



Stem Cells: Emerging Medical and Dental Therapies and the Dental Professional

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Full Speaker Notes

Introduction Recent exciting new discoveries place dentists at the forefront of helping their patients benefit from potentially life-saving therapies derived from a patient's own stem cells obtained from deciduous teeth and permanent teeth. We now stand at the threshold of a potential revolution in medical treatment for diseases and disorders in which organs stop working properly. At present, some such conditions, such as heart, kidney and liver disease, can be treated by transplantation of a replacement organ from another person. But demand for donor organs is far outstripping supply, and the failure rate of such surgery is quite high, mainly because of the problem of rejection. Many other disorders, such as stroke, diabetes and Alzheimer's disease, cannot presently be treated by transplantation. The great hope is that suitable stem cells, produced in large quantities through cell culture methods and injected into failing tissues and organs, will produce fresh, replacement cells to take over from lost or damaged ones. It is now known that adult stem cells taken from one area of the body can be transplanted into another area and grown into a completely different type of tissue. This ability to grow and regenerate tissues is the focus of the emerging field of personalized medicine which uses a patient's own stem cells for biologically compatible therapies and individually tailored treatments. The Dental professional will play an important role in both the recovery and the use of these stem cells in both Dental and Medical regenerative therapies.

What is a stem cell Stem Cells are the master cells of the body. There are two major types of Stem Cells, Embryonic and Adult stem cells. A single embryonic stem cell has the potential to differentiate into all 220 types of specialized cells that make up the human body. Adult stem cells are responsible for the regeneration and replacement of tissue damaged by disease or injury. There are two properties of stem cells that make it different from any other specialized cells in the body. These properties are self renewal - the ability to go through numerous cycles of cell division while maintaining their undifferentiated state and the ability to differentiate into a specialized cell type. Another unique property of stem cells is their ability to grow in vitro - outside of the body. In the right culture medium and under the right controlled conditions, stem cells are able to proliferate indefinitely. The expansion of Stem cells in this controlled tissue culture and by maintaining them in their undifferentiated state leads to the development of specific stem cell lines. These Stem cell lines are invaluable for present day Stem Cell research and medical therapies.

A stem cell is able to replicate itself or differentiate into many specialized cell types: Nerves, muscle, blood, fat, bone etc.

A unique property of stem cells is that they will proliferate in vitro in a cell culture medium. Days 1, 7, 14 and 21 of Stem Cell Proliferation in vitro.

There are two main types of stem cells. Embryonic that are harvested from the first 50 –150 cells of a 4 – 5 old fertilized egg or blastocyst. And there are the adult stem cells which are present in a fetus and in humans after birth. The adult stem cells are found deep within organs and tissues and are spread diffusely throughout.

Embryonic Stem Cells. The embryonic stem cells are harvested from the inner cell mass of a blastocyst and grown in culture medium. Under the proper environment stem cells can continue to proliferate indefinitely. When these stems cells are subjected to certain enzymes and environmental factors they differentiate into specialized cell types.

Embryonic Stem Cells

Embryonic Stem Cells are the result of the fertilization of an egg by a sperm. Cells produced by the first few divisions of the fertilized egg are **totipotent**. These cells can differentiate into embryonic and extraembryonic cell types. Embryonic stem cells are the cells that make up the human body and extraembryonic will make up the placenta. At about the 5th day this ball of cells becomes a blastocyst, an early stage embryo that is made up of 50 –150 cells.. **Pluripotent** Stem cells are those embryonic stem cells that are harvested after the 5th day from the inner mass of this blastocyst. These pluripotent Stem cells go on to differentiate into any of the tree types of cells that derive the germ layers: ectoderm, endoderm and mesoderm.

