Cell and Tissue Transplantation and Therapy

SHORT COMMENTARY

Cryopreservation and Cell Banking for Autologous Mesenchymal Stem Cell-Based Therapies

Adrian Harel

BrainStorm Cell Therapeutics, Petach Tikva, Israel. Corresponding author email: aharel@brainstorm-cell.com

Abstract: As cell-based therapies begin to progress through Phase III clinical trials, there is an increasing need for the development of comprehensive cell banking strategies. In order to achieve commercial viability, both autologous and allogeneic approaches must have a comprehensive, end-to-end cell banking model—including proper collection, manufacturing and release criteria, cryopreservation and storage of cells, shipping, delivery, and logistics management of the final cell product. By developing an understanding of industry standards and best practices across these areas, companies can be better positioned to reduce research costs, improve efficiencies, create revenue streams, decrease time to discovery and, ideally, increase the likelihood and number of approved marketed products. The focus of this paper will be on cell banking strategies for autologous-based cell therapies with mesenchymal stromal cells, which are the most widely used cell type in cell therapy clinical trials today.

Keywords: cryopreservation, cell banking, autologous, mesenchymal stem cells, cell therapy

Cell & Tissue Transplantation & Therapy 2013:5 1–7

doi: 10.4137/CTTT.S11249

This article is available from http://www.la-press.com.

Introduction

Stem cell-based therapies have tremendous promise for patients.[1] While there are a few cell-based products, which are commercially available, there are many more potential therapies in clinical development.[2,3] The two major types of stem cell-based products are those of allogeneic origin and of autologous origin. There are several differences between these two products, many of which impact the business model that is eventually deployed.[4,5] First and foremost is the purpose and approach to cell banking. In the allogeneic cell therapy model, cell banking is employed for manufacturing and storage of a large-scale inventory of a uniform, off-the-shelf product. In the autologous cell therapy model, cell banking is employed for manufacturing and storage of individual aliquots, to be used for the preparation of future, repeat doses for each patient. Nevertheless, there are several similarities in these business models, as both are highly dependent upon a comprehensive cell banking strategy in order to achieve commercial viability. This includes proper collection, manufacturing and release criteria, cryopreservation and storage of cells, shipping, delivery, and logistics management of the final cell product.[6] As cell-based therapies begin to progress through Phase III clinical trials, there is an increasing need to develop an understanding of industry standards and best practices across these areas. This will enable companies to reduce research costs, improve efficiencies, create revenue streams, decrease time to discovery and, ideally, increase the likelihood and number of approved marketed products.[7] In addition, it will drive further interest and investment from key stakeholders such as pharma companies.[8]

The focus of this paper will be on cell banking strategies for autologous-based cell therapies with mesenchymal stromal cells (MSCs), which are the most widely used stem cell type in cell therapy clinical trials today.[9]

MSCs are multipotent stromal cells capable of differentiating into a variety of cell types including osteoblasts, chondrocytes, and adipocytes. The most commonly used MSC tissue sources for autologous cell therapy are bone marrow (BM) and adipose tissue (AT). While the morphology and immune phenotype of the MSCs derived from both BM and AT sources are similar, there is a much higher concentration of MSCs in AT.[10] However, BM-derived MSCs have been more widely used and there is a much greater quantity of data regarding clinical safety and practice.

For autologous BM-derived stem cell therapies, the low colony frequency of MSCs and their long doubling time represent particular challenges, which are addressed with cell banking. For example, once the MSCs are isolated from the mononuclear cell component of the bone marrow tissue and they undergo a first stage of characterization with release criteria such as fluorescence-activated cell sorting, visual checks, sterility testing, and so on, these steps need not be repeated for the preparation of additional doses. With well characterized and well identified aliquots frozen and banked, preparation of repeat doses can begin with expansion of these cells, saving valuable time and resources.

