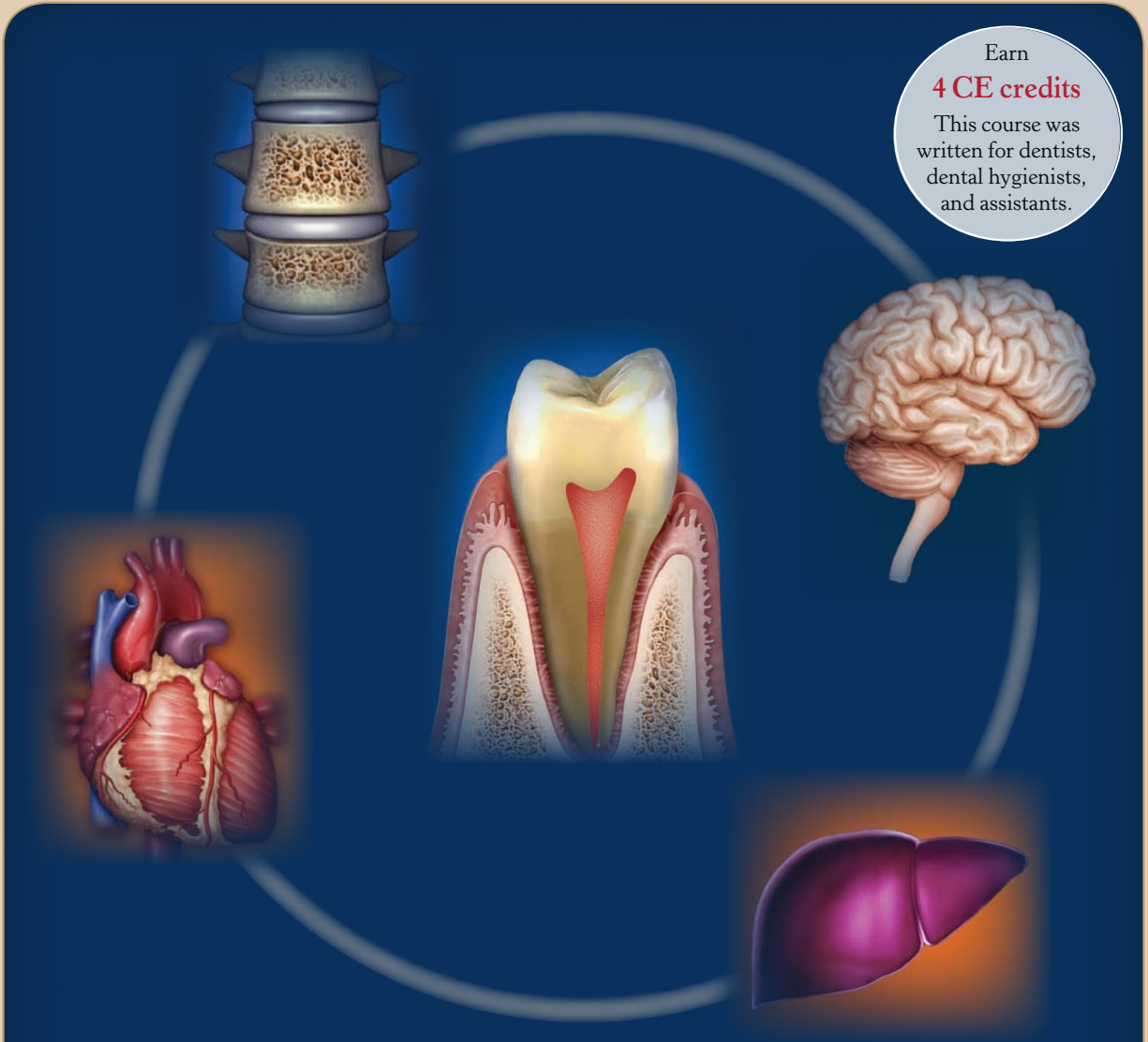


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# Stem Cells: Sources, Therapies and the Dental Professional

A Peer-Reviewed Publication  
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## Educational Objectives

Upon completion of this course, the clinician will be able to do the following:

1. Understand the range of diseases for which stem cell therapies are being investigated
2. Be knowledgeable about the various sources of stem cells and the advantages and disadvantages of each source
3. Understand the fundamental reasons for the effectiveness of stem cells and the meaning of tissue differentiation
4. Understand the basics of cryopreservation and the banking of stem cells

## Abstract

Recent exciting discoveries place dentists at the forefront of engaging their patients in potentially life-saving therapies derived from a patient's own stem cells located in deciduous and permanent teeth. Adult stem cells, including dental stem cells, have the potential, like bone marrow-derived stem cells and adipose-derived stem cells, to cure a number of diseases.