Ectoderm gives rise to the epidermis (skin), nails, hair, glands of the skin, the nervous system, the ears, the eyes (including the retina and lens), mammary glands, and the mucous membranes of the mouth and anus. The embryologic neural crest is also derived from ectoderm, which later gives rise to the cranial and spinal ganglia in the central nervous system. **Mesoderm** (Mesenchymal) gives rise to connective tissue, skeleton, bone, cartilage, skeletal muscles, blood and blood vessels, the lymphatic system, the linings of the major cavities of the body (pleura, pericardium, peritoneum), heart, spleen, kidneys and the sex organs. **Endoderm** gives rise to the respiratory tract (trachea, lungs, pharynx), thyroid and parathyroid glands, digestive tract (stomach, liver, pancreas, colon), the lining of the bladder, and the urethra. Stem cells from these germ layers later generate particular cell types as organs and tissues are formed.

As the embryo matures, and the parts of the body start to emerge, the individual stem cells within each future organ or tissue become further specialized so as to be capable of producing only a certain range of possible final cell types. These stem cells are then called **multipotent** or adult stem cells and form around day 14. At a certain stage in the development of these progenitor cells, one or both of the progenitor cells produced by the division of a stem cell becomes 'committed', that is, incapable of further division. These committed progenitor cells continue to *differentiate* and become the end lineage or the normal functional cells of the heart, skin, brain, kidney, and other organs.

Stem cells from the bone marrow are Mesenchymal, either Hematopoietic that produce blood cells or stromal that differentiate into other specialized cell types: fat, cardiac muscle, nerves, skeletal muscle bone etc.

After twenty years of research, there are no approved treatments or human trials using embryonic stem cells. Their tendency to produce teratomas and malignant carcinomas, cause transplant rejection, and form the wrong kinds of cells are just a few of the hurdles that embryonic stem cell researchers still face. Many nations currently have restrictions on either embryonic stem cell research or the production of new embryonic stem cell lines. Because of their combined abilities of unlimited expansion and pluripotency, embryonic stem cells remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

At around the 6th week as the embryo continues to advance in its development it is now considered a fetus. More and more of the pluripotential cells differentiate into multipotent cells that go on to differentiate into the specialized cells that become tissues and organs that make up the human body. These adult stem cells are responsible for the repair and replacement of diseased or damage tissue. Multipotent or adult stem cells are present in the fetus prior to birth but they are the same type of stem cell found at birth and throughout the rest of life.

Comparing the different Stem Cell types, Adult stem cells have the least amount of ethical concerns, are presently being used in medical therapies and are readily accessible.

Adult Stem Cells

To replace lost cells, stem cells typically generate intermediate cells called precursor or progenitor cells, which are no longer capable of self-renewal. However, they continue undergoing cell divisions, coupled with maturation, to differentiate into fully specialized cells.. Adult stem cells are usually designated according to their source and their potential. Adult stem cells are multipotent because their potential is normally limited to one or more lineages of specialized cells. However, a special multipotent stem cell that can be found in bone marrow and dental pulp called the mesenchymal stem cell can produce all cell types of bone, cartilage, fat, muscle, and connective tissues. However, a number of recent studies show that stem cells from one area may be manipulated to grow into cells types of a completely different tissue. This ability is called *transdifferentiation* or *plasticity*, and different types of adult stem cells have varying degrees of plasticity. Modifying the growth medium when stem cells are cultured in vitro or transplanting them to an organ of the body different from the one they were originally isolated from can induce plasticity. The finding of stem cell plasticity carries significant implications for potential cell therapy. For example, if differentiation can be redirected, stem cells of abundant source and easy access, such as blood stem cells in bone marrow or dental pulp cells from teeth, could be used to substitute stem cells in tissues that are difficult to isolate, such as heart and nervous system tissue.

Mesenchymal stem cells taken from the dental pulp and bone marrow are able to transdifferentiate into cartilage, bone and adipose cells.