Figure 1 depicts a representative cell banking and cryopreservation process for repeat dosing of an autologous, MSC-based therapy. Once the source tissue is harvested from the patient, it is transported to a dedicated cGMP (Current Good Manufacturing Processes)-compliant cell therapy processing facility, where the MSCs are isolated, cultured, and characterized to insure that only the desired cell type(s) has been cultured. Appropriate release criteria for fresh MSCs are required before moving to aliquot and cryopreserve the cells. Once cryopreserved according to the Food and Drug Administration (FDA) guidelines, the cells can be banked for subsequent doses for the patient. Thereafter, individual aliquots can either be sent frozen to a cell therapy processing facility at the medical center for thawing and for further processing into single doses, or they will more likely be thawed and processed within the external facility. In either case, after gradual thawing, the cells must be characterized using release criteria appropriate for thawed MSCs. The cells can then be expanded and activated, when applicable, into individual treatment doses, or recultured if needed, before being shipped to the medical center for injection into the patient. While autologous derived therapies have advantages over allogeneic derived therapies when it comes to immunogenicity, their primary disadvantage is in the potential for higher Cost of Goods (COGs) due to the individualized, rather than large-scale, processing and delivery.[11]

Optimization of the elements of the process can minimize the COGs and, therefore, improve overall

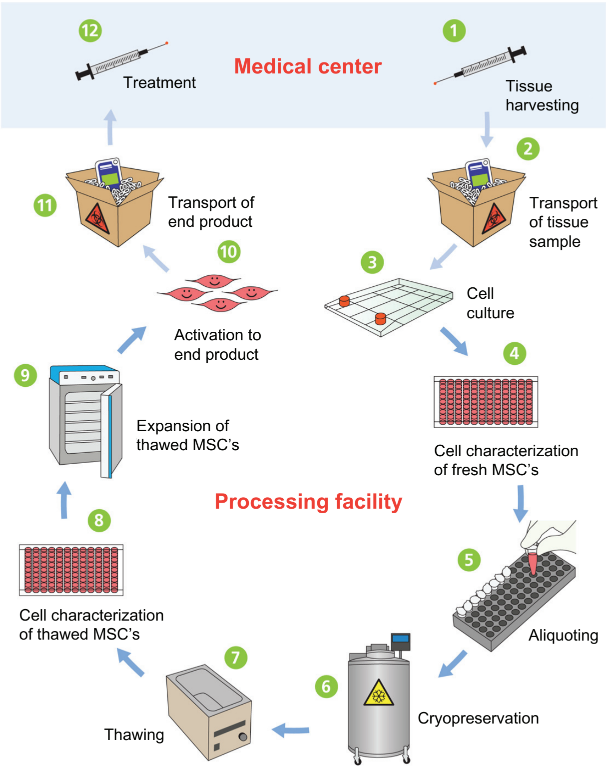

Figure 1. Cell banking process for autologous MSC therapy. (**1**) Tissue aspiration is performed at a medical center; (**2**) Shipping of the tissue sample at 4 °C in temperature controlled packaging, with a temperature recorder, to cell therapy processing facility; (**3**) MSC isolation and culturing in culture chambers (manual production) or bioreactor; (**4**) Cell characterization according to Release Criteria for Fresh MSC's; (**5**) Aliquoting of MSC samples with cryopreservation medium for future repeat doses; (**6**) Freezing and storage at −196 °C in Vapor Liquid Nitrogen Freezer as per FDA guidelines*; (**7**) Slow thawing at 37 °C in water bath; (**8**) Cell characterization according to Release Criteria for Thawed MSC's; (**9**) Expansion of thawed MSC's in incubator and/or bioreactor for processing into repeat dose; (**10**) Activation of MSC's into final cell therapy product; (**11**) Shipping of final product in optimized, approved delivery device to medical center at 4 °C in temperature controlled packaging with temperature recorder; (**12**) injection of the cell therapy product into patient.
Notes: *In the event that a cGMP compliant cell therapy processing unit exists within the medical center, frozen MSCs could theoretically be shipped to the medical center for thawing and further processing at that unit before delivery to the patient.

commercial viability of the product. However, optimization of the cell banking and cryopreservation processes must be based on industry requirements and/or standards of practice, which are constantly shifting as the field and regulators learn more about autologous cell-based therapies. This analysis will perform a deep dive into some of the key areas depicted in Figure 1 in order to highlight the key challenges and opportunities.

