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Challenges of stem cell-based pulp and dentin regeneration: a clinical perspective

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Abstract

There are two types of approaches to regenerate tissues: cell-based and cell-free. The former approach is to introduce exogenous cells into the host to regenerate tissues, and the latter is to use materials other than cells in an attempt to regenerate tissues. There has been a significant advancement in stem cell-based pulp and dentin regeneration research in the past few years. Studies in small and large animals have demonstrated that pulp/dentin-like tissues can be regenerated partially or completely in the root canal space with apical openings of 0.7-3.0 mm using dental pulp stem cells, including stem cells from apical papilla (SCAP) and subpopulations of pulp stem cells. Bone marrow mesenchymal stem cells (BMMSCs) and adipose tissue-derived MSCs (ADMSCs) have also been shown to regenerate pulp-like tissue. In contrast, the cell-free approach has not produced convincing evidence on pulp regeneration. However, one crucial concept has not been considered nor defined in the field of pulp/dentin regeneration and that is the critical size defect of dentin and pulp. Without such consideration and definition, it is difficult to predict or anticipate the extent of cell-free pulp regeneration that would occur. By reasoning, cell-free therapy is unlikely to regenerate an organ/tissue after total loss. Similarly, after a total loss of pulp, it is unlikely to regenerate without using exogenously introduced cells. A cell homing approach may provide a limited amount of tissue regeneration. Although stem cell-based pulp/dentin regeneration has shown great promise, clinical trials are difficult to launch at present. This article will address several issues that challenge and hinder the clinical applications of pulp/dentin regeneration which need to be overcome before stem cell-based pulp/dentin regeneration can occur in the clinic.

INTRODUCTION

Amputation of damaged tissue such as performing pulpectomy followed by disinfection and replacement of the lost tissue with gutta percha is a primitive medical treatment. The lack of understanding of the biology of cells, especially stem cells, is the key reason why little to no advancement of pulp/dentin regeneration has taken place until recently. Regeneration of pulp tissue has been considered to be difficult as the tissue is encased in dentin without a collateral blood supply except from the root apex. Approximately ten years after the discovery of dental pulp stem cells (1), pulp/dentin regeneration was demonstrated using exogenously transplanted dental stem cells in small and large animals including orthotopic *de novo* regeneration in the root canal space of mature teeth (2,3). While more preclinical research is needed to further validate the findings and establish a reliable clinical protocol, such demonstration suggests that preparation for clinical trials may be initiated. However, two major issues are likely to impede the progress of clinical applications: lack of recognition of cell-based therapy for pulp/dentin regeneration and shortage of affordable facilities for processing clinical grade stem cells. Furthermore, there is a lack of understanding of the concept of critical size defect in the context of human pulp/dentin

regeneration that causes confusion when considering pulp/dentin regeneration either with a cell-free or cell-based approach. This article will review the recent advancement of pulp/dentin regeneration research using either cell-free or cell-based approaches with an emphasis on the critical size defect concept. Special focus will be to address challenges facing cell-based therapy for pulp/dentin regeneration in patients.

Groundbreaking research on stem cell-based pulp/dentin regeneration

By definition, cell-based pulp/dentin regeneration means transplanting exogenous stem cells into the root canal system of the host to allow regeneration to take place (for a detailed review of the cell-based approach to tissue engineering, please refer to references 4 and 5). The transplanted cells have been removed from the host (autologous) or from other individuals (allogenic) and may have been either minimally processed (separation from tissues) or grown in cultures to expand their numbers. Stem cell-based therapy is effective for repairing extensive defects due to their potency in dividing and differentiating in response to microenvironmental cues. Most importantly, cells are the key for tissue regeneration. With this approach, we expect to regenerate pulp in the entire root canal space as well as in the pulp chamber, along with newly generated dentin on the dentinal walls. There has been a major concern that pulp regeneration is not a possibility because of the restriction of blood supply from the small apical foramen, which may also hinder the angiogenesis of the pulp (6). However, the recent reports described below show otherwise.

