Systematic Review

Umbilical Cord Tissue Offers the Greatest Number of Harvestable Mesenchymal Stem Cells for Research and Clinical Application: A Literature Review of Different Harvest Sites

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Purpose: Recent years have seen dramatic increases in the techniques used to harvest and isolate human mesenchymal stem cells. As the potential therapeutic aspects of these cells further develop, informative data on the differences in yields between tissue harvest sites and methods will become increasingly valuable. We collected and compared data on cell yields from multiple tissue harvest sites to provide insight into the varying levels of mesenchymal stem cells by tissue and offer primary and alternative tissue types for harvest and clinical application. **Methods:** The PubMed and Medline databases were searched for articles relating to the harvest, isolation, and quantification of human mesenchymal stem cells. Selected articles were analyzed for relevant data, which were categorized according to tissue site and, if possible, standardized to facilitate comparison between sites. **Results:** Human mesenchymal stem cell levels in tissue varied widely according to tissue site and harvest method. Yields for adipose tissue ranged from 4,737 cells/mL of tissue to 1,550,000 cells/mL of tissue. Yields for bone marrow ranged from 1 to 30 cells/mL to 317,400 cells/mL. Yields for umbilical cord tissue ranged from 10,000 cells/mL to 4,700,000 cells/cm of umbilical cord. Secondary tissue harvest sites such as placental tissue and synovium yielded results ranging from 1,000 cells/mL to 30,000 cells/mL. **Conclusions:** Variations in allogeneic mesenchymal stem cell harvest levels from human tissues reflect the evolving nature of the field, patient demographic characteristics, and differences in harvest and isolation techniques. At present, Wharton's jelly tissue yields the highest concentration of allogeneic mesenchymal stem cells whereas adipose tissue yields the highest levels of autologous mesenchymal stem cells per milliliter of tissue. **Clinical Relevance:** This comparison of stem cell levels from the literature offers a primer and guide for harvesting mesenchymal stem cells. Larger mesenchymal stem cell yields are more desirable for research and clinical application.

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Recent advances in stem cell technology have begun to realize the therapeutic regenerative potential of mesenchymal stem cells (MSCs).[1,2] As new experiments are performed in various fields of medicine, more and more physicians may be able to improve disease outcomes through the use of MSCs. In orthopaedic surgery, MSCs may present a unique opportunity to decrease recovery time[3] and reduce morbidity rates[4] among patients. Disorders such as osteoarthritis,[5-7] ligament and tendon repair,[8-10] and bone union[11] may all benefit from the therapeutic application of MSCs in humans. As evidence of the benefits of these procedures grows, more surgeons will look to provide cellular treatments to their patients. At present, there are 502 active human clinical trials involving the therapeutic use of MSCs,[12] a number that is only expected to increase.

With these innovations have come new developments for the harvesting and characterization of MSCs. Advances over the past several years have yielded promising avenues for collecting MSCs for potential surgical applications. As the technology for these applications develops, a direct comparison of the qualities, tissue harvest sites, and yields for different sources of MSCs will become valuable to treating surgeons.

To this end, we reviewed the established literature on MSC sources from different tissue harvest sites for human MSCs. The purpose of this study was to provide a

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consensus of opinion for the best site and tissue type for MSC harvest through review of established literature. We hypothesized that, despite wide variations in yields between anatomic sites and harvest techniques, placental tissue yields the greatest, most easily accessible quantity of MSCs for research or clinical application.

Methods

Search

For this study, the PubMed and Medline databases were used to conduct a comprehensive search of journal articles related to the qualities, classes, and harvest of human MSCs (Fig 1). The search terms used were as follows: placental stem cell, adipose stem cell, bone marrow mesenchymal stem cell, umbilical cord mesenchymal stem cell, amniotic stem cell, chorionic stem cell, mesenchymal stem cell isolation, mesenchymal stem cell harvest, progenitor cell harvest, and mesenchymal stem cell quantification. These searched terms yielded 25,063 results. Among these results, articles without the keywords "human" and "harvest" were excluded, yielding 1,075 articles. These articles were evaluated for quality and relevance to this study, after which 161 articles were selected for more detailed analysis. The bibliographies of these 161 articles were also searched for relevant publications, ultimately yielding 29 articles for review. In addition, the ClinicalTrials.gov database was reviewed for relevant clinical trials involving the use of human MSCs.