Cell Characterization Considerations for Fresh Cells

As described in Figure 1, aspiration of BM or other tissue is performed at the hospital and the aspirate is shipped in controlled temperature packaging to the manufacturing site for processing. The cells are then cultured and characterized using appropriate methods to ensure the cells are what they are supposed to be (ie, release criteria for fresh cells). In fact, generating robust release criteria is becoming a large area of focus for the industry.[12] According to the FDA guidance:[13]

> The final product is the final formulated product used for administration to human subjects. Final product release criteria testing should be performed on each lot of product manufactured. In some situations, each dose could be considered a single lot, depending on the manufacturing process. The results from final product release criteria testing should be available prior to administration to a human subject. We recommend that you provide, in table format, all of your proposed specifications (tests for safety, purity, potency, and identity as described in Section IV, test methods, and acceptance criteria), including test sensitivity and specificity, where appropriate, for the final product.[13]

As mentioned above, the key elements are the safety, purity, potency and identity tests that need to be employed for a particular product.[14] Safety refers to confirmation that the cell-based product is not contaminated with bacteria, fungus, or other microbes. Purity is done to confirm that the product does not have any contamination from cellular or acellular material (eg, processing material). Potency is a test to confirm that the cell-based product actually has the biological functions that are required to successfully treat the relevant patients. Finally, identity testing is used to confirm that the specific components, cellular and other, are both present and in the correct quantities. These are becoming such large issues for the industry that entities such as the Alliance for Regenerative Medicine have begun to tackle some of these challenges by providing more detailed guidance to industry players, as well as playing an active role for the industry in discussions with the relevant regulatory authorities.[15]

Cryopreservation Considerations

Cryopreservation has been successfully utilized for the long-term storage of several different cell types for many years, and is considered the most effective method for cell preservation.[16–18] Cryopreservation is based on the principle that chemical, biological, and physical processes are sufficiently stopped at cryogenic temperatures (−196 °C) because liquid water does not exist at such low temperatures. In fact, the only physical states that exist at cryogenic temperatures are crystalline. Furthermore, there is insufficient thermal energy for chemical and metabolic processes to proceed at biochemical relevant rates.

Cryopreservation, if done appropriately, will involve the following basic process. During cooling, ice nucleation occurs in the extracellular environment while the intracellular water super-cools. Because of extracellular ice formation, solution concentrations are higher outside the cells than inside and, hence, water diffuses out of the cell. As the temperature continues to decrease, the unfrozen solution within the cells and the extracellular unfrozen fraction subsequently solidify into a solid-like state. The viscosity is sufficiently high to reduce molecular motion on a practical time scale, thereby providing long-term stable storage of the cells.[19]

Given the fact that the current process is not yet optimized for cell-based products, there is a significant need to further improve cryopreservation techniques in this area. For example, an ideal cryo-protection solution should be non-toxic for cells and patients, non-antigenic, chemically inert, provide a high survival rate after thawing, and allow immediate implantation into the patient without washing. However, cryopreservation protocols still often rely on 10% dimethyl sulfoxide (DMSO) as a cryoprotectant, due to its marked ability to penetrate cell membranes and prevent cell rupture. It is well known that DMSO is potentially cytotoxic, and transplantation of DMSO-preserved human BM cells has been shown to cause severe adverse reactions.[20] Moreover, the use of human serum albumin, cerebrospinal fluid, fetal bovine serum, and bovine serum albumin as cryoprotectants is also problematic, due to the risk of contamination with human or animal viruses and the potential of infection with prions and other unidentified pathogens.[21] In addition, these are potentially dangerous due to the possible induction of an allergic response.