De novo regeneration of pulp/dentin in animal models—Our team first reported the complete regeneration of pulp by dental stem cells using a combination of orthotopic and ectotopic animal study models. The emptied and widened canals of human tooth root fragments were filled with scaffolds loaded with human stem cells from apical papilla (hSCAP) or dental pulp stem cells (hDPSCs) and transplanted into immunocompromised mice for the blood supply. We observed *de novo* synthesis of vascularized human pulp-like tissue as well as a new layer of dentin-like tissue on the canal dentinal walls. Subsequently, complete orthotopic pulp regeneration was also demonstrated by Iohara et al. in a large animal study model using dog DPSCs (3). In this report, mature dog teeth were used and the apical foramen was only enlarged to 0.7 mm. These findings indicate that even when the root canal is totally emptied, as long as it is filled with SCAP or DPSCs, pulp tissue can be regenerated in the canal space from scratch. Vascularization can successfully occur in the regenerated pulp. Additionally, newly deposited dentin-like structure can form on the canal dentinal walls.

Stem cell sources for pulp/dentin engineering and regeneration—Different subpopulations of DPSCs have been identified and used for specific regenerative purposes. A highly angiogenic subfraction of side population (SP) cells, CD31[−]/CD146[−], has been isolated from porcine dental pulp expressing CD34 and receptor for vascular endothelial growth factor-2 (VEGFR2)/Flk, which are similar to endothelial progenitor cells (7). These cells have MSC properties as well as angiogenic potential demonstrated by mouse hind limb ischemia study models. Furthermore, in a cerebral ischemic model, transplanting CD31[−]/CD146[−] SP cells or CD105⁺ cell fractions into the striatum of adult rats induced neovascularization of the ischemic zone and enhanced subsequent neuronal regeneration (8). These findings led to the utilization of these fractionated SP cells and CD105⁺ cells to enhance angiogenesis and reinnervation following pulpotomy and pulpectomy in large animal study models. In an experimental model of amputated pulp in dogs, autogenous transplantation of CD31[−]/CD146[−] SP cells or CD105⁺ cells, together with type I and type III collagen as a scaffold, resulted in regeneration of pulp tissue with capillaries and neuronal processes within 14 days. The transplanted cells expressed pro-angiogenic factors, implying trophic action on endothelial cells (9). Furthermore, the transplantation of CD105⁺

DPSCs in the dog root canal after pulpectomy resulted in complete pulp regeneration including nerves and vasculature by day 14, followed by new dentin formation along the dentinal wall by day 35 (3).

In addition to DPSCs, stem cells from human exfoliate deciduous teeth (SHED) and SCAP are also suitable cell sources for pulp/dentin regeneration because they are derived from pulp tissue or the precursor of pulp. Both DPSCs and SCAP are capable of generating an ectopic pulp/dentin complex *in vivo* when mixed with hydroxyapatite/tricalcium phosphate particles (1,10,11). However, SHED form mineralized tissue *in vivo* without a distinct pulp/dentin complex (12).

Non-dental MSCs such as BMMSCs and adipose tissue-derived MSCs (ATMSCs) have also been shown to be able to regenerate pulp (13), although obtaining these cells is quite traumatic. Nonetheless, these cell sources may be an alternative when dental MSCs are not available.

Cell-free approaches for pulp/dentin regeneration

The concept of critical size defect—The common practice when performing wound healing experiments is to use the concept of critical size defect, which refers to the defect of bone that, without introducing any supportive approaches, will not regenerate naturally during the lifetime of the animal (14). For example, to study bone healing, the frequently used standard test is the calvarial bone defect model. In mice, the critical size defect is 2 mm or larger in diameter (15).

The critical size defect has never been experimentally defined for dentin. When pulp tissue is exposed due to the loss of the overlying dentin, direct pulp capping therapy can allow the pulp to form new dentin, termed the dentin bridge. Stanley stated that the size of the dentin defect should not determine the ability of the underlying pulp to form a dentinal bridge as long as the management is properly performed (16), i.e. theoretically the entire pulp chamber roof dentin, if lost, may be regenerated with the dentin bridge. From this point of view, the clinical concept of pulp capping for pulp exposure cases should not be limited to sizes smaller than 1 mm. The use of various cement-based materials for pulp capping, such as calcium hydroxide and mineral trioxide aggregate (MTA), has been well documented and studied (17,18).

