregulation of genes responsible for the maintenance of genomic integrity and chromatin remodeling. The loss of epigenetic integrity (heritable changes in gene function) leads to functional attenuation and the risk of neoplastic transformation.[131] Reduced telomerase activity (the enzyme counteracting loss of telomeres) and consequent telomere shortening, that results in cell dysfunction, is also a feature of the ageing stem cell.[132] The influence of age on the tri-lineage differentiation potential of MSC has been investigated, with some studies reporting a loss of osteogenic potential and enhanced

adipogenic potential.[133] This phenomenon may reflect differential growth factor expression in senescence, with reduced BMP2/4 and TGF-β expression (reviewed[134]). The clinical implications of differential growth factor expression in senescent ADSC are unclear and likely depend on the indication for ADSC use. Aged MSC also express higher levels of pro-inflammatory cytokines yet cytokine and chemokine receptor expression is attenuated, impairing the ability of these cells to respond to injury hence the localized need for repair and regeneration.[135]

The influence of extrinsic factors on aged ADSC is difficult to interpret in the context of known intrinsic changes. Attritional damage to nucleic acids by reactive oxygen species, UV and ionizing radiation and chemical carcinogens results in cumulative errors in the replicative process which manifest with age and affect cell function.

Tumorigenicity

ADSC express cytokines that promote angiogenesis, cell migration, proliferation, renewal and mesenchymal to epithelial trans-differentiation.[136] Moreover, as discussed earlier, ADSC are immuno-modulatory. This combination of features is potentially tumorigenic. As discussed in the section on breast lipofilling, the reports are conflicting. There is some evidence to link MSC with tumor activated (myo)fibroblasts; a key contributor to tumor aggression and invasiveness.[137] BM-MSC have been shown to increase the tumorigenicity and invasiveness of breast cancer cells by induced *de novo* expression of CCL5, a chemokine which acts in a paracrine manner to increase cell migration (*in-vitro*) and extravascular translocation (*in-vivo*).[138] Similarly, induced *de novo* expression of the chemokine CXCL-12 (SDF-1) has been reported in ADSC which increased tumorigenicity and invasiveness and was reversible by inhibition of the corresponding receptor.[139] By contrast, ADSC and ADSC-conditioned supernatant have been shown to induce necrosis (without evidence of apoptosis) in a range of tumor cell lines *in-vitro* and *in-vivo*.[140] A recent study designed to evaluate whether fat engraftment offers a supportive environment for tumor growth concluded, emphatically, that it does not and may even be suppressive.[141] This conclusion appears to be supported by reviews of clinical studies of fat grafting for oncological breast reconstruction.[65,66] In summary, the theoretical association remains disputed within the experimental literature and unproven clinically. It seems reasonable to conclude that the *in-vitro* and pre-clinical models using immuno-deficient mice are not clinically representative.

The Translational Hurdles of Ex-Vivo Manipulation

As cells replicate, mistakes happen. The imperfect nature of cell replication has been exploited by nature for the purpose of natural selection for millennia. However, while some mistakes are

harmless or confer unexpected advantages, others induce cells to override their intrinsic cell cycle control. Such malignant transformation is countered by immune surveillance strategies that are evaded when cells are cultured *ex-vivo*. Moreover, *in-vitro* treatment of the cells with growth factors, cytokines and chemokines to expand and differentiate them, can result in unanticipated changes in cell properties. A much-cited paper from 2005 reported sarcoma formation in immuno-deficient mice following the injection of ADSC, transformed by multiple *in-vitro* passages into post-senescence, chromosomally unstable cells. However, this was not particularly surprising and, importantly, the paper was retracted after failure to replicate some results.[142] The use of fetal calf serum in cell culture presents a risk from prion exposure[143] although this remains, for now, a theoretical concern.

