

REVIEW

Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells

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Abstract

Adipose tissue is now recognized as an accessible, abundant, and reliable site for the isolation of adult stem cells suitable for tissue engineering and regenerative medicine applications. The past decade has witnessed an explosion of preclinical data relating to the isolation, characterization, cryopreservation, differentiation, and transplantation of freshly isolated stromal vascular fraction cells and adherent, culture-expanded, adipose-derived stromal/stem cells *in vitro* and in animal models. This body of work has provided evidence supporting clinical translational applications of adipose-derived cells in safety and efficacy trials. The present article reviews the case reports and phase I–III clinical evidence using autologous adipose-derived cells that have been published, to date, in the fields of gastroenterology, neurology, orthopedics, reconstructive surgery, and related clinical disciplines. Future directions and challenges facing the field are discussed and evaluated.

During the past decade, the body of literature relating to the isolation, characterization, differentiation, expansion, immunophenotype, immunomodulatory properties, and preclinical use of SVF cells and ASCs has increased dramatically (reviewed in [1–4]). These studies, conducted throughout the globe, have provided evidence on the safety and efficacy of SVF cells and ASCs *in vivo* using animal models. With these reproducible and independently generated data, regulatory authorities in multiple countries have permitted a limited number of clinical trials involving SVF cells and ASCs to move forward. The present concise review will focus on publications relating to these ongoing clinical trials, their outcomes, and the future clinical translation of adipose-derived, cell-based tissue engineering.

Background

Both ASCs and SVF cells have been used in preclinical models to treat acute and chronic diseases afflicting a range of tissues and organs as summarized in Table 1; this summary is by no means a complete survey of this extensive and emerging body of literature. The majority of preclinical studies using ASCs and SVF cells have been conducted in rodent models due to their size, cost, availability of antibody probes, and access to inbred, genetically modified, and transgenic strains. A small, but growing, number of experiments have sought to evaluate the impact of adipose-derived cells in large animal disease models; however, these studies in the dog, goat, horse, pig, rabbit, or sheep have been hampered by the lack of appropriate monoclonal antibody reagents for cell tracking and immunophenotypic characterization. Nevertheless, the data accumulated have been sufficient to allow clinical investigators to apply for regulatory approval of clinical trials in many countries around the world.

Before beginning clinical trials, it has been necessary to develop current good manufacturing practices for the production of clinical-grade human SVF cells and ASCs. The Food and Drug Administration [5], the European Medicines Agency [6], and other national regulatory authorities have developed guidelines for industry specifically for adult cell production that are generally considered biological products as opposed to devices or

Introduction/overview

Subcutaneous human adipose tissue is an abundant and accessible cell source for applications in tissue engineering and regenerative medicine. Investigators have explored methods to extract stromal/stem cells from the waste products of abdominoplasty, cosmetic surgery, and tumescent liposuction procedures. Routinely, the adipose tissue is digested with collagenase or related lytic enzymes to release a heterogeneous population of stromal vascular fraction (SVF) cells. The SVF cells can be used directly or can be cultured in plastic ware to select and expand an adherent population known as adipose-derived stromal/stem cells (ASCs).

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Table 1. Representative preclinical animal studies using adipose-derived stromal/stem cells and stromal vascular fraction cells

Tissue type	Defect	Species	References
Adipose/soft tissue	Fat pad generation	Murine, rat, ovine	[51-60]
	Lipodystrophy	Murine	[48,61]
	Burn/radiation trauma	Murine	[62-64]
Bone	Critical sized defect		
	Craniofacial	Murine, rat	[65,66]
	Long bone	Murine	[67]
Cardiac	Spinal fusion	Rat	[68,69]
	Myocardial infarction	Murine, rat	[42,43,70,71]
		Murine, rat	[44,46,72]
Central nervous system	Vascular injury/stroke	Rat	[47]
	Spinal cord trauma	Murine	[33]
	Multiple sclerosis		
Gastrointestinal tract	Crohn's disease/inflammatory bowel syndrome	Murine	[73]
Hematopoiesis	Bone marrow transplantation	Murine	[74,75]
Joint	Osteoarthritis	Canine, caprine, equine	[76-79]
Liver	Acute toxicity/regeneration	Murine	[80-88]
Pancreas	Type 1 diabetes mellitus	Murine, rat	[48,89,90]
Renal	Acute ischemia	Rat	(JMG, unpublished)
Skin	Wounds, burns	Murine, porcine	[62,64,91]
Tendon	Tendonitis	Equine	[92]
Urinary bladder	Incontinence	Rat	[93]
Vascular	Hind limb ischemia	Murine, rat	[36,41,45]