Eligibility Criteria and Data Extraction

This search was limited to articles published in the English language up to December 31, 2014. Relevant articles for this survey were studied, and their bibliographies were searched and evaluated for relevant data concerning MSC harvest. Articles were analyzed for information on different classes of MSCs, cell surface markers, and tissue harvest sites, as well as quantification of cells at these specific harvest sites, with relevant articles selected for this review based on inclusion of MSC harvest data. These data were organized by different harvest sites and tissue types to be presented in a clear and direct format.

Results

The results of this literature review yielded 4 major tissue sources of MSCs as defined by tissue localization, as well as multiple subclasses. These broad classes were placental tissue derived, adipose derived, bone marrow derived, and umbilical cord derived. The synovial membrane, peripheral blood, umbilical cord blood, periosteum, muscle, and trabecular bone have been studied as sources of MSCs, but comparative data are much less common. The results of this review are summarized in Table 1.

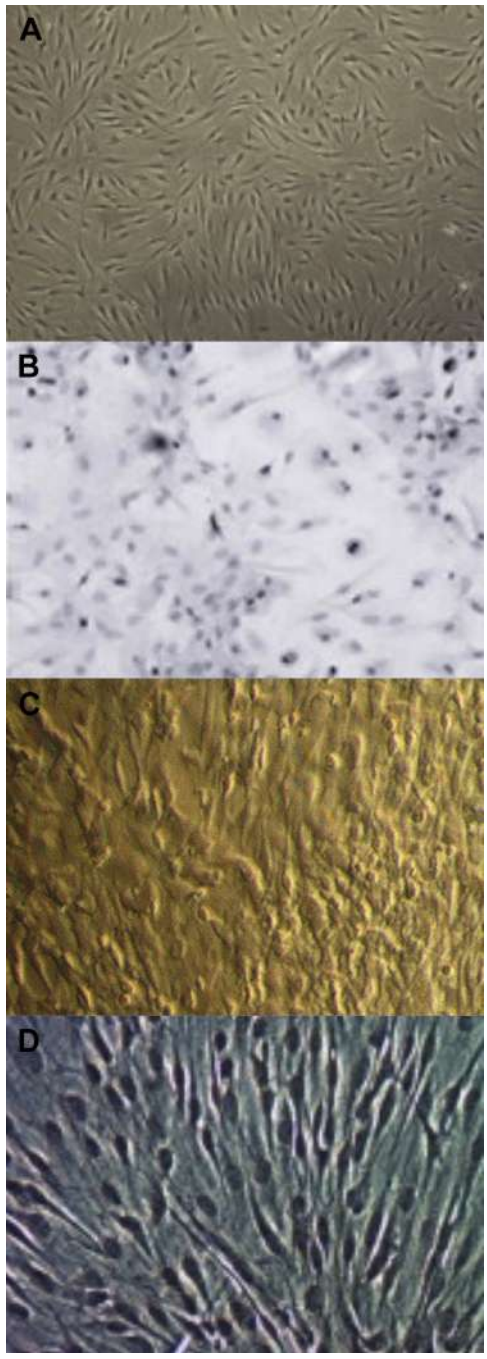

Fig 1. Comparative histological slides of various Human mesenchymal stem cell populations from various tissues. (A) Adipose tissue mesenchymal stem cell shown in inverted phase microscopy (magnification unknown; reprinted with permission[56]). (B) Bone marrow mesenchymal stem cells. (×10 magnification; reprinted with permission[57]). (C) Umbilical cord tissue mesenchymal stem cells shown in inverted phase (×200 magnification; reprinted with permission[58]). (D) Synovial tissue mesenchymal stem cells shown in alizarin red S stain (×200 magnification; reprinted with permission[59]).