When discussing transplantation of tissues, two terms are always mentioned and they are both of significant importance. These terms are autogenous and allogenic. Autogenous is the harvest of

tissue from an individual and reimplanting this tissue back into the same individual. The transplantation can be done during the same procedure i.e. iliac crest bone graft to repair a mandibular discontinuity or tissue can be cryopreserved, thawed and then reimplanted at a future date i.e. IVF. The other term is allogenic. Allogenic is the harvest of tissue from one individual and transplanted into another individual. This term is used for organ transplantations. No matter how close the cellular match of the donor is with the recipient there is always the potential for an immune reaction and host rejection of the transplanted tissue. In most instances the recipient requires immune-suppression drugs to prevent rejection and the recipient is always susceptible to host vs. graft disease. When applying these terms to stem cells and future stem cell therapies they would have the same meaning. In principle, some of the patient's own stem cells could be harvested (most likely from bone marrow or dental pulp), multiplied in culture and injected into a diseased or damaged region to produce new cells. Stem cells derived from the patient's own body would have the great advantage that they would not be rejected. This approach has already been successful in experimental animals, with stem cells from bone marrow used to replace damaged heart muscle. It may soon be used in humans to treat heart disease, diabetes, and other such diseases. However, it would not be appropriate for the replacement of tissues that are diseased because of a genetic disorder (such as Muscular Dystrophy or cystic fibrosis), since stem cells from the patient would have the same genetic mistake in their DNA. This strategy would also be inappropriate in acute conditions, demanding immediate treatment, because of the time needed for stem cells to multiply in culture.

Adult stem cells are found in most mature tissues. The Adult stem cells are present in the fetus and in individuals after birth. Like all stem cells, adult stem cells can self-replicate. Their ability to self-renew can last throughout the lifetime of individual organisms. But unlike embryonic stem cells, it is usually difficult to expand certain types of adult stem cells in culture. Adult stem cells reside in specific organs and tissues, but account for a very small number of the cells in tissues. These stem cells are buried deep in organs and tissues and are diffusely spread out, making the collection of these stem cells an invasive procedure in healthy individuals. Such stem cells have been identified in many types of adult tissues, including bone marrow, blood, skin, dental pulp, retina eye, skeletal muscle and brain.

Stem Cell Markers

Stem cells are found in very small populations in the human body and under a microscope they look like any other cell in the tissue or organ from which they are found. Researchers and scientist use stem cell markers to distinguish these rare cells. What are stem cell markers? Coating the surface of every cell in the body are specialized proteins, called receptors that have the capability of selectively binding or adhering to other "signaling" molecules. There are many different types of receptors that differ in their structure and affinity for the signaling molecules. These same cell surface receptors are the stem cell markers. Each cell type, for example a muscle cell, has a certain combination of receptors on their surface that makes them distinguishable from other kinds of cells. The uniqueness of these receptors allows scientists to mark these stem cells. The stem cells markers have are used by researchers to identify and characterize a number of adult stem cells including stem cells found in the pulp of teeth. Stem cell markers are given short-hand names based on the molecules that bind to the stem cell surface receptors. For the

Mesenchymal stem cells it is the Stro – 1+ antigen. Cell-surface glycoprotein on subsets Stro-1+ cells assists in isolating mesenchymal precursor cells, which are multipotent cells that give rise to adipocytes, osteocytes, smooth myocytes, fibroblasts, chondrocytes, and blood cells. Researchers use signaling cells that adhere to the surface receptors to identify the stem cells.

Most Stem cells are diffusely dispersed deep within organs and tissues. There are presently several commercial methods available for the collection of Adult Stem cells. Most of these procedures are time dependent, invasive and costly.

Bone marrow derived mesenchymal stem cells. Bone marrow transplants were the first successful stem Cell therapies. A bone marrow transplant involved a donor and recipient with a close cellular match. The harvest and transplantation were involved procedures that often required a bone marrow aspiration operation for the donor. When bone marrow is aspirated and cultured, a subset of adherent and mononuclear cells are mesenchymal stem cells (MSCs) 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 Bone marrow derived MSCs are currently being investigated in broad applications such as cartilage defects as in arthritis, bone defects, adipose tissue grafts, cardiac infarcts, liver disease and neurological regeneration. Mesenchymal stem cells are often viewed as a yardstick of adult stem cells. Presently peripheral blood Stem Cell collection is being used in place of bone marrow aspiration. A patient is given a Granulocyte Colony-Stimulating Factor (Neupogen) which releases the stem cells from the bone marrow and into the blood stream . Through aphaeresis blood is taken intravenously and circulated through a filtering device where the stem cells are collected.