As a result, efforts are being made to reduce the DMSO content of cryoprotection media as much as possible,[22] in some cases by replacing a portion of it with alternatives such as hydroxyethyl starch,[23] and, in some cases, by replacing it completely.[24] In addition, serum-free cryomedia are increasingly being developed.[25,26]

In addition to the above areas, efforts to optimize the cryopreservation process have focused on the use of more environmentally-friendly cryopreservation agents, lowering the overall cost of reagents, manual handling and storage, and using slow-rate cooling methods.[27,28] These efforts have resulted in good, reproducible yields, maintenance of similar phenotypes and cell surface markers, cells remaining untransformed, no microbiological contaminations, and comparable growth rates to non-cryogenically treated cells.[29–31]

Adhering to FDA Cell Banking and Storage Standards

Another area in which adherence to FDA guidelines is critical to the cell banking and storage process is that of cGMP storage. Key elements include: cGMP monitoring, access and backup, cGMP record-keeping inventory and proper identification, and cGMP in reuse of samples and discarding of surplus.[32] In addition, a related need is for traceability of the cells in the treatment, and follow-up with the donor and recipient, both of which are much easier for autologous products. Finally, these standards apply effectively to anything that will end up being used by, for, or on the cells during cryopreservation, storage, and subsequent thawing including equipment, reagents, and processes.[33]

The FDA guidance for cGMP is included in the “Compliance Program Guidance Manual.”[34] A key quote from this section highlights the FDA’s guidance and where it can be found:

> Biological drug products are subject to the applicable regulations promulgated under both Acts, including the Current Good Manufacturing Practice regulations (CGMPs), which are found in Title 21 Code of Federal Regulations (CFR), Parts 210 and 211, and the Biologics regulations, 21 CFR Parts 600–680. In addition to the above, human cells, tissues, and cellular and tissue- based products regulated as biological drug products are also subject to the Registration and Listing, Donor Eligibility, and Current Good Tissue Practice (CGTP) regulations in 21 CFR Part 1271. Section 501(a)(2)(B) of the FD&C Act requires that biological drug products be manufactured in compliance with CGMPs. CGMP regulations apply to the manufacture of biological drug products and CGMP principles apply for the manufacture of

biological intermediates and drug substances under Section 501(a)(2)(b) of the FD&C Act, and the Biologics regulations under 21 CFR Part 600."[34]

The European Medicines Agency has similar guidance to the FDA.[35,36] The key is that companies understand the similarities and differences between the two agencies and ensure GMP compliance across their product lifecycle.

Cell Characterization and Release Criteria for Thawed Cells

Once the cell-based product has been thawed, the final characterization prior to expansion, activation, when applicable, and delivery to the patient must be performed.[37] Post-thawing release criteria, like post-culturing release criteria, include parameters such as viability, recovery, doubling time, phenotyping, and differentiation capacity. In addition, analytical methods must be used to rule out the presence of residual growth media supplements. See above discussion on release criteria for more details.

These tests are focused on ensuring that the cells, post-thaw, have the same characteristics as they did prior to freezing and shipping. The time and costs involved in this step, as well as in the subsequent expansion and activation into the end product, could add significantly to COGs if performed within an on-site cell therapy processing unit of a medical center as opposed to an external processing facility, due to the need for specially trained and qualified personnel. If the banked cells can be thawed, characterized, expanded, and processed into the end-product before shipping to the medical center, this would be of significant benefit to both the COGs, as well as to the overall success of the product.

Packaging and Shipping Considerations

The ability to preserve the integrity of the tissue sample and the final, autologous MSC-based cell product is critical to the shipping method used between the manufacturing site and the medical center. Key factors are the minimization of: loss of cell viability and potency, time the cells are in transit, temperature fluctuations of the cells, and appropriate media for transporting the cells. There are a variety of possible approaches for transporting cells, primarily dependent on temperature. For example, one could ship cells either in a frozen state, at a variety of temperatures including liquid nitrogen, −80 °C, −20 °C, or non-frozen at either 4 °C or 20 °C. Each of these has unique benefits and disadvantages that must be considered, including ease of maintaining the temperature during transit to the hospital, time that the cells remain viable, ease of preparing the cells for the patient at the hospital, and storage of the cell-based product at the hospital.

