# Stem Cells and the Future of Dental Care

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**Abstract**

What are stem cells? As dentists, why should we be concerned with stem cells? How would stem cells change dental practice? Is it possible to grow a tooth or TMJ with stem cells? This article summarizes the latest stem cell research and development for dental, oral and craniofacial applications. Stem cell research and development will, over time, transform dental practice in a magnitude far greater than did amalgam or dental implants. Metallic alloys, composites and even titanium implants are not permanent solutions. In contrast, stem cell technology will generate native tissue analogs that are compatible with the patient's own.

STEM CELLS CAN BE DEFINED as cells that 1. self-replicate and 2. are able to differentiate into at least two different cell types. Both conditions must be present for a cell to be called a stem cell. For example, osteoblasts are not stem cells. Although osteoblasts differentiate into osteocytes, they typically do not differentiate into other cell types except osteocytes. Osteocytes are not stem cells; they are end-lineage cells that typically neither self-replicate nor differentiate.

## Different Types of Stem Cells

*Embryonic stem cells (ES)* refer to the cells of the inner cell mass of the blastocyst during embryonic development. ES are particularly notable for their two fundamental properties: the capacity to differentiate into any cell type in the body and the ability to self replicate for numerous generations (Lyons and Rao, 2007). One potential disadvantage of human ES, besides ethical issues, is precisely their virtually unlimited proliferation and differentiation capacity (Ryu et al., 2004). The clinically observed teratoma is an example of ES growing into wrong tissues. To date, little attempt has been made towards the use of ES in dental, oral and craniofacial regeneration.

*Amniotic fluid-derived stem cells (AFS)* can be isolated from aspirates of amniocentesis during genetic screening. An increasing number of studies have demonstrated that AFS have the capacity for remarkable proliferation and differentiation into multiple lineages, such as chondrocytes, adipocytes, osteoblasts, myocytes, endothelial cells, neuron-like cells and live cells (Barria et al., 2004; Prusa et al., 2004; De Gemmis et al., 2006; De Coppi et al., 2007; Kolambkar et al., 2007; Perin et al., 2007). The potential therapeutic value of AFS remains to be discovered.

*Umbilical cord stem cells (UCS)* derive from the blood of the umbilical cord. There is growing interest in their capacity for self-replication and multi-lineage differentiation (Laughlin et al. 2001). UCS have been differentiated into several cell types, such as cells of the liver, skeletal muscle, neural tissue and immune cells (Warnke et al.,

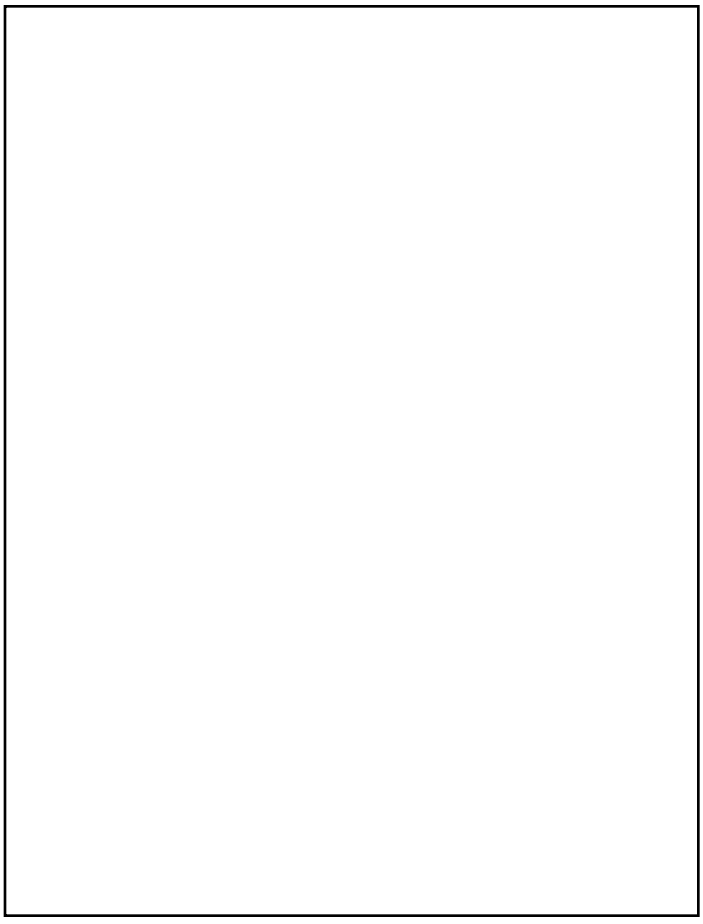
2004; Young et al. 2004). Their high capacity for multi-lineage differentiation is likely attributed to the possibility that UCS are chronologically closer derivatives of embryonic stem cells than adult stem cells. Several studies have shown the potential of UCS in treating cardiac and diabetic diseases in mice (Rebel et al. 1996; Tocci et al. 2003; Lee et al. 2005). UCS are neither embryonic stem cells, nor are they viewed as adult stem cells.