Adipose

Adipose tissue harvest by reviewed studies relied primarily on a lipoaspiration technique to isolate

Table 1. Reported Mesenchymal Stem Cell Yields From Various Harvest Sites

Authors	Tissue Type	Tissue Site	Reported Level	Converted Level: Cells per Milliliter of Tissue
Raposio et al.[44]	Adipose	Unknown	5.0×10^5 cells/80 mL adipose tissue	6,250 cells/mL
Minonzio et al.[45]	Adipose	Unknown	587,753 cells/75.3 g adipose tissue	7,395 cells/mL
Oedayrajsingh-Varma et al.[46]	Adipose	Abdomen, hip, thigh	6.3 ± 1.8% of harvested adipose SVF (mean ± SEM)	18,334-61,398 cells/mL
von Heimburg et al.[47]	Adipose	Unknown	80,000 to 350,000 cells/g adipose tissue	75,800-331,625 cells/mL
Policha et al.[48]	Adipose	Abdomen	259,345 ± 15,441 cells/g adipose tissue (mean ± SEM)	245,729 ± 14,630 cells/mL
Gruber et al.[49]	Adipose	Abdomen	471,000 cells/mL of adipose tissue	471,000 cells/mL
Aust et al.[50]	Adipose	Abdomen	404,000 ± 206,000 cells/mL lipoaspirate (mean ± SD)	404,000 cells/mL
Mitchell et al.[29]	Adipose	Unknown	308,849 nucleated cells/mL of lipoaspirate	19,303 cells/mL
Yoshimura et al.[14]	Adipose	Unknown	$1.31 \pm 0.5 \times 10^9$ and $1.55 \pm 0.79 \times 10^9$/L adipose tissue (mean ± SEM)	1,310,000 cells/mL and 1,550,000 cells/mL
Zhu et al.[36]	Adipose	Unknown	500,000 cells/1.5 mL of adipose tissue	333,333 cells/mL
Yu et al.[51]	Adipose	Unknown	$375 \pm 142 \times 10^3$/mL of lipoaspirate (mean ± SD)	375,000 cells/mL
Strem et al.[13]	Adipose	Unknown	5,000/g of adipose tissue	4,737.5 cells/mL
De Ugarte et al.[16]	Adipose	Unknown	2×10^5/g of adipose	189,500 cells/mL
De Ugarte et al.[16]	Bone marrow	Hip	3×10^5/g	317,400 cells/mL
Wexler et al.[33]	Bone marrow	Unknown	1 in 3.4×10^4 nucleated cells	
Hernigou et al.[32]	Bone marrow	Anterior iliac crest	612 ± 134 cells/mL of bone marrow (mean ± SD)	612 cells/mL
Hernigou et al.[52]	Bone marrow	Iliac crest	84 to 7,581 cells/mL	84 to 7,581 cells/mL
Pierini et al.[34]	Bone marrow	Posterior iliac crest	$269.3 \pm 185.1/10^6$ mononuclear cells (mean ± SD)	3,606.94 cells/mL
Pierini et al.[34]	Bone marrow	Anterior iliac crest	$166 \pm 133.8/10^6$ mononuclear cells (mean ± SD)	1,942.72 cells/mL
de Girolamo et al.[6]	Bone marrow	Iliac crest	0.04% of cells	
de Girolamo et al.[6]	Bone marrow	Subchondral knee	0.02% of cells	
Sakaguchi et al.[15]	Bone marrow	Tibia	$1:10^5$ to $1:10^6$ nucleated cells	1-30 cells/mL
Sakaguchi et al.[21]	Trabecular bone	Tibia	Approximately $1:10^3$ to $1:10^5$ nucleated cells	1,000-100,000 cells/g
Sakaguchi et al.[21]	Periosteum	Tibia	Approximately $1:10^2$ nucleated cells	30,000 cells/g
Sakaguchi et al.[21]	Synovium	Medial knee	Approximately $1:10^2$ nucleated cells	30,000 cells/g
Sakaguchi et al.[21]	Muscle	Semitendinosus muscle	Approximately $1:10^2$ nucleated cells	20,000 cells/g
Bongso and Fong[18]	UC	Wharton's jelly	4.7×10^6/cm of UC	
Tsagias et al.[53]	UC	Wharton's Jelly	0.65×10^6/cm of cord	
Chatzistamatiou et al.[54]	UC	Wharton's Jelly	$1.75 \times 10^5 \pm 0.94 \times 10^5 - 3.02 \times 10^5 \pm 0.66 \times 10^5$ cells/cm (mean ± SD)	
Karahuseyinoglu et al.[17]	UC	Wharton's jelly	10×10^3/cm of UC	
Weiss et al.[35]	UC	Wharton's jelly	1.5×10^4/cm UC	
Fu et al.[55]	UC	Wharton's jelly	50×10^3/cm of UC	
Lu et al.[21]	UC	Cord blood	Approximately $1:10^3$ to $1:10^4$ nucleated cells	
Kim et al.[18]	UC	Wharton's Jelly	$6.4 \pm 3.2 \times 10^4$ /g wet tissue (mean ± SEM)	1,000 cells/mL
Kim et al.[19]	Placental tissue	Chorion	$4.5 \pm 2.7 \times 10^4$/g of wet tissue	
Zvaifler et al.[20]	Blood	Peripheral	Approximately $1:10^3$ to $1:10^4$ nucleated cells	1-40 cells/mL