Today, many cell-based products, such as blood components, are shipped at −20 °C due to the current limitations of the manufacturing processes of early stage products, as well as due to the type of freezing equipment typically available in most hospitals. These limitations exist because companies with products in early clinical trials have yet to optimize the process given limited funds and the relatively early stage of the asset. Whether this temperature will be viable in the market in the future remains to be seen, but will likely depend on the indication and where the product is stored and administered. However, shipping autologous cell therapy products in non-frozen states is more likely to be adopted in the short-term until on-site cell therapy processing units become the norm in major medical centers.

Finally, a key consideration is the stability and viability of the autologous cell-based product during shipping, as this will impact the number and location of supplier manufacturing sites. For example, if the cell-based product is stable for 24 hours post-release from the manufacturing site, and assuming 8–12 hours of time is required on-site for delivery to the patient (less if shipped non-frozen, more if shipped frozen), then 12 hours of time is required to deliver the product to the medical center. This would likely require one to two manufacturing sites in the US, located at key airline hub metropolitan areas to enable coverage of the majority of the larger cities in the US. If the stability time is much less, then the number of processing sites must increase or the number of potential patients who could be treated will decrease (eg, limited to the east coast of Washington DC to the Boston corridor or California).

The actual logistics of moving the cells from one place to another can be challenging to ensure that the right patient gets the right cells at the right time.

Companies should look at entities that are expert in shipping blood/tissue/materials such as blood banks, specialized logistics companies, or companies that specialize in delivering peritoneal dialysis equipment and supplies to patients. While the logistics of shipping cell-based products can be challenging, many entities have already solved this.

Storage and Delivery of Cells to Patient

The cell-based product that has been shipped to the hospital for delivery to the patient could be stored on site, frozen or fresh depending on the product, in the existing blood/tissue bank of the hospital for a few days if needed until the patient is ready for treatment. But allowing the medical team to receive the final end-product ready for treatment without having to depend on the hospital cell processing unit to perform thawing, characterization, expansion, and possible differentiation procedures on-site would be optimal. In the perfect world, a preloaded syringe of the ready dose would be shipped to the medical center, ready for the physician to administer to the patient. There is some learning that companies trying to develop cell therapy products could derive from companies that have marketed monoclonal antibody-based products regarding optimizing product delivery to the patient.[38] While these are still early days for cell therapy products, logistical and cost-related benefits will determine commercial successes and differentiate between otherwise similar products.

Future Frontiers

From this discussion, it is clear that the provisioning of an autologous cell-based product is complicated and can be quite expensive using today's technology. Methods and techniques that both decrease the complexity and cost are welcomed and are being explored. In the future, an optimal product could actually resemble that of a biologic—where the ready-to-deliver end product is shipped for immediate administration to the patient with minimal complexity. This would be based on fully optimized cell banking protocols in which cryopreservation and storage of the patients' aliquots for future repeat dosing would be normalized, not unlike today's procedure for oocyte cryopreservation. In addition, significant advances might be made, perhaps in lyophilization of cells or even bone marrow, which could provide additional benefits such as significantly longer viability, simpler long-term storage, and less costly shipping.[39] Finally, this future is not likely to happen until several autologous products are launched and can optimize the business model by reinvesting profits from the product. Time will tell, but early data are encouraging that cell therapy products may become a mainstream medical treatment in the near future.

Author Contributions

Analyzed the data: AH. Wrote the first draft of the manuscript: AH. Agree with manuscript results and conclusions: AH. Developed the structure and arguments for the paper: AH. Made critical revisions and approved final version: AH. All authors reviewed and approved of the final manuscript.

Funding

The author discloses no funding sources.

Competing Interests

Author(s) disclose no potential conflicts of interest.