CONCLUSIONS

ADSC show enormous therapeutic potential. They exhibit reliable mesenchymal lineage differentiation and are relatively easy to harvest, with an assured high yield. Cell harvest is achieved using lipo-aspirate which is technically straightforward, associated with minimal donor site morbidity and, as a popular aesthetic therapy in its own right, acceptable to patients. They are immuno-modulatory and there is evidence, however disputed, that they can induce tumor cell arrest and necrosis. Both these features might be channeled for therapeutic exploitation. While evidence on ADSC long-term safety is awaited, current tissue engineering strategies are focused not only on standardizing isolation protocols but on dispensing with the need for whole cell therapeutics by focusing on the exosome to develop biocompatible, non-immunogenic and commercial therapeutic options. Improving *in-vivo* cell homing and differentiation by optimizing the molecular signals within the localized recipient environment is another important goal. Such strategies will enable the translational gap to be bridged, turning today's furtive efforts of direct injection of the SVF and lipo-aspirates into tomorrow's predictable tissue engineering strategies.

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Table 1 Characterizing ADSC Through Cell Surface Markers

ADSC surface markers				
International Society for Cellular Therapy (ISCT) position statement markers for MSC[12]		Additional published markers reported for ADSC		Equivocal markers
Positive	Negative	Positive	Negative	(±)
CD73	*CD34*	*CD10*[13]	*CD133*[8]	*CD31*[6,14]
CD90	*CD45*	*CD13*[6,11,13]		*CD106*[8,13]
CD105	*CD11b or CD14*	*CD29*[6,8,11,14]		*CD146*[6,14]
(HLA-ABC)	*CD79α or CD19*	*CD44*[6,8,11]		
	(HLA-DR)	*CD166*[6,11,14]		

ADSC, adipose tissue-derived stem cells; CD, cluster differentiation; HLA, human leucocyte antigen; MSC, mesenchymal stem cells

Table 2 The Abbreviated ADSC Secretome[2,34,144]

Classification	Factor	Purpose
Growth factor	*VEGF, PDGF, TGF-β, b-FGF,*	Angiogenesis, cell proliferation
Cytokines (pro-inflammatory)	*TNF-α, IL-1β, IL-6, IL-12, IFN-γ*	Recruitment and activation of cells of innate and adaptive immunity, fibroblasts and MSC
Cytokines (anti-inflammatory)	*IL-10, IL-13, Prostaglandin E2*	Immunosuppression
Chemokines	*IL-8, MCP-1 (CCL-2), MIP-1α (CCL-3), MIP-1β (CCL-4)*	Promotes migration of cells of innate and adaptive immunity, fibroblasts and MSC
Adipokines	*Leptin, IGF-1, Adiponectin, steroid hormones, resistin, PAI-1*	Adipose tissue homeostasis
Matrix proteins	*Collagen-1*	ECM synthesis
Matrix protease	*MMP-1, MMP-2*	ECM remodeling; permits cell transit

VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor; TNF, tissue necrosis factor; IL, interleukin; IFN, interferon; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; CCL, chemokine ligand; IGF, insulin-like growth factor; PAI, plasminogen activator-inhibitor; ECM, extracellular matrix; MMP, matrix metalloproteinase

Figure Legends

Figure 1. Mesenchymal stem cell lineage. The figure has been truncated, demonstrating only the mesenchymal stem cell lineage. While the arrows suggest differentiation proceeds inexorably from totipotency to terminal differentiation, this is an oversimplification as de-differentiation[145,146] and trans-differentiation[147] have been observed.

Figure 2. Tri-lineage differentiation potential of ADSC. (A) ADSC stained with crystal violet to demonstrate cell morphology in culture. (B) Stained with alizarin red after culture in osteogenic media for 28 days. Bone nodules are clearly demonstrated. (C) Stained with oil red "O", following culture in adipogenic media for 21 days. Lipid droplets are clearly demonstrated. (D) Stained with alcian blue, following culture in chondrogenic media for 21-28 days. Foci of cartilage (stained blue) are demonstrated.