NOTE. Values were reported in mL when reported in mL in the literature, or when accepted densities were available for conversion to mL. Values reported in grams of cm of tissue which could not be converted were reported in their original units.

SVF, stromal vascular fraction; UC, umbilical cord.

adipose tissue, and unprocessed lipoaspirate and simple adipose tissue were evaluated as equivalent substances. Levels for adipose-derived MSCs ranged from 4,737.5 MSCs/mL of lipoaspirate[13] to 1,550,000 MSCs/mL of lipoaspirate[14] (Table 1, Fig 1).

Bone Marrow

Bone marrow tissue harvest was primarily conducted through repeated aspirations through large-bore needles, ranging from 15- to 18-gauge sizes.[15] Levels for bone marrow–derived MSCs ranged from 1 to 30 MSCs/mL[15] to 317,400 cells/mL[16] (Table 1, Fig 1).

Umbilical Cord and Placental Tissue

Placental tissue– and umbilical cord–derived MSCs proved unique in their diverse harvest and tissue-specific harvest sites. Tissue cell levels for Wharton's jelly (umbilical cord connective tissue) ranged from 10,000 MSCs/mL of umbilical cord[17] to 4,700,000 MSCs/cm of umbilical cord.[18] Chorionic tissue cell levels were reported to be 45,000 MSCs/g of wet tissue (Fig 1).[19]

Peripheral Tissue

Peripheral blood, which was collected through peripheral blood draw and centrifugation, was reported to have MSC levels of 1 to 40 cells/mL.[20] Muscle tissue was harvested from the semitendinosus tendon, which was collected with a tendon stripper.[21] Similarly, periosteum tissue was collected from the tibial insertion of the same harvested semitendinosus tendon.[21] Synovial tissue was harvested during arthroscopic surgery from the medial joint capsule of the knee using a pituitary rongeur.[21]

Discussion

The advancement of stem cell transplant techniques over recent years has made the practical acquisition of these cells increasingly worthwhile for the purpose of reconstructive surgery. Autologous sources represent the most current, cost-efficient, and least controversial option to acquire and transplant MSCs in the clinical setting. Physicians and researchers exploring this emerging field will require resources concisely explaining the most efficient sites for MSC harvest, as well as the levels of cells available in different tissues. Determining the best and most consistent tissue source of human MSCs, as well as the cell levels typically harvested from related sites, offers a valuable resource for future clinical studies.

Given the diverse array of units used to report cell harvest levels among selected studies, values were converted to a standard measurement to allow direct comparison between studies and tissues. For adipose values, a common value for the density of adipose tissue was selected from previous studies as 0.9475 g/mL[22] to convert values from grams to milliliters. The standard density for bone marrow used for conversions was determined to be 1.058 g/mL.[23] Values from studies that did not include volume or mass data for bone marrow harvest could not be reported in milliliters and, consequently, were reported with MSCs as a percentage of total nucleated cells. Similar concerns arose in the reporting of umbilical cord tissue. Values from studies that did not include mass or volume data were instead recorded by length of cord and could not be converted.

