Banking of Adipose- and Cord Tissue-Derived Stem Cells: Technical and Regulatory Issues

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Abstract

Stem cells are found in all multicellular organisms and are defined as cells that can differentiate into specialized mature cells as well as divide to produce more stem cells. Mesenchymal stem cells (MSC) were among the first stem cell types to be utilized for regenerative medicine. Although initially isolated from bone marrow, based on ease and costs of procurement, MSC derived from adipose tissue (AT-MSC) and umbilical cord tissue (CT-MSC) are now preferred stem cell sources for these applications. Both adipose tissues and cord tissue present unique problems for biobanking however, in that these are whole tissues, not cellular suspensions. Although the tissues could be processed to facilitate the biobanking process, by doing so additional regulatory issues arise that must be addressed. This review will discuss the technical issues associated with biobanking of these tissues, as well as regulatory concerns when banking of utilizing MSC derived from these sources in the clinic.

Keywords

Stem cells • Cord tissue • Adipose tissue • FDA • Regulations • Biobanking • Regenerative medicine

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Abbreviations

MSCs	Mesenchymal stem cells
AT-MSC	Adipose tissue-MSC
CT-MSC	Cord tissue-MSC
BM	Bone marrow
MS	Multiple sclerosis
TNC	Total nucleated cells
SVF	Stromal vascular fraction
IND	Investigational new drug
BB	Obstetrician

12.1 Introduction

Stem cells are found in all multicellular organisms and are defined as cells that can differentiate into specialized mature cells as well as divide to produce more stem cells. Stem cells can be divided into embryonic/fetal stem cells, and adult stem cells, based on their origin. Stem cells can be further classified as totipotental capable of giving rise to all tissues in the organism including the organism itself; as pluripotential able to give rise to multiple lineages of tissues and cells from different germ lineages; as multipotential which can give rise to different cell types generally within the same germ lineage; or as progenitor cells which only give rise to more lineage-restricted cells and tissues from a single germ layer origin. This appreciation for the numbers, types and potentials of stem cells has given rise to the fields of regenerative medicine and tissue engineering which encompasses a variety of cellular therapies. Mesenchymal stem cells (MSC) were among the first stem cell types to be utilized for such applications. Initially, all MSC were bone marrow (BM) derived. However, based on the ease and costs of procurement, as well as the possibility of obtaining autologous MSC from most patients using these sources, MSC derived from adipose tissue (AT-MSC) and umbilical cord tissue (CT-MSC) are now beginning to replace BM as the preferred source of clinically applicable MSC. In fact, as of March 2016 there were 176 clinical studies listed on www.clinicaltrials.gov for AT-MSC and an additional 342 studies listed for CT-MSC, with thousands of patients having received such treatments. These studies encompassed such clinical applications as osteoarthritis, orthopedic reconstructions, autism, multiple sclerosis (MS), spinal cord injury, diabetes, wound healing, cardiovascular disease and pulmonary disease.

Work done over the past decade has demonstrated that subcutaneous adipose tissue (AT) is the richest source of MSC in the human body, containing 100–1000× more MSC/g or cc of tissue that either BM or CT [1, 2]. In fact, as many as 1 % of all total nucleated cells (TNC) contained within AT may be MSC [1, 2]. However, AT is a tissue, not a cellular suspension, posing unique constraints on its use. In fact, lipoaspirate, which is often the starting point for AT-MSC collection, is a viscous gelatinous tissue that is difficult to manipulate, even at room temperatures (see Fig. 12.1). Although the lipoaspirate can be digested and processed, such a procedure would necessarily require imposition of additional and somewhat onerous regulatory guidelines (see below). The physical characteristics of the biospecimen also present unique requirements when it comes time to thaw, wash and clinically use the banked sample. Although MSC in general have long been studied as a clinically relevant source of stem cells, and have been extensively studied in multiple regenerative medicine and tissue engineering applications [3–5], it was not until recently that the umbilical cord (specifically the Wharton's jelly contained within the tissue; CT) itself was recognized as an economical and readily available source of large numbers of MSC [6, 7]. Similar to adipose tissues, CT presents unique problems for biobanking in that it is a whole tissue, not a cellular suspension (see Fig. 12.2). Although the tissue could be processed to facilitate the biobanking process, by doing so additional regulatory issues arise that must be addressed. However, as one of the youngest sources of MSC available its inclusion into any stem cell banking program is worthwhile.