In medicine, stem cell-based treatments are being used and investigated for conditions as diverse as Parkinson's disease, neural degeneration following brain injury, cardiovascular disease and autoimmune diseases. Stem cells will be used in dentistry for the regeneration of dentin and/or dental pulp, biologically viable scaffolds will be used for the replacement of orofacial bone and cartilage, and defective salivary glands will be partially or completely regenerated.

Dental stem cells can be obtained from the pulp of the primary and permanent teeth, from the periodontal ligament, and from associated healthy tissues. Exfoliating/extracted deciduous teeth and permanent teeth extracted for orthodontic treatment, trauma or dental implant indications are all readily available sources of dental stem cells. The harvest of these dental stem cells results in minimal trauma. Dental professionals have the opportunity to make their patients aware of these new sources of stem cells that can be stored for future use as new therapies are developed for a range of diseases and injuries.

## Introduction/Overview

Recent exciting discoveries place dentists at the forefront of engaging their patients in potentially life-saving therapies derived from a patient's own stem cells located in deciduous and permanent teeth. In 2000, the National Institutes of Health (NIH) released two studies of research on human teeth detailing the discovery of adult stem cells in impacted third molars and even more resilient stem cells in deciduous teeth.

Dentistry and medicine are evolving into new forms, in which care is being delivered with increasing frequency through biologically based approaches. The first wave of this paradigm shift in health care is likely more imminent than anyone is willing to predict at present, and its impact will eventually be felt in every medical and dental office and setting. In medicine, stem cell-based treatments are being used and investigated for conditions as diverse as Parkinson's

Table 1: Baby boomers in the U.S. population (in millions)

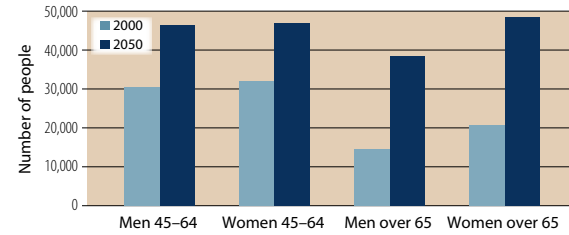


Table 2a: Prevalence of heart failure and stroke by age

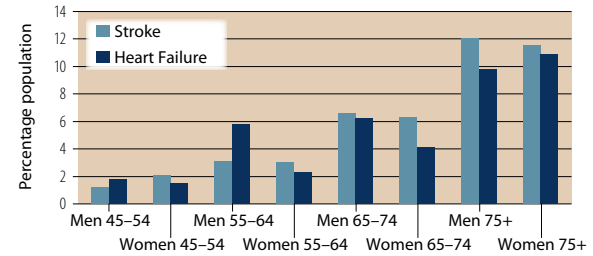
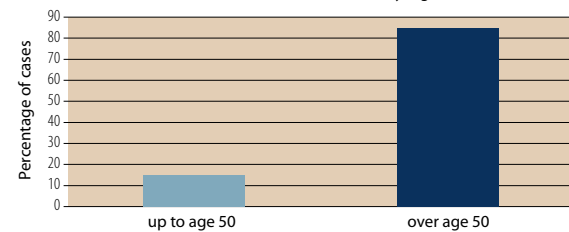