It should be noted that the regeneration conditions between bone and dentin are very different. Bone contains marrow space where stem cells reside and they are responsible for the regeneration of the adjacent bone that is lost. Dentin, however, does not contain any marrow space and its regeneration is dependent on the condition of the pulp. Therefore, having a healthy pulp is a prerequisite for dentin regeneration. As well, the critical size defect for human pulp tissue has never been tested and defined. Thus, it is difficult to determine how much pulp tissue loss exceeds the natural ability to regenerate. Limited evidence in a dog model showed that when approximately half of the pulp was lost, the pulp system did not regenerate (Fig. 1) (19). Recently there was a human case report of revitalization on a maxillary central incisor (20). As shown in Figure 2, the pulp tissue appears to have regenerated after treatment.

Current approaches to cell-free pulp/dentin regeneration—Pulp regeneration has been understood in the context of regenerating the entire pulp. Although tissue regeneration can be accomplished by endogenous cell homing instead of transplanting exogenous cells, in cases of the total loss of an organ, self-regeneration or cell homing regeneration is not expected since it would then have far exceeded its critical size defect. The concept of cell homing in the context of tissue engineering is defined as the active recruitment, by design,

of endogenous cells into an anatomic compartment (21,22). Using a scaffold that allows cell colonization and an appropriate balance of morphogens (growth factors), endogenous cells from a distant site will be recruited where regeneration is needed. It should be noted that there has been no report showing the regeneration of an entire organ via cell homing in humans. Nevertheless, the cell homing technique, which is not dependent on cell culture, seems to be simpler than the cell-based technique from a technical and economical point of view.

The current protocol of pulp revitalization used in Endodontics could be considered a type of cell homing approach. The blood clot created in the pulp canal acts as a scaffold and the growth factors in the blood clot could contribute to the recruitment of stem cells. It is still unclear to what extent the blood clot itself will attract endogenous stem cells. Lovelace et al. observed that the evoked blood itself during the revitalization procedure contains a significant amount of undifferentiated MSCs when compared with systemic blood (23). Therefore, at least some of the stem cells recruited during the revitalization technique are introduced into the pulp canal at the time of bleeding and not by later migration. One important point is that currently there is limited knowledge on cell markers that are for specific MSCs. It is difficult to identify MSCs from blood versus those from pulp. Thus, it is not clear whether the MSCs in blood introduced into the canal can regenerate pulp/dentin as do the DPSCs of SCAP. Furthermore, whether the revitalization approach can regenerate pulp that is entirely lost remains unclear.

One limitation of the current revitalization technique used in the clinic is that we cannot determine the exact nature of the tissues that recolonize the pulp space. A dog model study showed that revitalization procedures at best allowed the ingrowth of periodontal tissues including bone and periodontal ligament as well as the intracanal cementum, which contributed to the increased dentin wall thickness and root length (19). Many clinical case reports have shown good outcomes with pulp revitalization of immature teeth. However, the results vary in those case reports, especially when comparing the dentin wall thickness, the root length, and the size of the pulp canal after treatment. Andreasen & Bakland described four possible outcomes of pulp revascularization procedures on traumatized teeth: (i) accelerated dentin formation and pulp canal closure; (ii) ingrowth of cementum and periodontal ligament (PDL); (iii) ingrowth of cementum, PDL, and bone; and (iv) ingrowth of bone and bone marrow (24). They suggest that these scenarios are the results of different cell populations invading the pulp space.

A recent case report by Shimizu et al. (mentioned above) demonstrated the first histological finding of a human tooth after revitalization procedures (20). The histology showed well-vascularized pulp-like tissue in the canal with an odontoblast layer on the dentin wall (Fig. 3). There is also STRO-1 detection in the apical papilla. Because it is difficult to visualize and assess the condition of the pulp clinically before treatment, it is unclear how much regeneration actually took place in the canal after treatment. Nonetheless, this case report indicates a promising clinical outcome using revitalization protocols.

A concern in cell homing procedures to regenerate dental pulp is the size of the apex needed to allow proper revascularization. Kling et al. showed that apical sizes less than 1.0 mm do not allow pulp revascularization in re-implanted permanent incisors; therefore a minimum of 1.1 mm is necessary to obtain proper revascularization (25). Another study on replantation of avulsed teeth also showed that an increased apical diameter tends to promote more frequent pulp healing while apices smaller than 1.5 mm have the lowest rate of healing (26). However, using the stem cell-based method, pulp can regenerate in canals with 0.7 mm apical sizes as mentioned above (3).