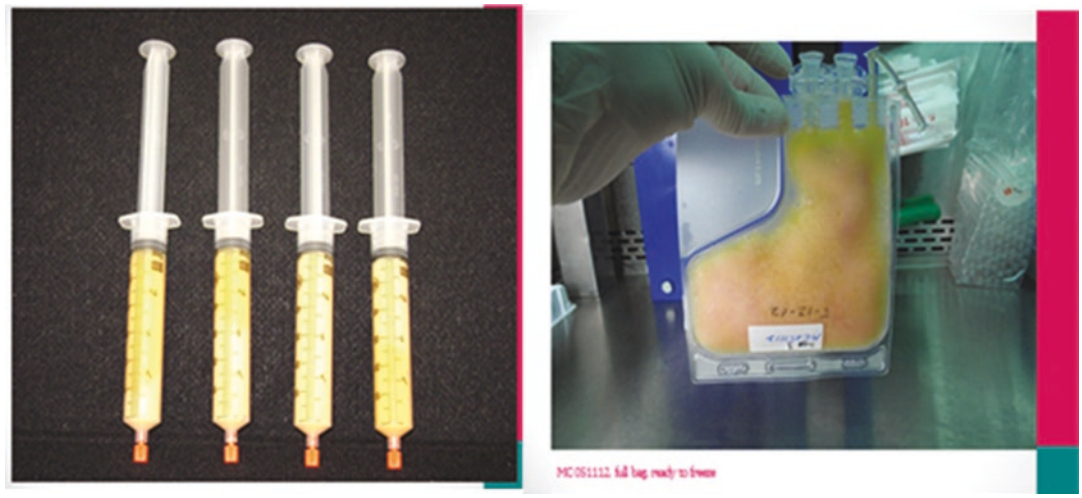

Fig. 12.1 Adipose tissue collection (*left*) and storage (*right*) using 60 cc syringes and 60 cc bags, respectively. Note the gelatinous nature of the stem cell source

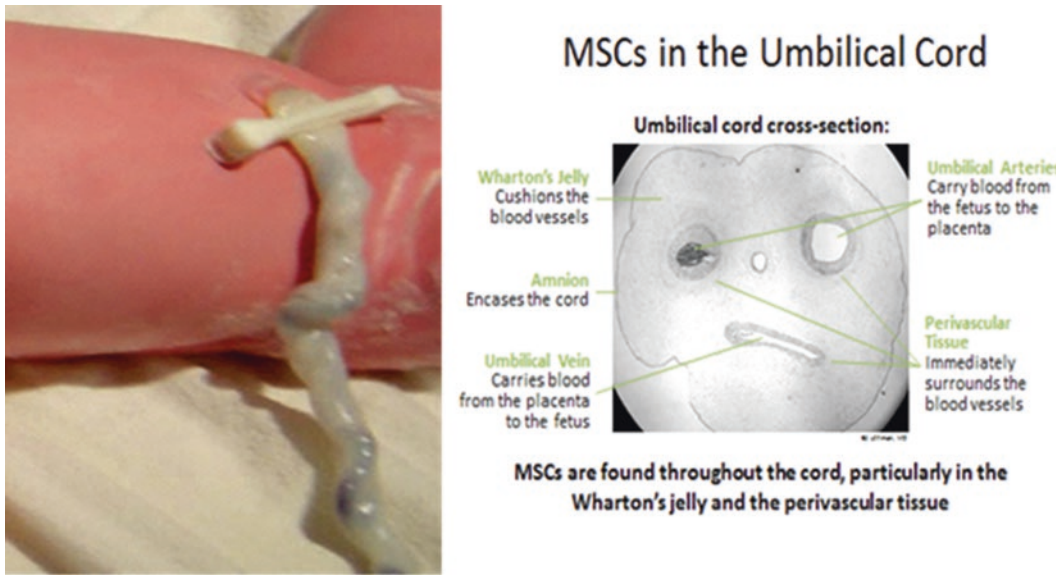

Fig. 12.2 The umbilical cord of a newborn (*left*) and a cross-sectional representation of the anatomy (*right*) demonstrating the unique structure of the stem cell source