Table 2b: Prevalence of Parkinson's disease by age

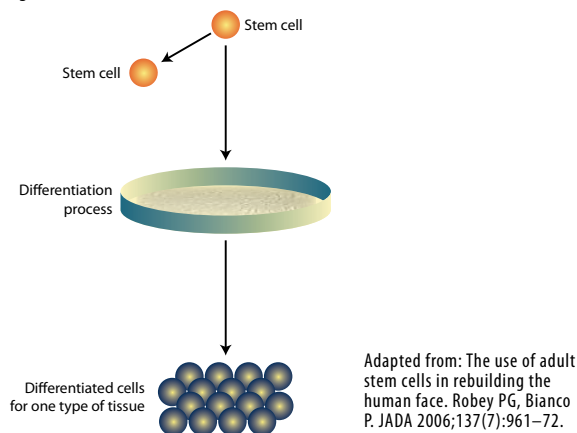


Source: Parkinson's Disease Foundation

disease, diabetes, liver disease, neural degeneration following brain injury, cardiovascular disease and autoimmune diseases. Biomolecules will be used in dentistry for periodontal regeneration, stem cells will be used in the regeneration of dentin and/or dental pulp, biologically viable scaffolds will be used for the replacement of orofacial bone and cartilage, and defective salivary glands will be partially or completely regenerated.<sup>1</sup> No longer will it be necessary to rely on the chance of finding a cellular match from a donor, and devastating and formerly incurable diseases could potentially be treated.

This is no longer science fiction. Some stem cell therapies have already been approved or are being reviewed by the U.S. Food and Drug Administration (FDA), while others are at various stages of development. Starting in the 1970s, it was discovered that cells taken from bone marrow postnatally had the ability to differentiate into bone, cartilage and marrow fat cells when they were transplanted.<sup>2,3</sup> Stem cell research has increased dramatically in recent years as the potential for use of stem cells has become better understood. Degenerative diseases increase in incidence with age, and as the baby boomer population ages it can be anticipated that the need for viable and improved treatment options for these diseases will increase. Patients are being treated using stem cells for cardiovascular, orthopedic, dental, oncological and other conditions.

Figure 1. Stem cell differentiation



New stem cell therapies will become available in the future, and in the next three years it is anticipated that stem cell product therapies for graft-versus-host disease, damaged heart muscle due to cardiac disease and knee cartilage repair will become available.<sup>4</sup> The stem cell market in 2006 represented approximately \$36 million, and it has been estimated that it will reach \$8 billion by 2015 with the advent of new therapies and increased treatment needs.<sup>5</sup>

### Stem Cell Types and Sources

Stem cells are immature, undifferentiated cells that can divide and multiply for an extended period of time, differentiating into specific types of cells and tissues. Autogenous stem cells are derived from the patient being treated, while allogeneous stem cells are derived from other individuals. Stem cells available commercially are currently mainly allogeneous (donor-derived). While it is believed that allogeneous stem cells will not produce an immune response, this is not known with certainty.<sup>6</sup> Autogenous stem cells, on the other hand, reduce the risk of rejection and, provided they are handled correctly, remove the risk of cross-infection from allogeneous transplanted tissue. In addition, autologous stem cell transplant recipients will not require immunosuppressive drugs to combat rejection.

Stem cells may be totipotent, multipotent or unipotent; i.e., able to differentiate into any tissue, several types of tissue or one type of tissue, respectively. The process by which stem cells are derived from one type of tissue and differentiate into other types of tissue is referred to as plasticity or transdifferentiation. Multipotent stem cells consist of three major types — ectodermal (skin and nerves), mesodermal or mesenchymal (bone, cartilage, muscle and adipose), and endodermal (intestines and other). The two main categories of stem cells are embryonic stem cells and adult stem cells, defined by their source.

### Embryonic stem cells

Embryonic stem cells (ESCs) are derived from the cells of the inner cell mass of the blastocyst during embryonic development. ESCs have the capacity to differentiate into any

cell type and the ability to self-replicate for numerous generations. A potential disadvantage of ESCs is their ability to differentiate into any cell lineage and to proliferate endlessly unless controlled.<sup>7</sup> The clinically observed teratoma is a tumor that is an example of ESCs growing into a “different and undesired tissue.” ESCs can be obtained only from embryos, and therefore are associated with ethical issues.

### Adult stem cells

Sources of adult stem cells include the umbilical cord, amniotic fluid, bone marrow, adipose tissue, brain and teeth.<sup>8,9</sup> Adult stem cells are not subject to the ethical controversy that is associated with embryonic stem cells; they can also be autologous and isolated from the patient being treated, whereas embryonic stem cells cannot.

### Induced pluripotent stem cells (iPS)

The newly discovered iPS cells are adult or somatic stem cells that have been coaxed to behave like embryonic stem cells.<sup>10,11</sup> iPS cells have the capacity to generate a large quantity of stem cells as an autologous source that can be used to regenerate patient-specific tissues. However, even the authors of these recent reports have cautioned that any carcinogenic potential of iPS cells should be fully investigated before any commercialization can be realized.