Autograft Tissue and Minimal Manipulation

Comparisons of yields between placental and autograft tissue invite clarification of the practical difference between autograph and allograph transplantation, as well as minimally manipulated tissues. Allograft tissue rarely presents with immune complications after transplantation. The lack of the human leukocyte antigen–A surface antigen confers an immune-privileged nature to placental tissue, allowing for comparable use of the 2 tissues without immune-modifying therapy.[24] Consequently, for the purposes of clinical use and this review, allograft placental tissue is comparable with autograft cells.

According to US Food and Drug Administration (FDA) regulations, only cellular products classified as "361 tissue" may be exempt from premarket review and regulation. Classification as 361 tissue requires cells to be "minimally manipulated," a criterion that excludes many common techniques used to harvest, isolate, and purify MSCs today. It should be noted that adipose tissue currently harvested for MSCs requires multistep processing, including enzymatic digestion, purification, and expansion in culture, which is considered more than "minimal manipulation," thereby excluding them from 361 cellular tissue classification by the FDA.[25] However, recent procedural and technologic advances have demonstrated efficient, non-enzymatic purification of human MSCs from lipoaspirate.[26] Further, recent studies have shown mechanically purified adipose-derived MSCs demonstrate greater pluripotent response compared to enzymatically isolated adipose stem cells.[27] Given recent FDA approval for marketing of this system and subsequent "361 cellular tissue" classification, the field of adipose-derived stem cells and their clinical application may greatly expand in the coming years. In addition, a 2013 update by the FDA Tissue Reference Group clarified that bone marrow MSCs, when expanded in culture, did not fall under the classification of 361 cells.[28] Consequently, the advancement of the field and therapeutic application of MSCs will likely rely on the ability to harvest cells in quantities suitable for implantation without digestion and expansion. A detailed understanding of the anatomic sites and tissue types yielding the highest levels and concentrations of cells by volume will prove crucial to these initial steps.

Technologic developments to further purify MSCs from harvest tissue without the use of expansion in culture will allow researchers to rapidly expand both the academic and clinical applications of these cells. Indeed, novel "non-manipulating" measures to efficiently extract MSCs from adipose tissue are currently being explored,[29] which will likely allow for the circumvention of 361 regulations for clinical study and application.

Our results indicate significant differences in the quantity and consistency of stem cell levels between adipose, bone marrow, and placental tissues. Studies performing harvest and isolation of MSCs from adipose tissue consistently showed higher cell yields than with MSCs from bone marrow and placental tissue. Furthermore, variations in harvest levels between different studies of the same tissue indicate notable differences. The highest reported yield for studies on adipose tissue showed an over 300-fold increase in cell harvest over the lowest reported values.[13,14] Bone marrow studies showed an over 1,000-fold increase between the highest and lowest reported yields.[15,16] This large variation must be noted.

Quantification of Cells

Pertinent to the analysis of cell yields from various tissues is the methods by which yields were quantified. Cellular quantification techniques proved relatively homogeneous across both tissue subtype and anatomic site. The primary method of cell harvest quantification was a limited-dilution colony-forming unit assay. Tissues were harvested and homogenized by serial centrifugation and suspension in liquid media according to techniques and concentrations specific to each anatomic site. Purification of MSCs was performed by serial replacement of cellular growth media and subsequent disposal of nonadherent cells using the innate cellular adhesion properties of MSCs.[30] Rough cell densities in liquid media were determined using cell counters and hemocytometers, after which cells were plated at densities ranging from 10^3 cells per plate[20] to 10^6 cells per plate.[31] After growth of fibroblast colonies, cells were stained and counted using light microscopy. Studies conducted by Mitchell et al.,[31] Wexler et al.,[32] Hernigou et al.,[33] Pierini et al.,[34] Sakaguchi et al.,[21] Weiss et al.,[35] and Lu et al.[22] all used the limited-dilution fibroblast colony-forming unit assay. Among these studies, notable variables included the time allowed for colony growth, which varied from 7 to 14 days; the number of cells determined to define a "colony," which ranged from 20 cells per colony[31] to 50 cells per colony[33]; and the number of serial dilutions conducted beforehand to purify the cells. Because the anatomic tissue source of each cell type necessitates different methods of initial preparation, comparison of homogenization and serial dilution is impractical, and this variable should be noted. Alternative quantification methods used serial dilution and cellular adherence, followed immediately by cell quantification using cell counters. This technique was used by Zhu et al.,[36] De Ugarte et al.,[16] Yoshimura et al.,[14] and Zvaifler et al.[20] Finally, de Girolamo et al.[6] used flow cytometry to quantify cellular harvest levels, incubating cells with commercial anti-CD45 and anti-CD271 antibodies after serial dilution and purification using cellular adherence. Differences in quantification are likely to yield significant variations in harvest levels. As shown by Cuthbert et al.,[37] Jones et al.,[38] and Tormin et al.,[39] roughly 1 in 17 CD271-positive cells yield a fibroblast colony during colony-forming unit assay. Although these potential differences did not influence our conclusions, in the future, consideration must be given to the method of cellular quantification.