*Bone marrow-derived mesenchymal stem cells*. When bone marrow is aspirated and cultured, a subset of adherent and mononuclear cells are mesenchymal stem cells (MSCs) (Alhadlaq and Mao, 2004; Marion and Mao, 2006). Bone marrow-derived MSCs can self-replicate and have been differentiated, under experimental conditions, into osteoblasts, chondrocytes, myoblasts, adipocytes and other cell types, such as neuron-like cells, pancreatic islet beta cells, etc. (Alhadlaq and Mao, 2004; Kim et al., 2006; Marion and Mao, 2006). Bone marrow-derived MSCs are currently being investigated in broad applications, such as cartilage defects in arthritis, bone defects, adipose tissue grafts, cardiac infarcts, liver disease and neurological regeneration. MSCs are often viewed as a yardstick of adult stem cells.

*Tooth-derived stem cells (TS)* are isolated from the dental pulp, periodontal ligament—including the apical region—and other tooth structures (Gronthos et al., 2000; Shi et al., 2001; Batouli et al., 2003; Miura et al., 2003; Mao et al., 2006). Craniofacial stem cells, including TS, originate from neural crest cells and mesenchymal cells during development (Zhang et al., 2006; Takashima et al., 2007). Neural crest cells share the same origin as progenitor cells that form the neural tissue. Conceptually, TS have the potential to differentiate into neural cell lineages. Indeed, TS from the deciduous tooth have been induced to express neural markers such as nestin (Miura et al., 2003). Similarly, bone marrow-derived stem cells also have been induced to express neural cell markers (Kim et al., 2006). The expression of neural markers in TS elicits imagination of their potential use in neural regeneration, such as in the treatment of Parkinson's disease. However, the expression of certain end cell lineage markers by stem cells only represents the first of many steps towards the treatment of a disease. In balance, the potential of TS in both dental and non-dental regeneration should be further explored. TS that have been isolated to date, either from deciduous teeth or permanent teeth, are considered postnatal stem cells or adult stem cells.

*Adipose-derived stem cells (AS)* are typically isolated from lipectomy or liposuction aspirates. AS have been differentiated into adipocytes, chondrocytes, myocytes, neuronal and osteoblast lineages (De Ugarte et al., 2003; Zuk et al., 2002; Peptan et al., 2006). AS can self-replicate for many passages without losing the ability to further differentiate (De Ugarte et al., 2003; Zuk et al., 2002; Gimble et al., 2007). Many believe that AS have advantages over other adult stem cell populations, for adipose tissue is abundant in certain individuals, readily accessible and replenishable. However, the ability to reconstitute tissues and organs by AS versus other adult stem cells has yet to be comprehensively documented.

*Induced pluripotent stem cells (iPS)* refer to adult or somatic stem cells that have been coaxed to behave like embryonic stem cells. Recent reports have shown that the transduction of a small number of genes or transcription factors, as few as four, transforms adult fibroblasts into cells that proliferate and differentiate into ES-like cells. The four genes are Oct3/4, Sox2, Klf4, and c-Myc in Takahashi et al. (2007),



but Oct4, Sox2, Nanog, and Lin28 in Yu et al. (2007). The biological and political implications of these studies are quite significant. On the biological front, the induced human somatic cells or iPS cells have the capacity to generate a large quantity of stem cells as an autologous cell source that can be used to regenerate patient-specific tissues. On the political front, iPS cells appear to minimize the need for human embryonic stem (ES) cells. However, even the authors of these recent reports have cautioned that any carcinogenic potential of iPS should be fully investigated before any commercialization can be realized.