### Amniotic fluid-derived stem cells (AFSCs)

AFSCs can be isolated from aspirates of amniocentesis during genetic screening. An increasing number of studies have demonstrated that AFSCs have the capacity for remarkable proliferation and differentiation into multiple lineages such as chondrocytes (for cartilage), adipocytes (for fat), osteoblasts (for bone), myocytes (for muscle), endothelial cells, neuron-like cells and live cells.<sup>12,13,14,15,16,17</sup> The potential therapeutic value of AFSCs remains to be discovered.

### Umbilical cord blood stem cells (UCBSCs)

UCBSCs derive from the blood of the umbilical cord.<sup>18</sup> There is a growing interest in their capacity for self-replication and multilineage differentiation, and UCBSCs have been differentiated into several cell types that resemble cells of the liver, skeletal muscle, neural tissue, pancreatic cells, immune cells and mesenchymal stem cells.<sup>19,20,21,22,23,24</sup> Several studies have shown the differentiation potential of human UCBSCs in treating cardiac<sup>25</sup> and diabetic diseases in mice.<sup>26</sup> The greatest disadvantage of UCBSCs is that there is only one opportunity to harvest them from the umbilical cord at the time of birth. Similarly, amniotic stem cells can be sourced only from amniotic fluid and are therefore subject to time constraints.

### Bone marrow-derived stem cells (BMSCs)

BMSCs consist of both hematopoietic stem cells that generate all types of blood cells and stromal cells (mesenchymal stem cells) that generate bone, cartilage, other connective tissues

Table 3. Comparison of stem cell sourcing

	Ethical issues	Harvest time limited	Autogenous an option	Invasive
<b>Embryonic stem cells</b>	Yes	Yes	No	Yes
<b>Adult stem cells</b>				
iPS	No	No	Yes	Yes
Amniotic fluid-derived	No	Yes	Yes	Yes
Umbilical cord-derived	No	Yes	Yes	Yes
Bone marrow-derived	No	No	Yes	Yes
Adipose-derived	No	No	Yes	Yes
Dental-derived	No	No	Yes	No*

\* Exfoliating teeth are non-invasive sources of stem cells. Extracted teeth are minimally-invasive sources of stem cells, involving no additional trauma if the teeth are already being extracted.

and fat. BMSCs are currently the most common commercially available stem cell.<sup>27</sup> They can be isolated from bone marrow aspiration or from the collection of peripheral blood-derived stem cells following chemical stimulation of the bone marrow, by means of subcutaneous injection, to release stem cells.<sup>28</sup>

#### **Adipose-derived stem cells (ASCs)**

ASCs are typically isolated from lipectomy or liposuction aspirates. They have been differentiated into adipocytes, chondrocytes, myocytes, and neuronal and osteoblast lineages, and may provide hematopoietic support.<sup>29,30,31,32,33,34</sup> ASCs express some, but certainly not all, of the cell markers that bone marrow MSCs express.<sup>35,36,37,38</sup> While ASCs have an advantage in that adipose tissue is plentiful in many individuals, accessible and replenishable, the ability to reconstitute tissues and organs using ASCs versus other adult stem cells has yet to be comprehensively compared and documented.

#### **Dental stem cells (DSCs)**

Dental stem cells (DSCs) can be obtained from the pulp of the primary and permanent teeth, from the periodontal ligament, and from other tooth structure.<sup>39</sup> Periodontal ligament-derived stem cells are able to generate periodontal ligament and cementum.<sup>40</sup> Extracted third molars; exfoliating/extracted deciduous teeth; and teeth extracted for orthodontic treatment, trauma or periodontal disease are all sources of dental stem cells from the dental pulp. The dental pulp offers a source of stem cells postnatally that is readily available, with a minimally invasive process that results in minimal trauma.

Exfoliating or extracted deciduous teeth offer extra advantages over other teeth as a source of stem cells. Stem cells from deciduous teeth have been found to grow more rapidly than those from other sources, and it is believed that this is because they may be less mature than other stem cells found in the body. Additional advantages of sourcing stem cells from exfoliating deciduous teeth are that the cells are readily available, provided they are stored until they may be needed later in life; the process does not require a patient to sacrifice a tooth to source the stem cells; and there is little or no trauma.