drugs. These guidance documents continue to be revised and updated as new evidence and products are evaluated. In general, cell products are divided into those that are minimally manipulated (such as autologous unfractionated circulating blood cells) or those that more than minimally manipulated (culture-expanded allogeneic bone marrow mesenchymal stem cells). Therapies involving minimally manipulated cells can advance more rapidly to the clinic. Nevertheless, both products face similar oversight requirements with respect to testing for contaminants.

All products must be evaluated for aerobic and anaerobic bacteria, endotoxin, and mycoplasma. If the cells are used for allogeneic purposes, either tissue donors or the cells themselves must be screened for a panel of viral agents, including cytomegalovirus, Epstein-Barr virus, hepatitis A and C, and HIV. The manufacturing process must define the distinct biological characteristics of the cells, and their stability throughout the isolation, storage, and shipping process, and must provide quality assurance/control of the various components used. The manufacturing facility must meet rigorous standards with respect to its construction. The facility must monitor air handling, ambient temperature, humidity, water source and quality. The operating site itself must meet construction standards concerning

flooring and wall materials, sealing between surfaces, traffic flow between designated clean and dirty rooms, and maintenance of positive air pressure gradients between such spaces. To insure that the facility meets these standards, the operators are required to monitor particle counts per cubic meter of air on a routine basis and to maintain records certifying the continuous operation of the equipment within designated environmental parameters. Likewise, the scientific equipment, instruments, and materials need to meet similar standards. The operation of all biological safety cabinets, incubators, water baths, and related equipment must be certified at least annually. All standard operating procedures must be documented. These documents must provide a detailed description of all routine laboratory methods as well as those activities required to support the manufacturing space, such as cleaning of biological safety cabinets and incubators, mopping of floors, walls and ceilings, and receipt and storage of perishable and nonperishable materials. By design, standard operating procedures should be written in a format that allows anyone knowledgeable in the field to conduct the procedures independently, with minimal oversight. Needless to say, the costs to meet the requirements for current good manufacturing practice certification are substantial.

Table 2. Published clinical case reports and clinical trials

Indication	Study type	Number of patients	Follow-up period	References
Soft tissue				
Breast augmentation	Case reports	403	Up to 6 years	[9-11] (K Yoshimura, personal communication, University of Tokyo)
Craniofacial	Case reports	58	9 to 13 months	[10,21] (K Yoshimura, personal communication, University of Tokyo)
Irradiation fibrosis	Case reports	20	31 months	[18]
Orthopedic				
Craniofacial	Case reports	2	>12 months	[25,26]
Immune				
Crohn's disease	Phase I	5 to 9	12 to 30 months	[27-31]
Multiple sclerosis	Case reports	3	7 months	[33]

There is a demand to make minimally manipulated adipose-derived cell products directly available to the surgeon in the operating room. To meet this need, Cytori Therapeutics (San Diego, CA, USA) and Tissue Genesis (Honolulu, HI, USA) have designed and manufactured devices with a single, closed, disposable container that is used to harvest, to collagenase digest, to centrifuge, and to separate the lipoaspirate into mature adipocytes and SVF cells within a short time frame (1 hour or less) [7,8]. These SVF cells, which fulfill many if not all criteria for a minimally manipulated product, can then be reintroduced into the donor for cosmetic, reconstructive, or other purposes by the surgeon. Tissue-processing devices are now marketed in Europe and Asia, and are under regulatory review for distribution in the US. It will be important to monitor outcomes from procedures employing these devices in the next decade. Although these devices are designed to guarantee consistent and reproducible results, it remains to be determined whether the operator technique introduces any variability with respect to surgical procedures using SVF cells.