Variations in Yields

Differences in yields among tissue sites are likely a result of 2 principal factors: harvest techniques and patient demographic characteristics. Adipose tissue–derived MSC yields have been shown to be only minimally affected by age differences among patients.[40] Given the multistep process of harvesting and isolating adipose tissue–derived MSCs, differences in yields may be principally a consequence of variations in harvest techniques. Procedural variations in enzymatic digestion, buffer selection, and centrifugation can all have significant impacts on MSC yields.[41] Despite this, analysis of our results indicates that in addition to higher levels of cells, adipose tissue maintains decidedly greater consistency in stem cell density as compared with alternative primary harvest sites. We believe this consistency results from both the more homogeneous nature of the tissue as compared with bone marrow and, paradoxically, the more procedurally involved manner of its harvest. The complex nature of MSC harvest from adipose tissue necessitates following or adapting proven procedures. Consequently, large mechanical differences in harvesting which lead to variations in yield, such as marrow aspiration technique, were largely eliminated. Concurrently, smaller differences were increased through the introduction of variations in enzyme and buffer concentrations.

Bone marrow–derived MSC yields showed significant variation likely because of differences in both the anatomic harvest site and patient demographic characteristics. Studies by Pierini et al.[34] and de Girolamo et al.[6] showed up to a 2-fold differences in yields between various marrow sites in the body. In particular, Pierini et al. concluded that the posterior iliac crest was the optimal harvest site for MSCs, above both the anterior iliac crest and the subchondral knee. Furthermore, evidence has shown that the use of the iliac crest as a harvest site, a common site in our review, predisposes harvest samples to significant dilution by

peripheral blood,[37] resulting in both depressed values and increased variation in harvest yields. In addition, increased age, particularly among women, has been shown to have a significant impact on bone marrow–derived MSC harvest yields, with numerous studies having shown bone marrow–derived MSC yields to decrease with age.[42,43] Because selected studies used a diverse range of donor ages, decreased yields compared with other studies are likely affected by increasing donor age and should be considered by physicians planning future MSC harvests. Finally, these data on harvest numbers of MSCs by anatomic site and tissue type offer no predictive information about the cellular activity of the individual MSCs. Differences in stem cell biology between and among these tissue sources must be evaluated in the laboratory and clinic as we proceed with this new field of biology.

Limitations

Limitations to our study primarily concerned issues of tissue comparability and scope of the initial search. As mentioned previously, conversion to common units (milliliters) for direct comparison of tissues was dependent on the existence of accepted values for tissue density. Consequently, umbilical cord stromal tissues, as well as certain reported bone marrow values, could not be converted to common units for direct comparison.

Initial development of the search criteria excluded articles that had not been translated into English. In addition, articles that were not accessible through the PubMed or Medline databases were excluded from our initial search. Although bibliographies of initially selected articles were evaluated for relevant publications and data, this limitation must be acknowledged.

Conclusions

Large variations in cell harvest yields remain for each major tissue site for MSCs as reported in the literature to date. Reviewed research supports the understanding that placental tissue provides the highest concentration of cells whereas adipose tissue offers the highest levels of autologous cells. Consequently, considerations must be made regarding the non-autologous nature of umbilical cord–derived stem cells, as well as the increased post-harvest processing required for adipose-derived stem cells, for the purposes of research and clinical application.

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