The structures of interest to the dental profession are the enamel; dentin; dental pulp; cementum; periodontal ligament; craniofacial bones; temporomandibular joint, including bone, fibrocartilage and ligaments; skeletal muscles and tendons; skin and subcutaneous soft tissue; salivary glands; and so forth. Without exception, neural crest-derived and/or mesenchymal cells form all these dental, oral and craniofacial structures during native development. Several populations of adult stem cells have been explored for the regeneration of dental, oral and craniofacial structures, including BMSCs, ASCs and DSCs,<sup>41,42,43,44,45,46</sup> which, despite important differences between them, are likely the subfamily of mesenchymal stem cells.<sup>47,48</sup>

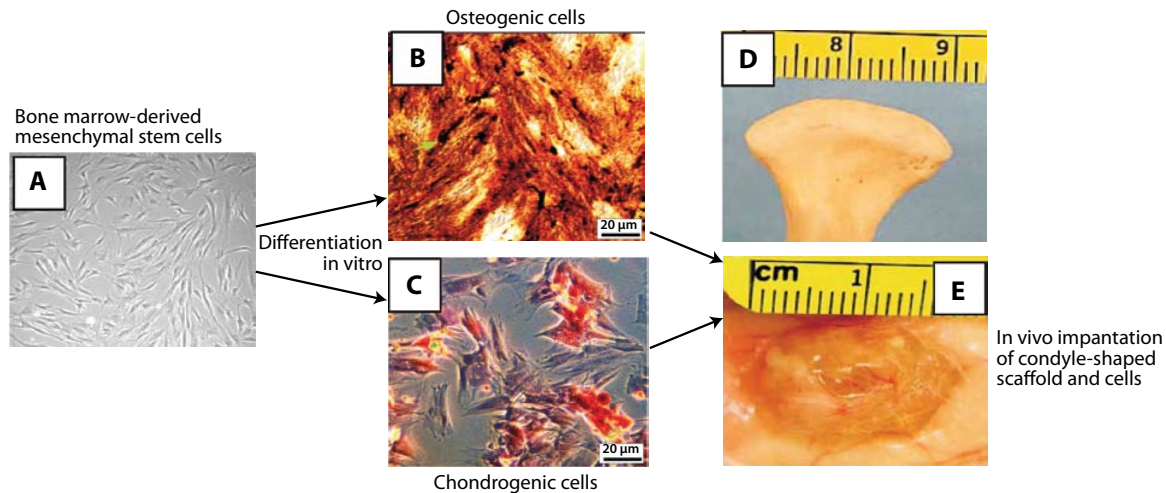
#### **Mesenchymal stem cells**

Mesenchymal stem cells (MSCs) in general have several important properties: adherence to cell culture polystyrene, self-replication to multiple passages and differentiation into multiple cell lineages. Mesenchymal cells natively form connective tissue, including bone, cartilage, adipose tissue, tendon and muscle, and participate in the formation of many craniofacial structures.<sup>49,50,51,52,53,54,55,56,57,58,59,60</sup> MSCs can differentiate into multiple cell lineages, including but not limited to chondrocytes, osteoblasts, myoblasts and adipocytes.<sup>61,62,63,64,65</sup> MSCs have been viewed as the yardstick by which to measure the regeneration of musculoskeletal tissues and have been utilized in the regeneration of non-musculoskeletal tissues such as cardiac and neural tissues. MSCs can be isolated from the patient who needs the treatment, and therefore can be used autologously without concern for immunorejection. MSCs have also been used allogeneically and been shown to heal large defects.<sup>66,67,68,69</sup>

### **Applications/Tissue Engineering With Stem Cells**

Stem cells from a tiny amount of tissue, such as the dental pulp, can be multiplied or expanded to potentially sufficient numbers for healing large, clinically relevant defects. Stem cells differentiating into multiple cell lineages offer the possibility that a common (stem) cell source can heal many tissues

Figure 2. Tissue engineering of the synovial joint condyle from mesenchymal stem cells



The tissue engineered articular condyle retained the shape and dimensions of the adult human cadaveric mandibular condyle (D).

in the same patient, as opposed to harvesting healthy autologous tissue to heal like tissue. Finally, stem cells can be seeded in biocompatible scaffolds in the shape of the anatomical structure that is to be replaced.<sup>70</sup> Scaffolds must provide support and should be resorbed when healing has occurred.