Acknowledgements

The author would like to thank Devyn Smith for his assistance in the preparation of this manuscript, Cheryl Singer for reviewing and editing the manuscript, and Suzan Breedveld for the illustration.

Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

References

1. Mason C, Brindley DA, Culme-Seymour EJ, Davie NL. Cell therapy industry: billion dollar global business with unlimited potential. *Regen Med*. 2011;6(3):265–72.
2. Buckler RL. Opportunities in regenerative medicine: the global industry and market trends. *Bio Process International*. 2011;9(S1):14–9.
3. Mason C, Manzotti E. Regenerative medicine cell therapies: numbers of units manufactured and patients treated between 1988 and 2010. *Regen Med*. 2010;5(3):307–13.
4. Smith DM. Assessing commercial opportunities for autologous and allogeneic cell-based products. *Regen Med*. 2012;7(5):721–32.
5. Caine B, Montgomery SA. Building a bridge to commercial success. *Bio Process International*. 2011;9(S1):1–55.
6. Mason C, Dunnill P. Assessing the value of autologous and allogeneic cells for regenerative medicine. *Regen Med*. 2009;4(6):835–53.
7. Brandenberger R, Burger S, Campbell A, Fong T, Lapinskas E, Rowley J. Cell therapy bioprocessing integrating process and product development for the next generation of biotherapeutics. *Bio Process International*. 2011;9(Suppl 1): 30–7.
8. McKernan R, McNeish J, Smith D. Pharma's developing interest in stem cells. *Cell Stem Cell*. 2010;6(6):517–20.
9. Buckler RL. *Opportunities in Regenerative Medicine*. etc.
10. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. 2006;24(5):1294–301.
11. Smith DM. Assessing commercial opportunities for autologous and allogeneic cell-based products. *Regen Med*. 2012;7(5):721–32.
12. Polchow B, Kebbel K, Schmiedeknecht G, et al. Cryopreservation of human vascular umbilical cord cells under good manufacturing practice conditions for future cell banks. *J Transl Med*. 2012;10:98.
13. Food and Drug Administration. Guidance for FDA reviewers and sponsors: content and review of chemistry, manufacturing, and control (CMC) information for human somatic cell therapy Investigational new drug applications (INDs) [webpage on the Internet]. Silver Spring, MD: Food and Drug Administration. Available from: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Xenotransplantation/ucm074131.htm. Accessed Oct 25, 2012.
14. Carmen J, Burger SR, McCaman M, Rowley JA. Developing assays to address identity, potency, purity and safety: cell characterization in cell therapy process development. *Regen Med*. 2012;7(1):85–100.
15. Alliance for Regenerative Medicine. Alliance for Regenerative Medicine Regulatory Committee [webpage on the Internet]. Washington, DC: Alliance for Regenerative Medicine. Available from: http://alliancerm.org/regulatory. Accessed Oct 25, 2012.
16. Pruksananonda K, Rungsiwiwut R, Numchaisrika P, Ahnonkitpanit V, Isarasena N, Virutamasen P. Eighteen-year cryopreservation does not negatively affect the pluripotency of human embryos: evidence from embryonic stem cell derivation. *Bio Research Open Access*. 2012;1(4):166–73.
17. Wu X, Goodyear SM, Abramowitz LK, et al. Fertile offspring derived from mouse spermatogonial stem cells cryopreserved for more than 14 years. *Hum Reprod*. 2012;27(5):1249–59.
18. Vosganian GS, Waalen J, Kim K, et al. Effects of long-term cryopreservation on peripheral blood progenitor cells. *Cytotherapy*. 2012;14(10): 1228–34.
19. Yokoyama WM, Thompson ML, Ehrhardt RO. Cryopreservation and thawing of cells. *Curr Protoc Immunol*. 2012;Appendix 3:3G.