## Stem Cells and Dental, Oral, Craniofacial Structures

Structures of interest to the dental profession include the enamel; dentin; dental pulp; cementum; periodontal ligament; craniofacial bones; the temporomandibular joint, including bone; fibrocartilage and ligaments; skeletal muscles and tendons; skin and subcutaneous soft tissue; and salivary gland. Without exception, all these dental, oral and craniofacial structures are formed by neural crest-derived and/or mesenchymal cells during native development.

Since cells are the centerpiece of growing tissue or organs, the immediate question is how to get hold of the cells that generate dental, oral and craniofacial tissues? Among all possible stem cell sources, adult stem cells have a number of advantages over embryonic stem cells, umbilical cord stem cells and amniotic fluid stem cells for regeneration of many dental, oral and craniofacial structures. Adult stem cells are chronologically closer to the target dental, oral and craniofacial structures than embryonic stem cells, umbilical cord stem cells and amniotic fluid stem cells. Adult stem cells are not subjected to the ethical controversy associated with embryonic stem cells. Adult stem cells can be autologous and isolated from the patient, whereas embryonic stem cells cannot be autologous. It is also impossible for amniotic fluid stem cells or umbilical cord stem cells to be used as autologous cells until these cells are banked. The risk of immune rejection is present for non-autologous cells, whereas autologous stem cells are free from immune rejection.

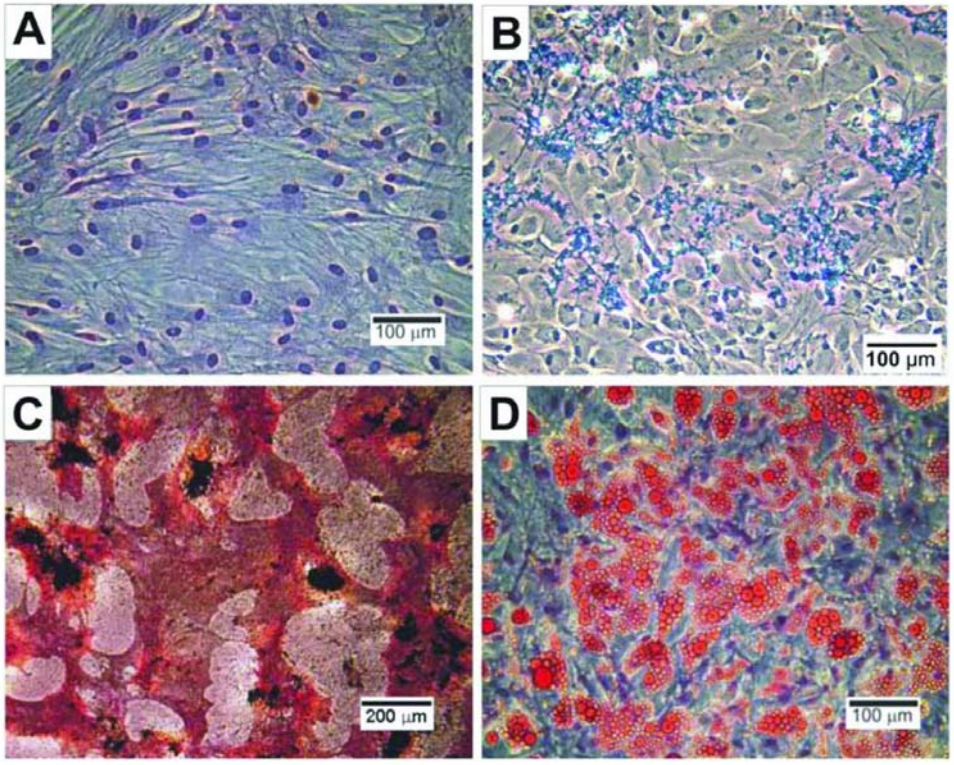



**Figure 1.**
A: Human mesenchymal stem cells (MSCs) isolated from anonymous adult human bone marrow donor following culture expansion (H&E staining). Further enrichment of MSCs can be accomplished by positive selection using cell surface markers, including STRO-1, CD133 (prominin, AC133), p75LNGFR (p75, low-affinity nerve growth factor receptor), CD29, CD44, CD90, CD105, c-kit, SH2 (CD105), SH3, SH4 (CD73), CD71, CD106, CD120a, CD124, and HLA-DR or negative selection (Alhadlaq and Mao, 2004; Marion and Mao, 2006).