Published clinical study reports

Soft tissue cosmetic and reconstructive surgery

Soft tissue repair is a logical application – and, theoretically, the simplest application – for adipose-derived cell therapies since the isolated cells presumably do not need to display any transdifferentiation potential. Both ASCs and SVF cells have been approved and employed in clinical trials involving soft tissue defects (summarized along with other published clinical studies in Table 2). Breast reconstruction or augmentation trials have enrolled the greatest number of patients. Plastic surgeons at the University of Tokyo have reported pioneering results using SVF cells in combination with intact lipoaspirate tissues. Yoshimura and colleagues have combined SVF cells with lipoaspirates from equal volumes of tissue to augment breast volume [9-11]. In

their clinical population of >70 subjects, these authors note improved fat grafting in the presence of the SVF cells with retention of volume for >1 year without evidence of fibrosis or adhesions. Complications such as cyst formation or microcalcifications occur in less than 2 to 3% of patients. Clinical trial reports in abstract form conducted by surgeons in Spain, South Korea, and elsewhere suggest similar outcomes [12].

It will be necessary to monitor patients for an extended period postoperatively. Some clinicians have raised concerns that morphological changes resulting from SVF cell/fat graft implants will complicate mammographic surveillance for breast cancer in recipients [13]. In addition, multiple studies have demonstrated that ASCs release growth factors/cytokines that attenuate the innate immune response [14-16]. Because of this immunosuppressive function, it is possible that the introduction of ASCs into breast tissue will create a microenvironment that favors or promotes the growth of rare tumor cells [17]. Long-term studies will therefore be required to compare the incidence of breast cancer in SVF cell-implanted and ASC-implanted patients with the incidence in untreated controls.

Breast cancer patients often undergo radiation treatment to the axilla post mastectomy, and this can be complicated by progressive fibrotic lesions of the exposed skin and dermis, resulting in an impaired range of motion in the upper extremity. In pilot clinical studies, surgeons have noted significant improvements following subcutaneous autologous fat transplants [18]. Recipients with chronic open lesions of the skin prior to treatment healed over time, characterized by enhanced microcapillary growth at the histological level. The irradiated skin at the transplantation sites became more supple, allowing the patients to recover an increased range of motion in their arm. These changes have been attributed to the presence of SVF cells and ASCs in the fat graft. It is likely that similar approaches may benefit subjects with chronic

open wounds from other causes, such as decubitus ulcers in bedridden patients and diabetic patients.

While plastic surgeons have used autologous fat grafts for over a century, there is substantial variability between surgeons with respect to the success of these procedures [19,20]. The fat grafts often lose volume, undergo fibrosis, or lead to necrosis. The University of Tokyo group used SVF cell/fat graft transplantation to treat craniofacial lipodystrophic and cosmetic defects with a high degree of success [21]. This suggests that the presence of the SVF cells, possibly due to their release of cytokines, improves the autologous fat graft viability and implantation. This hypothesis may reflect the ability of the SVF cells/ASCs to improve angiogenesis/vascularity around the implant, to scavenge reactive oxygen species, and/or to enhance adipocyte progenitor survival, proliferation, and differentiation.

Orthopedics

The use of SVF cells and ASCs for bone repair has been a target for many investigators. There are close developmental links between adipose tissue and bone, and it has been postulated that an inverse or reciprocal relationship exists between adipogenesis and osteogenesis at the cellular level [22-24].

Investigators at the Universities of Tampere and Helsinki (Finland) published exciting clinical translational outcomes using autologous ASCs to repair a craniofacial defect [25]. The patient was a 68-year-old male who had lost part of the hard palate of the mouth following removal of a cyst. The resulting defect left the subject without the ability to chew or drink effectively. To address this defect, the surgical team harvested 200 g autologous subcutaneous adipose tissue, isolated and culture expanded the ASCs in autologous serum, and seeded them in the presence of bone morphogenetic protein 2 onto a β -tricalcium phosphate scaffold fashioned into the shape of the lesion/defect. The ASC/scaffold construct was implanted into the patient's rectus abdominis muscle and followed for evidence of mineralization radiographically over an 8-month period. At that time, the construct was resected and transplanted to the maxillofacial defect, where its intact epigastric artery blood supply was re-anastomosed to the facial artery. Subsequently, the ASC/scaffold implant integrated into the bone site with the formation of a normal oral epithelium and achieved sufficient structural stability to allow for the implantation of prosthetic teeth. The patient recovered full oral function and remained complication free for at least 1 year.

This case represents the first peer-reviewed report of SVF cells or ASCs employed for the complete clinical repair of a craniofacial or orthopedic defect [25]. Since this case report, the surgical team has conducted similar

surgeries on >20 subjects with >90% positive outcomes. This initial approach has used ASCs for bone repair in a nonweight-bearing site. It will be necessary to develop techniques that reduce the number of invasive surgeries required for each procedure. In this context, a case report was published in 2004 describing the repair of a chronic calvarial defect in a single operation using autologous ASCs in combination with an autologous bone graft and fibrin glue; the reproducibility of this surgical approach needs to be validated [26]. Furthermore, it remains to be determined whether satisfactory outcomes can be achieved using ASCs or SVF cells in the repair of critical-sized defects in long bones.