20. Hunt CJ. Cryopreservation of human stem cells for clinical application: a review. *Transfus Med Hemother*. 2011;38(2):107–23.
21. Wang Y, Han ZB, Song YP, Han ZC. Safety of mesenchymal stem cells for clinical application. *Stem Cells Int*. 2012;2012:652034.
22. Smagur A, Mitrus I, Giebel S, et al. Impact of different dimethyl sulphoxide concentrations on cell recovery, viability and clonogenic potential of cryopreserved peripheral blood hematopoietic stem and progenitor cells. *Vox Sang*. Oct 9, 2012. doi: 10.1111/j.1423-0410.2012.01657.x. [Epub ahead of print.]
23. Naaldijk Y, Staude M, Fedorova V, Stolzing A. Effect of different freezing rates during cryopreservation of rat mesenchymal stem cells using combinations of hydroxyethyl starch and dimethylsulfoxide. *BMC Biotechnol*. 2012;12:49.
24. Nishigaki T, Teramura Y, Nasu A, Takada K, Toguchida J, Iwata H. Highly efficient cryopreservation of human induced pluripotent stem cells using a dimethyl sulfoxide-free solution. *Int J Dev Biol*. 2011;55(3):305–11.
25. Germann A, Schulz JC, Kemp-Kamke B, Zimmermann H, von Briesen H. Standardized serum-free cryomedia maintain peripheral blood mononuclear cell viability, recovery, and antigen-specific T-cell response compared to fetal calf serum-based medium. *Biopreserv Biobank*. 2011;9(3):229–36.
26. Freimark D, Sehl C, Weber C, et al. Systematic parameter optimization of a Me(2)SO- and serum-free cryopreservation protocol for human mesenchymal stem cells. *Cryobiology*. 2011;63(2):67–75.
27. Coopman K. Large-scale compatible methods for the preservation of human embryonic stem cells: current perspectives. *Biotechnol Prog*. 2011;27(6):1511–21.
28. Healy L, Young L, Stacey GN. Stem cell banks: preserving cell lines, maintaining genetic integrity, and advancing research. *Methods Mol Biol*. 2011;767:15–27.
29. Janz Fde L, Debes A, Cavaglieri Rde C, et al. Evaluation of distinct freezing methods and cryoprotectants for human amniotic fluid stem cells cryopreservation. *J Biomed Biotechnol*. 2012;2012:649353.
30. Costa PF, Dias AF, Reis RL, Gomes ME. Cryopreservation of cell/scaffold tissue-engineered constructs. *Tissue Eng Part C Methods*. 2012;18(11):852–8.
31. Naaldijk Y, et al. *Effect of different freezing rates*, etc.
32. Polchow B, Kebbel K, Schmiedeknecht G, et al. Cryopreservation of human vascular umbilical cord cells under good manufacturing practice conditions for future cell banks. *J Transl Med*. 2012;10:98.
33. George B. Regulations and guidelines governing stem cell based products: Clinical considerations. *Perspect Clin Res*. 2011;2(3):94–9.
34. Food and Drug Administration. *Compliance Program Guidance Manual Chapter—45 Biological Drug Products Inspection of Biological*. Silver Spring, MD: Food and Drug Administration. Available from: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ComplianceActivities/Enforcement/CompliancePrograms/UCM095419.pdf. Accessed Oct 25, 2012.
35. Fekete N, Rojewski MT, Fürst D, et al. GMP-compliant isolation and large-scale expansion of bone marrow-derived MSC. *PLoS One*. 2012;7(8):e43255.
36. Oancea G, Wagner B, Henschler R. Cell therapy regulations from a European perspective. *Regenerative Therapy Using Blood-Derived Stem Cells Stem Cell Biology and Regenerative Medicine*. 2012:191–204.
37. Stacey GN, Masters JR. Cryopreservation and banking of mammalian cell lines. *Nat Protoc*. 2008;3(12):1981–9.
38. Mason C, Hoare M. Regenerative medicine bioprocessing: the need to learn from the experience of other fields. *Regen Med*. 2006;1(5):615–23.
39. Coopman K. Large-scale compatible methods for the preservation of human embryonic stem cells: current perspectives. *Biotechnol Prog*. 2011;27(6):1511–21.