12.2 Technical Issues in Tissue Stem Cell Banking

Whenever possible it is always preferable to utilize a closed and sterile system for stem cell collection and processing, which simplifies and/or eliminates many regulatory requirements. If the stem cell collection device and the cell processing device can be combined into a single system design, then the methodological approach becomes more attractive to the end-user and more likely to be utilized. As most stem cell collections (and eventually their utilizations) will be performed by clinicians who may or may not have extensive experience in stem cell collection or cell manipulations, the employed systems should

be easy to use without extensive previous training. Although most collection systems are generally sterile, the commercially available options are constrained by the particulars of the stem cell source.

Neither CT nor AT are single cell solutions similar to blood that are easily manipulated and banked. Rather, both AT and CT are intact tissues that require extensive manipulation to obtain/retrieve the MSC contained within the tissue. This observation is true whether the tissues are to be used immediately in the clinic (e.g., point of care use) or banked for future use. These constraints and the caveats discussed below should be remembered carefully in light of marketing efforts by numerous commercial entities that entreat parent and patients to pay for AT-MSC and CT-MSC biobanking with the promise of having viable and potent stem cells available in the future whenever needed. Not everything is as simple as it looks, or as good a deal as it might appear. Many of these advertised approaches will not result in clinically utilizable stem cell products if ever needed.

12.3 Adipose-Derived Mesenchymal Stem Cells

Adipose tissue may be collected either as a by-product of a liposuction procedure (under general anesthesia), or as an independent stand-alone procedure (using local anesthetics such as lidocaine). Both approaches utilize syringes (either large 60 cc syringes that can connect to a liposuction canister or small 20 cc syringes with a cannula for the manual procedure). Both collection systems can be considered "closed". The syringes can also be sterilely connected in a closed fashion to large (commercially available) bags for processing and storage (depending on volumes desired). As an add-on to a liposuction procedure it is possible to obtain several liters of lipoaspirate (although most adipose banks collect and store no more than 2000 cc). As a stand-alone procedure done under local anesthetic, harvests are normally no larger than 20–100 cc.

AT-MSC have unique processing requirements in that the MSC are not obtained from a cellular suspension like BM, but are contained within a fairly viscous tissue composed of multiple cell types. Initial processing (often called "enhancement") removes as much tumescent fluid as possible along with any contaminating blood via the use of several low speed centrifugations (300×g). This process leaves the adipose tissue containing the MSC amenable for immediate cryopreservation or for clinical use, or as a source of MSC after enzymatic digestion. However, in each of these examples it can be processed sterilely in a closed system using either manual or automated methods. Methodology has been developed that allows for economical, closed system processing that meets FDA requirements for minimal manipulation utilizing modified syringes [8]. Automated approaches to processing can be found with companies such as BioSafe, GDP Inc., Cytori, TissueGenesis and American Cryostem. The automated approaches tend to be more expensive, requiring the purchase of machinery and/or expensive consumables. However, these approaches do increase throughput and are very reproducible. In addition, being a closed system these methodologies are compliant with (some) FDA regulations and minimize the risks of sample contamination. Generally, the machines have received a 510 k registration with the FDA for clinical use, and even for point-of-care applications, but none of the devices have received FDA approval for AT-MSC banking or later clinical applications after thawing at some time in the future. Each one of these systems is closed from start to finish, harvest to cryopreservation, including the thawing of the sample after storage. However, the major and significant difference between the manual systems and the automated systems is that the automated systems produce what is referred to as a stromal vascular fraction (SVF) after enzymatic digestion of the "enhanced fat" which by definition is "more than minimally manipulated" and requires an Investigational New Drug (IND) application from the FDA before clinical use (see www.fda.gov and guidance on adipose tissues). The manual system discussed in [8] only produces