The fundamental reasons for the effectiveness of stem cells are as follows:

- Unlike end-lineage cells, stem cells can be expanded ex vivo (outside the body). Thus a small number of stem cells can be sufficient to heal large defects or to treat diseases. In contrast, a large number of end-lineage cells need to be harvested for tissue regeneration, necessitating donor site trauma and defects.
- Stem cells may elaborate and organize tissues in vivo, especially in the presence of vasculature.
- Stem cells may regulate local and systemic immune reactions of the host in ways that favor tissue regeneration.
- Stem cells may provide a renewable supply of tissue-forming cells.

### Tissue Differentiation

Experimental data has shown that a single population of mesenchymal stem cells can differentiate into chondrocytes, myoblasts, osteoblasts and adipocytes. Stem cell-derived chondrocytes can be used for the reconstruction of orofacial cartilage structures such as the nasal cartilage and the temporomandibular joint. Stem cell-derived osteoblasts can be used to regenerate oral and craniofacial bones. Stem cell-derived myocytes can be used to treat muscular dystrophy and facial muscle atrophy. Stem cell-derived adipocytes can be used to generate soft tissue grafts for facial soft tissue or breast reconstructions, and may eliminate the need for autologous tissue grafting. Why bother deriving end-lineage cells from stem cells, instead of using end-lineage cells such as chondrocytes or adipocytes?

The short response is that end-lineage cells have a limited life span and cannot self-renew, whereas stem cells, by definition, self-renew and can replenish the supply of end-lineage cells.

### Bone and Craniofacial Regeneration

An estimated 1.6 million bone grafts are performed annually in the United States, of which approximately 6 percent are craniofacial grafts.<sup>71</sup>

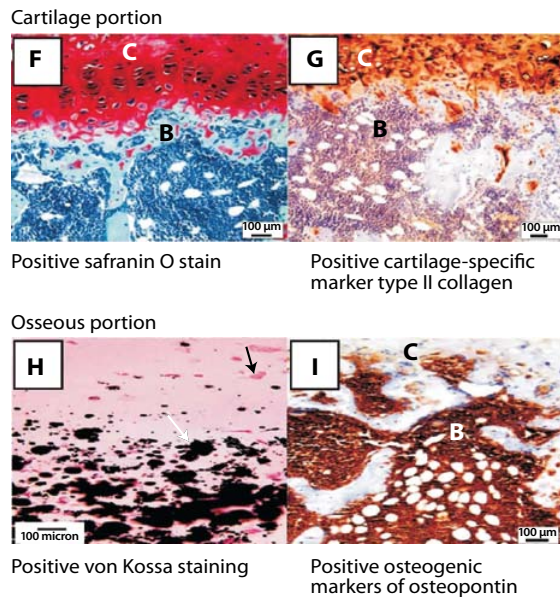
Bone grafts can be autologous, allogeneous or xenogeneous in nature. Autologous bone grafting is often considered the clinical gold standard. The use of stem cells is superior to autologous bone grafting. Why? Autologous tissue grafting involves harvesting healthy bone from the patient's iliac crest, rib bone, femur, chin or retro-molar area. Thus, the key drawback is donor site trauma and morbidity.<sup>72</sup> In contrast, stem cell-based therapeutic approaches could circumvent this drawback. Clinical studies are being conducted using stem cells for alveolar ridge augmentation<sup>73,74</sup> and long-bone defects. Vascularized bone grafts are also in development using stem cells, and reconstruction of a patient's resected mandible has been carried out using this technique.<sup>75</sup>

### Craniofacial regeneration

As an example of craniofacial regeneration, stem cells have been used in the tissue engineering of a human-shaped temporomandibular joint.<sup>76,77,78,79</sup> MSCs were first isolated from bone marrow and exposed separately to either chondrogenic or osteogenic supplemented culture medium (Figure 2).<sup>80,81</sup> MSC-derived cells were encapsulated in a poly(ethylene glycol) diacrylate (PEGDA) hydrogel that was molded into an adult human mandibular condyle in stratified yet integrated layers of cartilage and bone. The osteochondral grafts in the shape of human TMJs were implanted in immunodeficient mice for up to 12 weeks.