Figure 3. An example of a method for ADSC isolation. Fat may be acquired by a number of methods, the most common of which employs dry or tumescent-assisted liposuction, using a mechanical or ultrasound-assisted liposuction cannula. The fat sample is enzymatically digested at 37°C using collagenase or trypsin dissolved in Dulbecco's modified Eagle's medium (DMEM), before being filtered to remove undigested particles. The filtrate is essentially a heterogeneous cell suspension which is then centrifuged (to enable the enzymatic solution to be discarded) and the cell pellet (stromal vascular fraction) is re-suspended in culture medium such as DMEM with bovine calf serum, L-glutamine and 1% penicillin/streptomycin before plating on plastic. As ADSC adhere to the plastic dish a further culture cycle removes most of the non-ADSC cellular component of the SVF.

Figure 1.

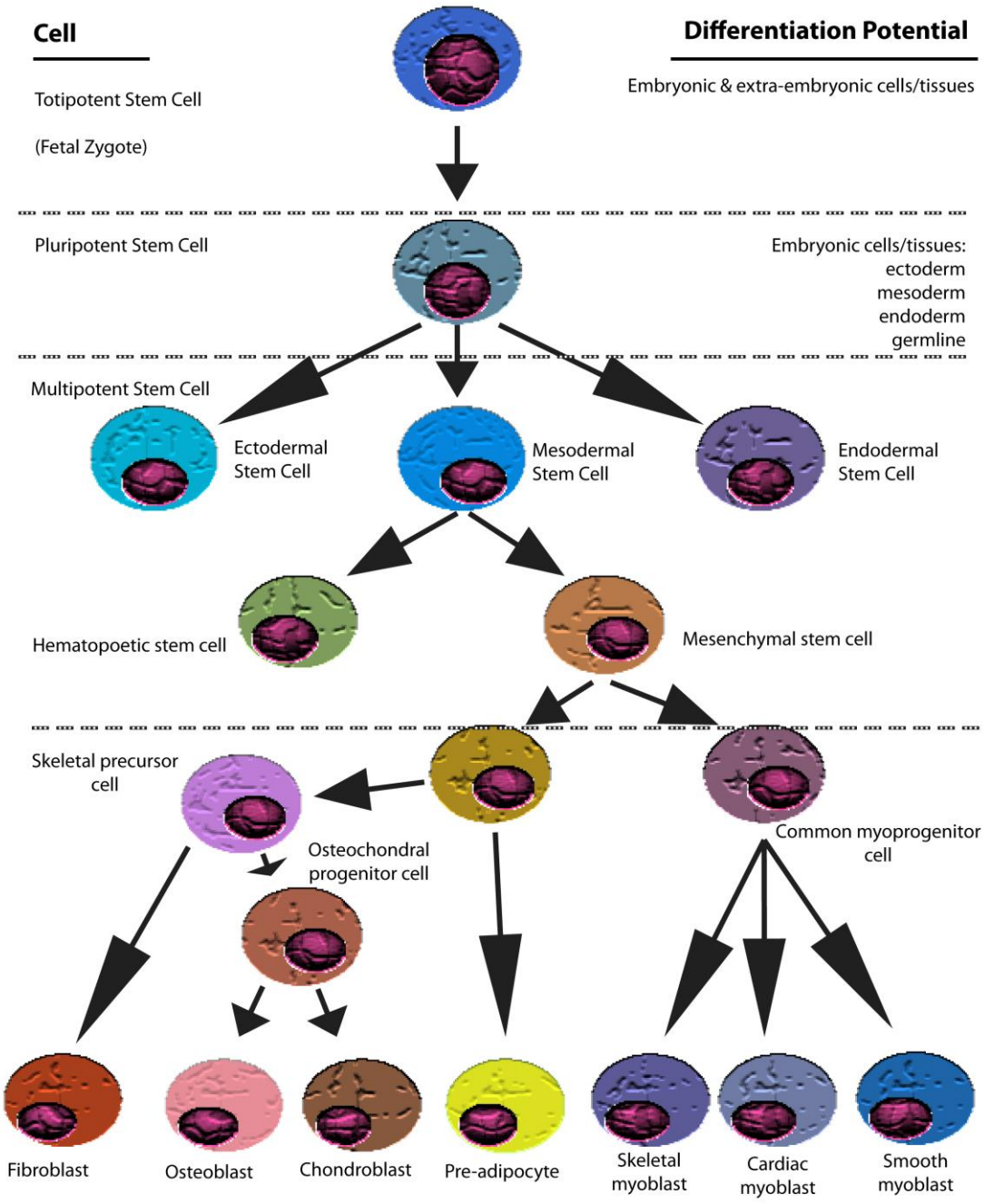

Figure 2a.