Adipose derived adult stem cells

Adipose-derived stem cells have also been isolated from human fat, usually by method of liposuction.. This cell population seems to be similar in many ways to mesenchymal stem cells (MSCs) derived from bone marrow. However, it is possible to isolate many more cells from adipose tissue and the harvest procedure itself is less painful than the harvest of bone marrow. Human Adipose-derived stem cells have been shown to differentiate in the lab into bone, cartilage, fat, and muscle, while Adipose-derived stem cells from rats have been converted to neurons which makes Adipose-derived stem cells a possible source for future applications in the clinic. In support of this, current studies in animals suggest that Adipose-derived stem cells might be able to repair significant bony defects and Adipose-derived stem cells have been recently used to successfully repair a large cranial defect in a human patient.

Umbilical cord stem cells derive from the blood of the umbilical cord. There is a growing interest in their capacity for self replication and multi-lineage differentiation. Umbilical cord stem cells have been differentiated into several cell types such as cells of the liver, skeletal muscle, neural tissue and immune cells. Their high capacity for multi-lineage differentiation is likely attributed to the possibility that Umbilical cord stem cells are chronologically closer derivatives of embryonic stem cells than adult stem cells. Several studies have shown the potential of Umbilical cord stem cells in treating cardiac and diabetic diseases in mice Umbilical cord stem cells is neither embryonic stem cell, nor viewed as adult stem cells.

Amniotic fluid-derived stem cells can be isolated from aspirates of amniocentesis during genetic screening or collection at the time of delivery, an increasing number of studies have demonstrated that Amniotic fluid-derived stem cells has the capacity for remarkable proliferation and differentiation into multiple lineages such as chondrocytes, adipocytes, osteoblasts, myocytes, endothelial cells, neuron-like cells and live cells. The potential therapeutic value of Amniotic fluid-derived stem cells remains to be discovered.

Induced pluripotent stem cells derived from epithelial cells

These are not adult stem cells, but rather reprogrammed epithelial cells with pluripotent capabilities. Using genetic reprogramming with protein transcription factors, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue. Shinya Yamanaka and his colleagues at Kyoto University used the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 in their experiments on cells from human faces. Junying Yu, James Thompson, and their colleagues at the University of Wisconsin-Madison used a different set of factors, Oct4, Sox2, Nanog and Lin28, and carried out their experiments using cells from human foreskin. Although this is an exciting breakthrough it still has many years of research to prove that these particular cells can be controlled to prevent tumor formation.

Dental Stem Cells are the most accessible stem cells; they are isolated from the dental pulp of healthy teeth, periodontal ligament including the apical region of developing teeth and other tooth structures. Craniofacial stem cells, including Dental Stem Cells, originate from neural crest cells and mesenchymal cells during development. Neural crest cells share the same origin as progenitor cells that form the neural tissue. Adolescents have two excellent opportunities for banking their stem cells from extracted teeth: following extraction of bicuspid teeth for orthodontic treatment and when their wisdom teeth are extracted. The bicuspid teeth are not fully formed until between the ages of 12 to 14 years. The apex of the root is not fully closed, which ensures a good blood supply and more proliferation of stem cells within the pulp. Typically, these teeth are extracted for orthodontic reasons before the roots are fully formed, which ensures a better chance for success of harvesting viable stem cells. These stem cells at the apical region of the developing root are responsible for producing Cementum and the periodontal ligament. The same scenario is true with wisdom teeth (third molars). The roots of the wisdom teeth are not fully formed until after the age of 18 years; extracting these teeth during the teenage years helps to ensure the greatest abundance of proliferative stem cells.

The follicular tissue of an unerupted tooth may also prove to be a valuable source for stem cells. The follicle is found at the coronal portion of impacted teeth. This soft tissue lining is ectomesenchymal origin and is responsible for producing the enamel portion of the tooth. This soft tissue is removed and discarded along with the impacted tooth.