Upon harvest, the tissue-engineered mandibular joint condyles retained their shape and dimensions. The chondrogenic and osteogenic portions remained in their respective layers, as demonstrated by positive staining using chondrogenic and osteogenic markers (Figure 3). Lastly and most importantly, there was mutual infiltration of the cartilaginous and osseous components into each other's territory that resembled mandibular condyle.<sup>82</sup>

Figure 3. Histology of the tissue engineered articular condyle



The cartilage portion and the bone portion remained integrated

### Medical Applications

Craniofacial stem cells, including dental stem cells, originate from neural crest cells and mesenchymal cells during development.<sup>83,84</sup> Conceptually, tooth-derived stem cells have the potential to differentiate into neural cell lineages. Indeed, deciduous dental stem cells<sup>85</sup> and bone marrow-derived stem cells<sup>86</sup> both express neural markers.

The expression of neural markers in dental stem cells elicits imagination of their potential use in neural regeneration, as in the treatment of Parkinson's disease, for which there is currently no cure. Adult dental stem cells have been and are being investigated to treat Parkinson's disease, which currently affects an estimated 1 million people in the United States, as well as to treat related neurological diseases and spinal cord injuries. In addition, they appear to replace dead neural cells and support degenerating neural cells.<sup>87</sup>

Stem cells are also being investigated for the development of myocardial cells to repair damaged heart muscle following cardiac infarct. Heart failure affects more than 5 million people in the United States alone. Mesenchymal stem cells have been found to be able to differentiate into myocardial cells and vascular epithelium,<sup>88</sup> as well as to release molecules that are protective for cardiac cells.<sup>89</sup> Patients have already

been treated with MSCs following cardiac infarcts to regenerate heart muscle and improve function.<sup>90,91</sup> It has also been possible to engineer functioning bladders using autologous adult stem cells.<sup>92</sup>

Stem cells are believed to modulate the immune system and are currently being investigated for use in the treatment of graft-versus-host disease, Crohn's disease and lupus.<sup>93,94</sup> DSCs isolated from dental pulp have been found to exhibit immunoregulatory and immunosuppressive properties.<sup>95</sup> Other potential uses include stem cell-derived insulin-producing cells to treat diabetes and MSCs for tissue regeneration following radiation-induced damage.<sup>96</sup> Clinical manipulation of stem cells' DNA is also leading to the development of gene therapies.

Table 5. Medical stem cell applications under investigation

<ul style="list-style-type: none"> <li>• Parkinson's disease</li> <li>• Other neurological diseases</li> <li>• Spinal cord injuries</li> <li>• Bone grafting</li> <li>• Cardiac disease</li> <li>• Urological</li> <li>• Immune system modulation</li> </ul>	<ul style="list-style-type: none"> <li>• Crohn's disease</li> <li>• Lupus</li> <li>• Diabetes</li> <li>• Genetic disorders (gene therapy)</li> <li>• Radiation-induced damage</li> </ul>
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The expression of certain end-cell lineage markers by stem cells represents only the first of many steps toward the treatment of a disease. The potential of dental stem cells in both dental and non-dental regeneration should be further explored.

### Dental Applications

Patients come to the dentist because of infections, trauma, congenital anomalies or diseases such as orofacial cancer and salivary gland disorders. Whereas native tissue is missing in congenital anomalies, conditions such as caries or tumor resection result in tissue defects. For centuries, dentistry has been devoted to the healing of defects with durable materials or the patient's own (autologous) tissue. However, amalgam, composites and even titanium dental implants can fail, and all have limited service time.<sup>97</sup>

Why are stem cells better than durable implants such as titanium dental implants? A short response to this question is that stem cell therapy could potentially lead to the regeneration of tooth roots, with periodontal ligaments that can remodel with host bone, which would be functionally superior to titanium dental implants.

Table 6. Dental applications under investigation

<ul style="list-style-type: none"> <li>• Craniofacial regeneration</li> <li>• Cleft lip and palate</li> <li>• Tooth regeneration</li> <li>• Pulp regeneration</li> </ul>	<ul style="list-style-type: none"> <li>• Periodontal ligament regeneration</li> <li>• Enamel and dentin production</li> </ul>
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Tooth root and supporting periodontal ligaments have been regenerated from dental stem cells in research.<sup>98</sup> Cells

from tooth buds can also differentiate into a small tooth structure when used with a carrier and transplanted in vivo.<sup>99</sup> Pulpal and dentin repair using dental stem cells is also under investigation, and the mesenchymal stem cells from the pulp are under investigation for their ability to produce dentin.<sup>100</sup> The follicle associated with third molars is under investigation for its ability to produce enamel.<sup>101</sup>

### Stem Cell Handling and Cryopreservation

Stem cells are released from small amounts of tissue, in the case of dental stem cells from dental pulp. The tissue is placed in an enzyme solution that releases the stem cells, which are then cultured to multiply. This can be accomplished using serum-free medium, removing the need for use of animal serum. Differentiation then occurs and the cells are transplanted – either alone or with a scaffold or other biomaterials, depending on the application.