Immune disease

Crohn's disease is an inflammatory bowel disorder characterized by bloody stools, diarrhea, weight loss, and autoimmune-related symptoms. A particularly troubling complication for many patients is the development of fistulas between the bowel and the genitourinary tract. The fistulas display a poor rate of healing and often recur. Clinicians at the Autonomous University of Madrid have explored both autologous SVF cell-based and autologous ASC-based therapies for fistula repair [27-32]. Their approach has been to inject 50% of their recovered cells 2 mm under the epithelium along the fistula track. The remaining cells are then combined with fibrin glue and used to fill in the fistula directly. In multiple publications reporting on phase I trials involving four or five patients each, the Madrid team was able to heal 75% of chronic fistulas using culture-expanded ASCs. In a single trial of four patients, however, they only achieved a 25% repair of fistulas with nonexpanded SVF cells. They postulated that this might be related to the difference between the reported immunogenic properties of ASCs and SVF cells. While ASCs display immunosuppressive features and low expression of immunoreactive surface antigens *in vitro*, SVF cells lack any immunosuppressive function and stimulate T-cell proliferation in parallel assays. In light of the autoimmune etiology of Crohn's disease, these immunomodulatory features could account for the relative success or failure of the two adipose-derived cell populations.

Multiple sclerosis is a progressive inflammatory disease affecting the myelinated cells of the central nervous system. Over time, this disease leads to degenerative changes and loss of cognitive, motor and sensory function; often, these changes occur in a waxing and waning manner. While the etiology of the disease remains in question, there is compelling evidence supporting a role for an autoimmune component. This has been supported by a preclinical model of the disease known as experimental autoimmune encephalitis, whereby a multiple sclerosis-like symptomatology is induced by injection of

animals with myelin basic protein, proteolipid protein, or myelin oligodendrocyte glycoprotein [33]. Based on this autoimmune association, clinical investigators in Costa Rica have begun phase I safety trials treating multiple sclerosis patients with intravenous administration of autologous SVF cells [34]. In their initial report, the investigators provide case reports on three individuals who each received two intravenous infusions, each of 25 to 75 million SVF cells. All patients tolerated the treatment without complications, and some experienced an improvement in symptoms.

These preliminary trials need to be followed rigorously with appropriate controls to insure both safety and efficacy. It remains to be determined whether the immunomodulatory functional differences between SVF cells and ASCs have relevance. It is possible that ASCs, which show evidence of immunosuppressive function, may be capable of suppressing the inflammatory features of multiple sclerosis more effectively than SVF cells. Additional preclinical modeling may help to address this issue.

Future directions and challenges

Peripheral vascular disease provides a promising target for adipose-derived cell therapies. Pioneering preclinical studies simultaneously published independently by groups in Indianapolis, Toulouse, and Frankfurt – and later confirmed by others in Japan and Korea – have demonstrated the protective effects of both SVF cells and ASCs against acute vascular injuries [35-41]. Using rodent hind-limb ischemia models, these investigators unequivocally demonstrated that intravenous infusion or intramuscular injection of adipose-derived cells accelerated recovery of blood supply to the damaged tissues and prevented necrosis over a 1-week period. The investigators concluded that production of the angiogenic/vasculogenic cytokines hepatocyte growth factor and vascular endothelial growth factor accounted, in part, for the mechanism underlying the ASC and/or SVF cell actions [37,40]. These preclinical studies suggest that ASCs and SVF cells could be used to treat claudication and chronic vascular insufficiency in the lower extremities, such as that seen in patients with diabetes and related small-vessel diseases. Consequently, doctors at academic medical centers have begun to submit protocols to their national regulatory authorities for translational clinical trials; however, to date, no phase I safety or efficacy results have been published.