B: Chondrocytes derived from human mesenchymal stem cells showing positive staining to Alcian blue. Additional molecular and genetic markers can be used to further characterize MSC-derived chondrocytes (Alhadlaq and Mao, 2004; Marion and Mao, 2006).

C: Osteoblasts derived from human mesenchymal stem cells showing positive von Kossa staining for calcium deposition (black) and active alkaline phosphatase enzyme (red). Additional molecular and genetic markers can be used to further characterize MSC-derived chondrocytes (Alhadlaq and Mao, 2004; Marion and Mao, 2006).

D: Adipocytes derived from human mesenchymal stem cells showing positive Oil Red-O staining of intracellular lipids. Additional molecular and genetic markers can be used to further characterize MSC-derived chondrocytes (Alhadlaq and Mao, 2004; Marion and Mao, 2006).

Bone marrow-derived, tooth-derived and adipose-derived stem cells, despite important differences among them, likely belong to subfamilies of mesenchymal stem cells (Marion and Mao, 2006; Gimble et al., 2007). Most dental, oral and craniofacial structures are connective tissue. During native development, dental, oral and craniofacial connective structures are formed by neural crest-derived and mesenchymal cells. Postnatally, clusters of mesenchymal cells continue to reside in various tissues and are the logical sources of adult mesenchymal stem cells (Marion and Mao, 2006).

MSCs can be isolated from the patient who needs treatment, and, therefore, they can be used autologously without immunorejection. MSCs have also been used allogeneically to heal large defects (Alhadlaq and Mao, 2004; Marion and Mao, 2006; Barrilleaux et al., 2006; Prockop, 2007). Figure 1 provides experimental data showing that a single population of mesenchymal stem cells can differentiate into chondrocytes, osteoblasts and adipocytes (Marion and Mao, 2006). Each of the differentiated cell lineages has implications in the treatment of a corresponding disorder. For example, MSC-derived chondrocytes can be used for reconstruction of orofacial cartilage structures, such as nasal cartilage and the temporomandibular joint. MSC-

derived osteoblasts can be used to regenerate oral and craniofacial bones. MSC-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 reconstruction and augmentation.

## Stem Cells and Dental Practice

Patients come to the dentist because of infections, trauma, congenital anomalies or other diseases, such as orofacial cancer and salivary gland disorders. Caries and periodontal disease remain highly prevalent disorders among humans. Whereas native tissue is missing in congenital anomalies, diseases such as caries or tumor resection result in tissue defects. For centuries, dentistry has been devoted to healing defects with durable materials or the patient's own (autologous) tissue. But we now realize that metallic alloys or synthetic materials are not permanent solutions (Rahaman and Mao, 2005). Amalgam, composites and even titanium dental implants can fail; and all have limited service time (Rahaman and Mao, 2005).

Why are stem cells better than durable implants such as titanium dental implants? A short answer to this question is that stem cells lead to the regeneration of teeth with periodontal ligament that can remodel with the host.

Why are stem cells superior to autologous tissue grafts? Autologous tissue grafting is based on the concept that a diseased or damaged tissue must be replaced by like tissue that is healthy. Thus, the key drawback of autologous tissue grafting is donor site trauma and morbidity. For example, we currently harvest healthy bone from the patient. We might take from the iliac crest, rib bone, chin or retro-molar area for bone grafting needs in cleft palate, ridge augmentation, sinus lifting, and maxillary and mandibular reconstruction.