### Cryopreservation

Stem cells must be derived from living tissue and must be preserved. This is achieved by cryopreservation. The cells are rapidly cooled to subzero temperatures as low as  $-196^{\circ}$  Celsius, stopping any cellular or biochemical activity. Rapid freezing is necessary to prevent ice from forming around or inside the cells and to prevent dehydration, as these would cause cell damage and death.

Extracted permanent and deciduous (including exfoliating) teeth can be preserved for future use with cryopreservation. Research has demonstrated that stem cells derived from the dental pulp of extracted third molars retain the ability to differentiate into multiple cell types following thawing after cryopreservation using liquid nitrogen.<sup>102</sup> Stem cells derived from the periodontal ligament are viable following cryopreservation.<sup>103</sup> After two years of cryopreservation, stem cells have been able to differentiate and to proliferate, and it has been concluded that DSCs can undergo long-term cryopreservation.<sup>104</sup> Companies are currently engaged in the collection and cryopreservation of deciduous teeth for patients' potential use in later life (StemSave, BioEden, Baby Teeth Cell Bank).

Figure 4. Banking Teeth



### Summary

Physicians and scientists have recommended that umbilical cord stem cells and amniotic fluid stem cells be banked for potential applications in the treatment of trauma and pathological disorders. Similarly, it is now possible to cryopreserve healthy teeth as sources of autogenous stem cells, either as they are exfoliated or by extraction, should these be needed in future years to treat diseases and/or conditions that the patient develops. With all we have learned about stem cells and tissue engineering of dental, oral and craniofacial structures, we are in a position to bring the awareness to patients regarding proper storage of their extracted teeth in conditions that will preserve craniofacial stem cells, including dental stem cells.

Banking teeth and dental stem cells offers patients a viable alternative to using more invasive or ethically problematic sources of stem cells, and harvesting can be done during routine procedures in adults and from the deciduous teeth of children. Now, dental professionals have the opportunity to make their patients aware of these new sources of stem cells that can be conveniently recovered and remotely stored for future use as new therapies are developed for a range of diseases and injuries.

Our understanding of mesenchymal stem cells in the tissue engineering of systemic, dental, oral and craniofacial structures has advanced tremendously.<sup>105,106,107,108,109,110</sup> The conservative treatment of life-threatening and disfiguring de-

### Glossary of Terms

- Allogeneous** — Refers to cells or tissues transplanted from a different individual.
- Autologous** — Refers to cells or tissues that are reimplanted in the same individual they come from.
- Biocompatible scaffold** — A structure that is compatible biologically with the cells and tissue and that acts as a structure or support for the shape of the part being engineered.
- Cryopreservation** — The process by which cells are frozen at very low temperatures under controlled conditions to store them until they are needed.
- Differentiation** — The process by which unspecialized stem cells acquire the features of specialized cells such as heart, liver or muscle cells.
- Hematopoietic stem cells** — Stem cells from which all red and white blood cells develop.
- Pluripotent** — Ability of a single stem cell to develop into many different cell types of the body.
- Regenerative or reparative medicine** — A treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or depleted adult cell populations or tissues.
- Mesenchymal stem cells** — Stem cells that have the ability to differentiate into many cell types, including bone, cartilage, fat, muscle, cardiac and neural (nerve) tissues. Also known as stromal stem cells.
- Transdifferentiation or plasticity** — The ability of stem cells from one adult tissue to generate the differentiated cell types of another tissue.
- Xenogeneos** — Derived from one species and used to treat a patient of a different species (for instance, bovine bone used to treat a human patient).

fects and diseases and the ability to treat currently incurable diseases are becoming a reality. The impact of this paradigm shift in health care will eventually be seen in every medical and dental office and setting.

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