"enhanced fat" that is minimally manipulated and can be immediately used for cosmetic and reconstructive purposes, or processed further under IND for regenerative medicine applications. Oftentimes, the SVF must be cultured in vitro to purify the AT-MSC before clinical use in order to remove the contaminating cell types. In vitro expansion (although not as often needed with AT-MSC as with other MSC sources) requires adherence to another and different set of FDA regulations. As AT contains the greatest number of MSC per gm or cc of tissue, and since most individuals have plenty of AT to harvest, it is generally more a matter of MSC purification or enrichment through in vitro culture than designed MSC expansion by culturing. A recent presentation has described a short term panning procedure that allows for almost 100 % enrichment with less than one to two cell doublings over the course of 3–5 days [9] that may be applicable to most clinical settings where MSC numbers are sufficient but non-MSC cells need to be removed. However, this methodology would still be considered a manipulation under FDA law requiring IND clearance before clinical use. It should be noted that AT, unlike CT as discussed below, is the only one of these two MSC sources that can be processed in a closed system, biobanked without manipulation (by FDA definitions), and later thawed with 80 % or greater cellular recovery for research or clinical use [8].

12.4 Umbilical Cord Tissue-Derived Mesenchymal Stem Cells

Collection of umbilical cord tissue (CT) is a recent development in the stem cell field (within the last 5 years or so). By its nature, CT is not sterile (at least the exterior which recently exited the birth canal and is generally covered with blood and mucus from the birth) and cannot be collected in a closed system, as its connected on one end to the baby and on the other end to the placenta. Normally, the physician, obstetrician (OB), or other caregiver will cut a 6–10 in. segment of the umbilical cord after birth of the child and ligation of the cord. The segment of umbilical cord is then placed into a sterile capped cup (e.g. sterile, screw-capped urine specimen cup) that contains a transport buffer (nutrients as well as antibiotics and antifungals). Care must be taken to insure that the CT is not exposed to excessive air and is kept "wet" (i.e., submerged) during transport.

Due to the nature by which CT is harvested and its structural composition, CT must be processed in an "open" fashion. Generally this means that it is either stored as whole minced tissue or the CT is enzymatically processed to its cellular components before use or banking (this latter approach would be classified as "more than minimally manipulated" for regulatory purposes). Therefore, extensive sterility testing (bacterial, fungal and mycobacterial) is necessary, as is testing of the donor for a variety of viral infections. The stem cells of interest, the MSC, lie within the cord tissue either as perivascular cells or contained within the Wharton's jelly [3, 4], and this anatomical distribution must be taken into consideration when processing and banking the stem cells. If one attempts to cryopreserve the whole, intact unprocessed tissue the MSC will be damaged and will not survive the procedure. Thus, one must either finely mince the tissue before cryopreservation or enzymatically digest the tissue and then freeze the isolated MSC as is typically done for a single cell suspension [6, 7]. Finely minced tissue requires the slow infusion of DMSO over prolonged periods of time to insure homogenous distribution of the cryoprotectant throughout the tissue [10]. The finely minced tissue may also be used for explant cultures although the time required to obtain sufficient numbers of MSC for use can be several weeks or more. Isolated MSC obtained from an enzymatic digest (or from in vitro expansion) can be frozen as for any single cell suspension. Two problems present themselves however, with either methodology. If one enzymatically digests the cord tissue before cryopreservation it is now considered more than minimally manipulated by the FDA, requiring an IND prior to clinical use.

Cryopreserved whole, minced CT meets the regulatory definition of minimal manipulation, but one generally only recovers approx. 10 % of starting MSC population, requiring extensive ex vivo expansion prior to clinical use (again requiring an IND prior to clinical utilization), as compared to 80 % or higher recoveries for frozen adipose tissues [11]. CT is problematic to thaw as the tissue itself serves as a "sink: for the cryoprotectant DMSO, making it difficult to thoroughly remove which results in a loss of viable cells. Thus, CT is a less than optimal stem cell source for clinical applications due to the regulatory oversight that is necessary for its clinical use.