Figure 2b.

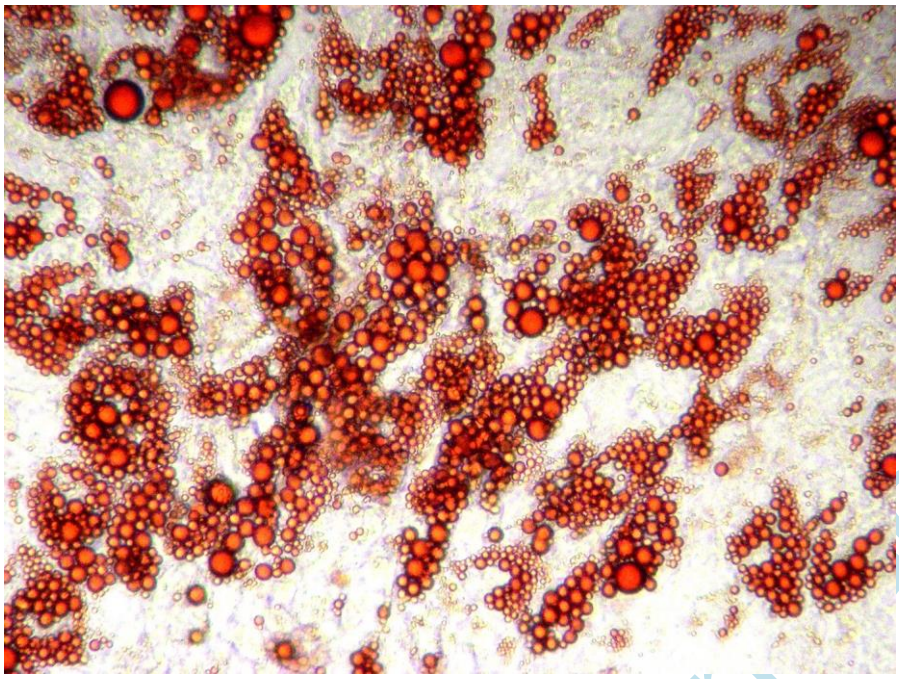

Figure 2c.

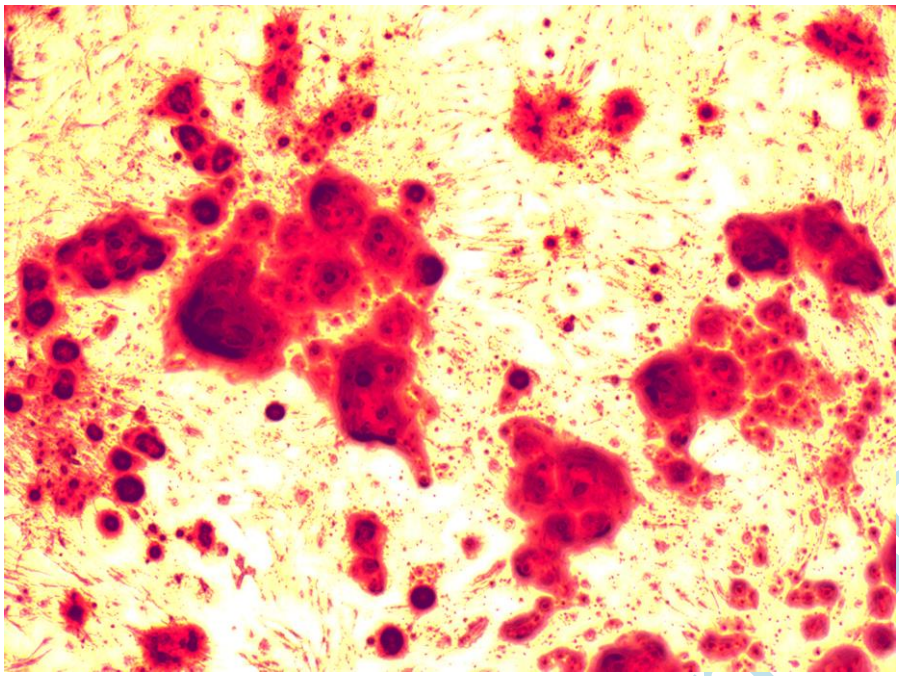

Figure 2d.