To achieve pulp regeneration via a cell-free approach, using an appropriate scaffold combined with the right combination of growth factors has been proposed and tested *in vitro* and in animal models. The growth factors used should possess three functions: (i) promote angiogenesis inside the root canal; (ii) promote migration of endogenous stem cells; and (iii) promote mineralization. Regarding angiogenesis, Mullan et al. observed that vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) both promote angiogenesis in severed dental pulp (27). VEGF was more potent than FGF-2 under their experimental conditions. Increased microvessel density was also obtained *in vivo* in the pulp of tooth slices exposed to VEGF and transplanted subcutaneously in mice. To study the chemotactic effect of different cytokines on dental pulp stem cells, Suzuki et al. tested the recruitment of DPSCs in 3D collagen gel exposed to stromal-derived factor-1 α (SDF1), basic fibroblast growth factor (bFGF), and bone morphogenic protein-7 (BMP-7) (28). SDF1 and bFGF were both able to recruit DPSCs into the collagen gel as demonstrated by the increased DNA content inside the gel. However, such an augmentation was not observed with BMP-7, which might have initiated cell differentiation at the expense of cell migration. The same group has also induced tissue regeneration via a cell homing technique using human teeth transplanted in a mouse model (21). They used combinations of different growth factors adsorbed on a collagen scaffold: VEGF (chemotactic, angiogenic, and mitogenic), bFGF (chemotactic and angiogenic), platelet-derived growth factor (PDGF) (angiogenesis), nerve growth factor (NGF) (promotes survival and growth of nerve fibers), and BMP-7 (promotes mineralized tissue formation). VEGF and bFGF alone or in combination promoted re-cellularized and re-vascularized connective tissue integrated to the dentin wall. This proof-of-principle study suggests that a cell homing technique may be considered for pulp regeneration; however, the nature of the regenerated soft tissue using this approach is unclear. As with revitalization procedures, further investigation is needed.

Facilities involved in cell-based therapy

Any clinical therapy involving the use of exogenous live cells is considered cell-based therapy. The complexity of cell-based therapy is determined by how much *ex vivo* manipulation is involved. The common practice of cell-based therapy has been the isolation of bone marrow or blood cells followed by centrifugation to separate out a specific population, which will then be injected back into the patient; for example, the use of bone marrow cells to enhance tissue regeneration (29). Often, this type of clinical cell-based therapy does not require any regulatory oversight because the doctors can define the therapy as a variation or modification of a commonly employed standard operation.

A certain level of *ex vivo* manipulation of a cell-based therapy that has been practiced for a long time is the treatment of hematopoietic diseases (30). Stem cell/bone marrow transplants have been used to treat patients with leukemia and lymphoma as a standard practice in many hospitals with these treatment programs (e.g. <http://www.stjude.org/stjude/v/index.jsp?vgnextoid=ff2abfe82e118010VgnVCM1000000e2015acRCRD>). Any cells that will be handled in culturing conditions in the laboratory will require a special protocol, which includes a facility that is qualified for such a process. This facility followed a guideline called current good manufacturing practice (cGMP). Additionally, any materials that will be in contact with the extracted human cells need to be qualified by the guideline (<http://www.fda.gov/cosmetics/guidancecomplianceregulatoryinformation/goodmanufacturingpracticegmpguidelinesinspectionchecklist/default.htm>).

MSCs require extensive *ex vivo* culturing. Normally, it takes approximately two weeks or longer from receipt of the tissue (e.g. bone marrow) to expansion in cultures and screening of potential contamination or pre-existing pathogens before it is ready to deliver back to the patient (for autologous cells) or to another patient (allogeneic cells). The overall process is summarized in Figure 4. Currently, the processing of MSCs for clinical applications is still

at its inception. The National Heart, Lung, and Blood Institute (NHLBI) (part of NIH) has sponsored a network of cGMP services called Production Assistance for Cellular Therapies (PACT) (<http://www.pactgroup.net/>), which supports a Coordinating Center—The EMMES Corporation—and five facilities for the production and testing of novel cell therapies. The facilities are: (i) Baylor College of Medicine Center for Cell and Gene Therapy (CAGT); (ii) Center for Human Cell Therapy (CHCT), Boston; (iii) City of Hope—Center for Applied Technology Development (CATD), Duarte, CA; (iv) University of Minnesota Molecular & Cellular Therapeutics (MCT) Facility; and (v) University of Wisconsin, Madison—Waisman Biomanufacturing (WB). These institutions form the PACT group and provide investigators with production assistance in areas ranging from translational development to supplying clinical grade products intended for use in human clinical trials. The facilities are also committed to providing investigators with the data needed to support an Investigational New Drug (IND) application (<http://www.fda.gov/Drugs/DevelopmentApprovalProcess>). Although cells are not a regular drug, to initiate any clinical trials using cells in order to test their effectiveness, approval of an IND application is required by the Food & Drug Administration (FDA).