Oftentimes thawing of whole minced CT results in few viable MSC being obtained, and many fewer than needed for most clinical applications [10]. However, thawed tissue can be grown in vitro as explants rather than digested with much more successful results. Unfortunately, in vitro culture and expansion is also considered a manipulation and requires extensive regulatory oversight along with an IND application. Although fresh CT can be easily digested with combinations of fairly benign enzymes (such as collagenase and hyaluronidase), the numbers of MSC obtained per gram of tissue is often quite low (often 50–100 times fewer MSC per gm of tissue for CT as compared to AT; [10, 11]). Thus, CT-MSC will most likely always require some in vitro expansion before clinical use, mandating federal oversight and need to obtain an IND.

12.5 Regulatory Issues

As with any type of cellular therapy, regenerative medicine application or tissue engineering procedure, there are always concerns and confusions about which guidelines mandated by the FDA (see 21CFR regulations) must be followed. Stem cell therapies are more in the spotlight these days than ever before with the seemingly nonstop construction of stem cell clinics and the rapid commercialization of this medical field (endeavor) by individuals that are neither qualified by training nor experience. Generally, the FDA is concerned with several basic issues such as sample identity (does the stem cell sample come from the person that is going to use it in an autologous setting, or is it suitably matched if unrelated?); sample purity (has it been adulterated by foreign substances which may be harmful or which may obliterate its usefulness?); the potency of the sample (is there a standard against which the sample can be compared to measure its potential biological function?); is the sample efficacious (will the sample have any beneficial effects after administration?), and is the sample free of exogenous disease causing organisms (i.e., what is the risk of disease transmission?). In order to address these issues and to determine the level of oversight required the FDA has instituted about 10 years ago two sets of guidelines termed "351" and "361" regulations. Generally the "361" guidelines are less onerous and sometimes apply to therapies carried out under the practice of medicine but often requiring an IRB approval. However, the "351" guidelines require an IND prior to clinical use (21 CFR Part 1271, human cells, tissues, and cellular and tissue-based products or HCT/Ps) in that cells and tissues (including stem cells) falling under these guidelines are considered "biological drugs". For a procedure to qualify under the "361" regulations one must use autologous tissues and cells, do no more than minimally manipulate the cells and tissues, and then use the cells and tissues in a homologous fashion. Under strict FDA interpretations, it is difficult to qualify under the less strenuous "361" guidelines.

The FDA's view on AT-MSC procedures has been clearly enunciated as can be found at (http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/cellularandgenetherapy/ucm427692.htm). However, there is surprisingly little guidance when it comes to CT-MSC. Autologous use of cells and tissues is fairly straightforward although sometimes the definition has been expanded to include first and second degree relatives (e.g., parents, siblings, aunts and uncles, and cousins). In terms of