In contrast, stem cell-based therapeutic approaches may circumvent the key deficiencies of autologous bone grafting (Rahaman and Mao, 2005). Stem cells from a tiny amount of tissue, such as the dental pulp, can be multiplied or expanded potentially to sufficient numbers for healing large, clinically relevant defects. Stem cells can differentiate into multiple cell lineages, thus providing the possibility that a common (stem) cell source can heal many tissues in the same patient, as opposed to the principle of harvesting healthy tissue to heal like tissue in association with autologous tissue grafting (Moioli et al., 2007). Stem cells can be seeded in biocompatible scaffolds in the shape of the anatomical structure that is to be replaced (Rahaman and Mao, 2005). Stem cells may elaborate and organize tissues *in vivo*, especially in the presence of vasculature. Finally, stem cells may regulate local and systemic immune reactions of the host in ways that favor tissue regeneration.

*The progress of stem cell-based technologies also depends on the regulatory pathways of the FDA in the United States and equivalent regulatory agencies elsewhere.*

When will each stem cell-based technology be available for dental and oral surgery practice? Some of the near-term applications, such as growth factor delivery, are approved or are being reviewed by the FDA, whereas others are being investigated at various stages of product development. However, it is impossible to provide the precise timeline of clinical application for a myriad of dental, oral and craniofacial diseases. Science does not progress linearly, and breakthrough is not always predicted.

Furthermore, the progress of stem cell-based technologies also depends on the regulatory pathways of the FDA in the United States and equivalent regulatory agencies elsewhere. What can be predicted is that stem cell-generated tissue analogs will be available for clinical use for certain tissues before others. The first wave of this paradigm shift in dental health care is upon us now. The impact of this paradigm shift will eventually be present in every dental practice.

Physicians and scientists have recommended that umbilical cord stem cells and amniotic fluid stem cells be banked for potential application in the treatment of trauma and pathological disorders. Our understanding of mesenchymal stem cells in the tissue engineering of dental, oral and craniofacial structures has advanced tremendously (Krebsbach et al., 1999; Pittenger et al., 1999; Bianco et al., 2001; Alhadlaq and Mao, 2004; Mao et al., 2006; Marion and Mao, 2006). We have witnessed tissue engineering of the tooth, temporomandibular joint condyle, cranial sutures, soft tissue grafts, craniofacial bone, and other dental, oral and craniofacial structures in animal models (review: Mao et al., 2006).

With all that we have learned about stem cells and tissue engineering of dental, oral and craniofacial structures, we are in a position to bring awareness to our patients regarding the proper storage of their extracted teeth in conditions that will preserve craniofacial stem cells, including tooth-derived stem cells. These include, but are not limited to, extracted wisdom teeth, extracted deciduous teeth, any teeth extracted for orthodontic purposes and any non-infected teeth extracted.

Among postnatal tissues that are sources of stem cells that are obtainable without substantial trauma are extracted wisdom teeth, exfoliating or extracted deciduous teeth, teeth extracted for orthodontic treatment, trauma or periodontal disease.

Craniofacial stem cells, including tooth-derived stem cells, have the potential, as do bone marrow-derived stem cells and adipose-derived stem cells, to cure a number of diseases that are relevant to dentistry as well as medicine, among them, diabetes, Parkinson's disease and cardiac infarct.

## Is it Possible to Grow a Tooth or TMJ with Stem Cells?

As an example of craniofacial regeneration, we have used stem cells in the tissue engineering of a human-shaped temporomandibular joint using MSCs (Alhadlaq and Mao, 2003; Alhadlaq and Mao, 2005; Marion and Mao, 2006; Troken et al., 2007). Given that the mandibular condyle consists of two stratified layers of cartilaginous and bone tissues,

MSCs were first differentiated into chondrogenic and osteogenic cells (Alhadlaq and Mao, 2003; Alhadlaq et al., 2004). MSC-derived chondrogenic and osteogenic cells were encapsulated in a biocompatible hydrogel in two stratified layers molded into the shape and dimensions of an adult human mandibular condyle (Alhadlaq and Mao, 2003; Alhadlaq et al., 2004).

Following *in vivo* implantation in immunodeficient mice for up to 12 weeks, the retrieved mandibular joint condyles retained the shape and dimensions of the native condyle. The chondrogenic and osteogenic portions remained in their respective layers (Alhadlaq and Mao, 2005). The chondrogenic layer was positively stained by chrondrogenic marker, safarnin O, and contained type II collagen. In the interface between cartilaginous and osseous layers, there is a presence of hypertrophic chondrocytes that express type X collagen (Alhadlaq and Mao, 2005). In contrast, only the osteogenic markers, such as osteopontin and osteonectin, stained the osseous layer, but not the cartilage layer.