Acute diseases

Since the adipose-derived cell secretome contains angiogenic and vasculogenic cytokines with regenerative and neurotrophic properties, there has been considerable interest in testing the impact of ASCs and SVF cells on

acute disorders. Transient ischemic injury underlies the pathophysiology of myocardial infarction, stroke, renal ischemia, obstruction of the small intestine by intussusception or volvulus, and related conditions associated with trauma to the brain, spinal cord, and extremities. Because these conditions develop rapidly, it follows that there is a narrow window of opportunity following the ischemic event to attenuate any pathological consequences. Theoretically, this window of time may close as reactive oxygen species are generated when postischemic reperfusion is established. In preclinical animal myocardial infarction models, ASC treatment shortly after the event improves ventricular function as compared with untreated controls or experimental groups where treatment was delayed [42,43]. In stroke models, ASC infusion during the immediate postischemic period accelerated recovery of motor function in rats [44-46]. Additional studies have explored the regenerative capacity of ASCs in models of spinal cord injury [47]. Nevertheless, while phase I trials using ASCs or SVF cells have been registered for each of these clinical diseases, outcome data have been limited to abstract presentations at national and international meetings.

Chronic diseases

Theoretically, acute diseases present situations where rapid intervention can most efficiently prevent the development of long-term pathologies. In contrast, chronic diseases may be less amenable to cell-based therapies. Preclinical animal models suggest that adult stem cells of bone marrow origin can be used to treat type 1 diabetes mellitus, chronic heart failure, and neurodegenerative diseases due to inborn errors of metabolism (Krabbe's disease, among others). Since these chronic diseases account for the majority of healthcare costs in most national economies, there is interest in using ASCs and SVF cells for their treatment.

There is particular interest in the use of ASCs and adipose transplantation therapy for lipodystrophy and associated metabolic disorders such as diabetes [48]. A single group in India has reported improved insulin sensitivity in group of five diabetic patients co-infused with ASCs and unfractionated cultured bone marrow cells [49]; however, the majority of studies have been limited to animal models. For example, our group has begun to explore the use of ASC therapy in murine and nonhuman primate models of Krabbe's disease (BA Bunnell and JM Gimble, unpublished data). After such small and large preclinical animal model data supporting the safety and efficacy of these approaches are reviewed, it will be possible to begin testing adipose-derived cells in these important human conditions. A positive outcome in a single disease alone could have substantial economic and public health benefits.

Conclusions: opportunities or roadblocks?

The application of ASCs and SVF cells is still in its infancy and the field has made progressive advances towards clinical applications. In general, the clinical investigators who have published their findings have taken pains to address the important regulatory questions before advancing from animal models to patients. These researchers are to be applauded for treating the regulatory guidelines not as roadblocks but as opportunities for documenting the safety of their cell-based products. Still, there have not been many published safety studies addressing the legitimate concerns of regulatory authorities that include the following questions.

Can human ASCs and SVF cell implants cause tumors either directly through transformation or indirectly by promoting the growth of endogenous tumor cells? At least one manuscript has reported that human ASCs expanded long term *in vitro* will cause sarcomas when implanted *in vivo* in an immunodeficient mouse [50].

Has a current good manufacturing practice scheme of manufacture been developed for the cell products? While this information must be available to support existing clinical trials, details of standard operating protocols have not been made public. International standardization of ASCs and SVF cells would significantly advance clinical translation. Published definition of the cell identity with a panel of surface antigens and establishment of universally applied quality assurance/quality control testing criteria would benefit the fledgling adipose stem cell community.

As discussed above, there is no shortage of diseases – both acute and chronic – where adipose-derived cell therapies could have a potential clinical impact. The next steps will include documenting the reproducibility of the current preclinical and clinical findings, and controlled testing of the safety and efficacy of ASCs and SVF cells in a range of human conditions. In another decade, we postulate that the cells will prove beneficial to some, but not all, of the disease models tested. We expect initial successes to come in applications relating to acute disorders; however, we are hopeful that chronic diseases amenable to SVF cell therapy or ASC therapy will also be identified.

Abbreviations

ASC, adipose-derived stromal/stem cell; SVF, stromal vascular fraction.

Competing interests

JMG has consulted for and collaborated with a number of companies pursuing ASC-related technologies, including Cognate Bioservices, Toucan Capital, Vesta Therapeutics, Vet-Stem, and Zen-Bio. He has co-founded two companies exploring the tissue engineering and regenerative medical applications of ASCs: Artecel Sciences and LaCell, LLC. FG is the founder of Cytx Therapeutics, Inc. BAB has no competing interests to declare.

Author contributions

JMG prepared the outline and wrote the initial draft of the manuscript. BAB and FG edited and revised both the outline and the draft manuscript, providing additional references and insights.

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