The PACT Coordinating Center monitors and manages the organizational and regulatory aspects of the program. PACT has an Education Training Program to promote the field of cellular therapy and provide new investigators in the field with recommended applications of the current GMP and Good Tissue Practice (GTP) guidelines. An investigator who is planning to launch a clinical trial on cellular therapy can apply for assistance from the PACT. If the application is approved, one of the PACT facilities will provide the necessary service at no charge in order to assist the investigator in obtaining IND approval and providing safe, tested, clinical-quality cells for therapy. However, there are specific scopes and areas of interest that the PACT supports including heart, lung, hematopoietic, cerebrovascular, cardiovascular, and immunodeficiency diseases. Applications outside of the areas of interest and scopes will be declined. Currently, the intramural research group at NIH has a Bone Marrow Stromal Cell Transplantation Center where there are cGMP facilities to process MSCs for cell-based therapies. However, the projects are restricted to intramurally initiated and those that are outside of NIH are yet to be supported at present.

Challenges and hurdles for cell-based therapy

Although cell therapy has been a common practice, to this day, most clinical cell therapies deal with blood-related diseases such as leukemia and lymphoma using hematopoietic stem cells (HSCs). In the 1960s, Till & McCulloch published a series of breakthrough papers that lead to the discovery of HSCs. As mentioned above, human HSCs have been utilized for the treatment of various blood diseases and are usually expanded in suspension cultures (31). However, MSCs, which are adherent cells, require extensive expansion in cultures. At present, we can summarize the following key challenges and hurdles for using MSC-based therapy. The solutions to the challenges are discussed.

(i) Lack of affordable cGMP facilities—Other than the PACT group, there have been only a number of private companies or programs in university based-institutions that provide this service for a fee. These include Osiris Therapeutics, Inc. (Columbia, MD), Advanced Cell Technology (Marlborough, MA), Lonza Walkersville, Inc. (Walkersville, MD), Aastrom Biosciences, Inc. (Ann Arbor, MI), NeoStem, Inc. (New York, New York), PCT/ New Jersey and California, (Allendale, NJ; Mountain View, CA); and UC Davis Stem Cell Program (Sacramento, CA). One approach for an investigator wishing to launch cell therapy research or clinical trials would be to convince the research department of one of these companies to collaborate, thereby obtaining the cell processing service without cost. Otherwise, to process bone marrow into MSCs from one patient may cost as much as

\$12,000 to \$20,000; in addition, technology transfer for the IND application costs another \$10,000 to \$300,000. This issue has limited the feasibility of mounting clinical trials to test cell-based pulp/dentin regeneration.

Currently the market system contradicts the dissemination of advanced medical technologies. Medical researchers are encouraged to work with industry to develop not just advanced medical technologies but “profitable medical technologies.” New drugs (patented) that can help millions are so expensive that most people cannot afford them or their insurance does not cover them and thus they have to wait ten years before a generic drug is produced. The concept of developing new and affordable technology is basically a fairy tale. The price of technology is not determined by the availability of material or labor resources, but by the corporations that sell it and the underlying economic system. Nonetheless, over time, cGMP facilities will become more popular and the prices will drop.

(ii) Outcome of allogenic dental MSC for pulp/dentin regeneration is not known—Due to the immunosuppressive properties of MSCs including dental MSCs, allogenic MSCs may be well tolerated by the recipients. In fact, such properties have been utilized to treat autoimmune diseases such as acute graft-versus-host disease and systemic lupus erythematosus, and to enhance engraftment (32-34). Dental MSCs have been shown to have immunosuppressive properties including DPSCs, SHED, SCAP, and PDLSCs (35-38). A recent report using minipigs as a study model has demonstrated that allogenic PDLSCs can regenerate periodontal defects as effectively as autologous PDLSCs without immunological rejections (39), indicating that it is likely that pulp/dentin regeneration can be achieved by allogenic DPSCs and SCAP.