Lastly and most importantly, there was mutual infiltration of the cartilaginous and osseous components into each other's territory, which resembles mandibular condyle (Alhadlaq and Mao, 2005). Therefore, the proof of principle has been established to regenerate the human-shaped TMJ condyle.

The tooth is a highly complex structure, with a level of complexity equal to that of internal organs, from the perspective of tissue engineering. Dental epithelial and mesenchymal cells isolated from rat or pig teeth have been seeded onto biodegradable scaffolds and implanted in immunodeficient mice. Several studies have shown that a tooth crown has been formed with different layers of enamel, dentin and pulp-like structures (Young et al., 2002; Duailibi et al., 2004; Sumita et al., 2006; Nakao et al., 2007). In vitro-generated tooth germ cells or stem cells have been transplanted into the adult tooth socket, leading to the formation of a tooth crown or root (Nakao et al., 2007; Sonoyama et al., 2007). Current efforts are occurring in several diverse directions, such as the use of sophisticated scaffold materials (Zhang et al., 2005, Moioli et al., 2007); the use of enriched dental stem cell populations (Laino et al., 2006; Shi et al., 2005; Sonoyama et al., 2006; Yen and Sharpe, 2006); and the use of specific dental epithelial and mesenchymal cell ratios and seeding (Hu et al., 2006; Honda et al., 2007).

Overall, the proof of concept has been established to generate biologically derived tooth structures from stem cells. The remaining challenges are along several fronts, including scale up, accelerated tissue maturation and development of viable commercialization approaches.

## Summary

In the dental profession, we treat a myriad of trauma, congenital anomalies and diseases, including tissue defects resulting from dental caries, periodontal bone defects or facial bone defects. These defects not only lead to physical trauma and pain, but they also are detrimental to the psychosocial well-being of patients, given that the oral cavity and the face are intimately involved in self identity, communication and the expression of emotion.

Current treatment approaches utilize the patient's own tissues, allogeneic grafts, metallic alloys or synthetic implants. Much of what we know as dentists is evolving into a new dentistry in which dental care is delivered increasingly by biologically based approaches. For example, biomolecules will be used for periodontal regeneration; stem cells will be used in the regeneration of dentin and/or dental pulp; biologically viable scaffolds will be used to replace orofacial bone and cartilage; the defective salivary gland will be partially or completed regenerated (Rahaman and Mao, 2006; Mao et al., 2006; Mao et al., 2007).

The challenge for the dental professional in the anticipated era of stem cells and tissue engineering is imminent. What would be a dentist's response when patients ask whether they can get their own stem cells if they have their wisdom teeth banked? What are the odds that tooth stem cells will grow a new tooth or be used to treat diabetes? Should I use a growth factor called PDGF or BMP2 to treat my periodontal bone defects or have a bone graft? Should my son's baby teeth be banked for stem cells, and, if so, what are the odds that these baby teeth stem cells will cure a bone fracture he may get during a soccer game?

The dental professional needs to be prepared to provide continuing education courses. Dental schools should consider the addition of stem cells and tissue engineering courses to the existing curriculum. Several textbooks are now available in the area of stem cells, tissue engineering and regenerative medicine (e.g. Mao et al., 2007). Without these and similar measures, dental students, postgraduate students and dental practitioners are likely to be ill-prepared for the upcoming era of stem cell-based technologies.

Several well-established dental supply companies have established, or are establishing, R&D efforts in the area of stem cells and tissue engineering. Federal funding agencies, such as the National Institutes of Health, have been providing research and training grants on a competitive basis to the external research community in the area of stem cells, tissue engineering and regenerative medicine, including regenerative dental medicine, for over a decade (Wang et al., 2007). Strategies for education, training, research, development, commercialization and practice models need to be formulated and implemented. ■

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*Editor's Note: Queries about this article can be sent to Dr. Mao at jmao@columbia.edu. Copies of the extensive references that accompanied Dr. Mao's manuscript are available upon request to The NYSDJ Managing Editor.*