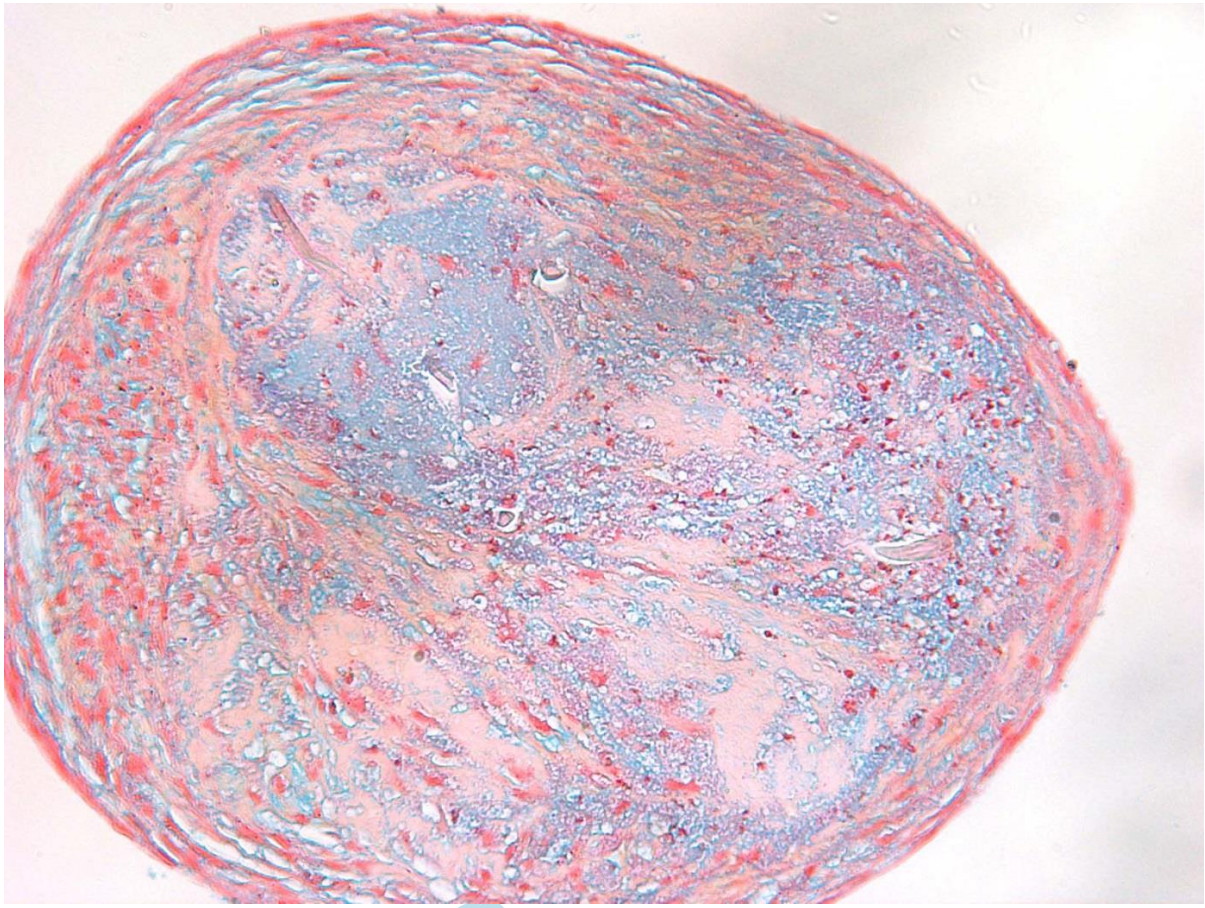

Figure 3.