(iii) Lack of dental stem cell banking system—As mentioned, the availability of dental MSCs for cell-based pulp/dentin regeneration is an issue. This hurdle may be resolved if a stem cell banking system were well established. While a good number of private tooth banks have been formed to provide service in banking extracted teeth, none are processing clinical grade dental MSCs and banking them for future use. Fortunately, more and more banking companies are emerging all over the world. Eventually, there should be a sufficient number of such services accessible to the public. At that time, either autologous or allogenic cells that have been stored in the stem cell banks will be available for the timely supply of clinical needs.

(iv) Lack of recognition of cell-based pulp/dentin regeneration by the medical field—Currently cell-based therapy is only considered important in the treatment of serious medical conditions such as hematopoietic, cerebrovascular, cardiovascular, and immunodeficiency diseases. Certainly there are risks involved in using cell-based therapy, for example, mutations accumulated in cultured cells, contamination, originally infected cell sources, etc. The common perception is that these risks, although controllable, may override the benefits of cell-based pulp/dentin regeneration. Researchers and educators in the dental field should be more actively spreading the knowledge to and educating the public, industry, and medical colleagues of their understanding in the advancement of regenerative dentistry.

CONCLUSION

It is inevitable that Medicine including Dentistry is moving toward regeneration instead of restoration. A cell-free approach is desirable at the present time for its simplicity; however, it is unlikely that this approach can handle large defects in general and in particular *de novo* regeneration of pulp/dentin in humans. Although there are challenges ahead in stem cell-based therapy for pulp/dentin regeneration, the main issues listed above are resolvable with

time. The challenge we least wish to face is the denial of the advancements in pulp/dentin regeneration that may act as a barrier to progress.

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Fig. 1.

Critical size defect in the pulp of a dog tooth. An immature dog tooth was accessed, a pulpectomy performed, and the canal infected by microbes. After disinfection of the root canal, the space was filled with blood induced from the periapical tissue and the tooth sealed. Three months later the tooth with the surrounding tissues was removed and processed for histological analysis. It was found that some pulp tissue was not removed but healed after disinfection (blue arrow). The odontoblast layer (arrowhead) on the left side of the canal space remained intact. The right side of the pulp tissue was removed and the space filled in by periodontal tissue including soft connective tissue and intracanal cementum (white arrow). The dashed line separates healed pulp tissue (left) and ingrown periapical connective tissue (right). Adapted with permission from Wang et al., 2010 (19).

Fig. 2. Revitalization and regeneration of human pulp: clinical case. Clinical radiographs showing treated tooth #9: (a) preoperative and (b) fractured 3.5 weeks after revitalization treatment. Adapted with permission from Shimizu et al., 2012 (20).

Fig. 3.

Histology of a section of extracted revitalization tooth #9. (a) Loose connective tissue with few collagen fibers has filled the canal space up to the coronal MTA plug (hematoxylineosin). (b) High magnification of the square in (a) (the apical root canal). Flattened odontoblast-like cells line the predentin (solid arrows). Many blood vessels filled with red blood cells (open arrows). No mature nerve-like bundles along the blood vessels are observed. Most cells are spindle-shaped. (c) High magnification of the rectangle in (a) (the apical foramen). There are fewer blood vessels (arrow) and cellular components at the apical foramen than in the canal. (d) High magnification of the square in (c) (part of the root apex). Layers of epithelial-like Hertwig's epithelial root sheath (arrow) surround the root apex. Spaces in the tissue are artifacts caused by histological preparation. Adapted with permission from Shimizu et al., 2012 (20).

Fig. 4.

Flowchart of processing mesenchymal stem cells for clinical applications. MSC harboring tissue is first obtained from the host. It is then either processed (freeze down or cut into small pieces) or sent to a cGMP facility directly for MSC isolation. Following a standard protocol, MSCs are isolated and expanded in cultures. Isolated MSCs will be screened and tested for the absence or presence of pathogens through established/approved protocols. Normally MSCs are passaged minimally (usually ~passage 3) before they are prepared for shipping to the clinic. MSCs may be frozen down and saved in a stem cell bank for future use. Depending on the distance between the GMP facility and the clinic, the MSCs may be shipped at low but not freezing temperature or frozen down and kept on dry ice for shipping to the clinic. If frozen, the clinical personnel will thaw the cells following established protocols before transplanting into the patient.