AT-MSC the FDA defines "minimal manipulation" for structural tissue such as AT as "processing that does not alter the original relevant characteristics of the tissue relating to the tissue's utility for reconstruction, repair, or replacement". By definition then, enzymatically digested AT to generate SVF would be considered "more than minimally manipulated" and would require an IND. An IND is undesirable not just because of regulatory paperwork and inspections that accompany it, but also because non-academic establishments are not allowed to charge a profit for any cellular endeavor until it is proven safe and efficacious which may take up to a decade or more at an investment that is often in the hundreds of millions of dollars range. In addition, with regard to AT the federal guidelines have sometimes been interpreted to mean that MSC isolated from various anatomical locations throughout the body cannot be used at any other dissimilar location in the body, although there is no data to support that assumption [2]. In regard to CT these guidelines could be interpreted to mean that CT-MSC will always require an IND in that the umbilical cord and those MSC contained within only exist during gestation whose only purpose is to protect the fetal blood supply during pregnancy. Homologous use of cells and tissues seems to pose the greatest confusion for practitioners. A strict definition would imply use only in the same context as the cells and tissues were originally found. For example, AT-MSC used to reconstruct atrophied facial fat after chemotherapy or used to reconstruct structural tissue in a different part of the body or a limb would be homologous use. However, utilization of AT-MSC or CT-MSC for treatment of stroke, myocardial infarction, or Multiple Sclerosis most probably would be considered non-homologous use. Many stem cell clinics and physicians claim to be exempt from such guidelines under the practice of medicine. However, nothing could be further from reality, as the practice of medicine does not allow for the routine application of unproven or unsafe medical practices, particularly for profit. It should be noted that several such stem cell clinics have been closed and physicians have been arrested and their medical license suspended for continuing to perform such unauthorized and unproven practices, particularly when they are not qualified by training or experience (see for example, http://www.ipscell.com/2015/01/stem-humanexperiment/; http://www.nejm.org/doi/full/10.1056/NEJMp1504560 and http://www.ipscell.com/tag/celltex/). As an example, wouldn't you prefer a licensed professional to perform your liposuction and do you really want an everyday GP to be injecting your joints or spinal cord (see http://www.scripps.edu/friedlander/docs/Graefe's%20Arch%20Clin%20Exp%20Ophthalmol.pdf versus https://www.ipscell.com/2016/01/us-stem-cell-clinic-sued-for-injection-into-patients-eyes-landmark-case/ for example).

For some reason CT has really not been regulated or even discussed in this context at all despite a large and successful commercial endeavor on the part of numerous cord blood banks. Many consumers are misled by these evangelists, opportunists and charlatans to believe that their CT-MSC will survive banking and be immediately available for clinical use when needed in the future. Those banks freezing minced whole tissue will recover few cells upon thaw that require extensive expansion before use which will require weeks to months (see above and reference [11]). Those banks that digest the CT before banking in order to cryopreserve the isolated MSC may not be able to use the samples upon thaw due to improper methodology that runs afoul of regulations. In both cases the numbers of cells obtained is generally below clinically useful levels and will require expansion before use. Perhaps the FDA has concluded that since there will always be a requirement for in vitro expansion before clinical use they will wait to have regulatory oversight at that time. Or perhaps the cord blood banking industry is more influential than the AT-MSC industry (e.g., lobbying).

12.6 Conclusions

Stem cells are found in different locations throughout the body, with each anatomical site generally containing a mixture of stem cell types. However, the most frequently utilized sources due to ease of accessibility and reduced costs are those stem cells found in adipose tissue, bone marrow (similar to mobilized peripheral blood), umbilical cord blood, and umbilical cord tissue. Each of these stem cell sources has different requirements when it comes to collection, processing, cryopreservation and storage. Cord tissue and adipose tissue are unique in that both are (semi) solid tissues that require enzymatic digestion before the MSC contained within can be obtained, purified and stored or utilized. CT is a preferred MSC source in that it represents the youngest gestational source of MSC for regenerative medicine and tissue engineering, having demonstrated superior proliferative capacity to other MSC types. AT is a preferred source of MSC for many applications in that it generally can be obtained from all potential subjects (autologous) removing concerns of immune rejection, and AT is the richest source of MSC in the body with more than 500,000 MSC/g of tissue [2, 10, 11], eliminating the need to expand MSC before use, which can induce cellular senescence [12]. However, for both CT and AT the need to use enzymatic digestion to obtain the MSC prior to banking or utilization falls under the "more than minimally manipulated" category, even in an autologous setting, mandating FDA oversight and the need to obtain an IND approval before clinical use. This point cannot be emphasized or overstated enough in that although both CT and AT are sources of MSC with significant potential for use in regenerative medicine and tissue engineering, without adherence to the regulatory guidelines (including oversight and an understanding of proper methodology) that have been instated for such tissues and their clinical use, it is only a matter of time before a patient is injured or is killed. If that were to happen, the fields of regenerative medicine and tissue engineering could be set back years with patients in need not having access to lifesaving and life-altering medical advances.

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