The Stem Cells that are found in the pulp of deciduous and permanent teeth are adult multipotent mesenchymal Stem Cells. The central region of the pulp contains large nerve trunks and blood vessels. This area is lined peripherally by a specialized odontogenic area which has **three layers** (from innermost to outermost) 1. **Cell rich zone**; innermost pulp layer which contains fibroblasts and **undifferentiated mesenchymal Stem Cells**. These cells are dispersed diffusely throughout this layer and the number of Stem Cells depends upon the quantity of pulpal tissue. (Age, the type of tooth, incisor vs. molar, deciduous vs. permanent, stage of development or resorption) 2.

Cell free zone (zone of Weil) which is rich in both capillaries and nerve networks. The nerve plexus of Rashkow is located in here 3. **Odontoblastic layer;** outermost layer which contains odontoblasts and lies next to the predentin and mature dentin. Other Cells found in the dental pulp include fibroblasts (the principal cell), odontoblasts, defense cells like histiocytes, macrophages, granulocytes, mast cells and plasma cells. Pulpectomies on vital pulps is another accessible means to collect viable stem cells.

Anatomically the pulpal volume is the greatest during Developmental stage of tooth development. As a person increases in age, the pulpal chambers decrease in size. Even with the decreased pulp chamber size stem cells have been identified and expanded in vitro in patients greater than 60 years old. All extracted teeth with healthy pulp should be considered candidates for stem cell collection. Common sources of Dental Stem Cells in an adult are all healthy teeth needing extraction: Impacted wisdom teeth, Bicuspid recommended for extractions for orthodontic indications and supernumerary teeth.

As a patient increase in age the pulpal chamber decreases in size. Comparison of pulp chamber in an 18 year old and a 60 year old.

Sources of Dental Stem Cells. In a child the most accessible stem cells are from the oral cavity. For deciduous teeth, the best candidates are moderately resorbed canine and incisors with the presence of healthy pulp. Most Deciduous Molars are not candidates because of their resorption pattern. In children, other sources for easily accessible stem cells are supernumerary teeth, mesodens and over retained deciduous teeth associated with congenitally missing permanent teeth. Prophylactic removal of deciduous molars for orthodontic indications.

Conceptually, Dental Stem Cells have the potential to differentiate into neural cell lineages. Recently, investigators have discovered a unique type of mesenchymal stem cell in the dental pulp of deciduous teeth. Stem cells from deciduous teeth, which are formed during the embryonic phase of human development, Nicknamed ‘SHED’ (Stem Cells from Human Exfoliated Deciduous teeth), scientists believe that these stem cells behave differently than adult stem cells. SHED cells are capable of extensive proliferation and differentiation, which makes them an important resource of stem cells for the regeneration and repair of craniofacial defects, tooth loss and bone regeneration. Given their ability to produce and secrete neurotrophic factors, SHED cells may also be beneficial for the treatment of neurodegenerative diseases and the repair of motoneurons following injury. Indeed, Dental Stem Cells from the deciduous tooth has been induced to express neural markers such as nestin. Similarly, bone marrow derived stem cells also have been induced to express neural cell markers The expression of neural markers in Dental Stem Cells elicits the 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 Dental Stem Cells in both dental and non-dental regeneration should be further explored. Dental Stem Cells that have been isolated to date, either from deciduous teeth or permanent teeth, are considered postnatal stem cells or adult stem cells.

Teeth that are in the “Harvest Zone” are excellent candidates for Stem Cell recovery. These are deciduous teeth from canine to canine in both the maxilla and mandible. They become mobile with a good portion of their root and pulp still intact.

Exfoliating deciduous teeth are an excellent source of highly proliferative mesenchymal stem cells. The stem cells are located diffusely throughout the pulpal chamber of the tooth. The ideal deciduous tooth for collection is one that still has a measurable amount root that contains healthy pulp tissue.

The pulp of the tooth is nourished by the collateral blood supply from around the apex of the resorbing root. When a deciduous tooth becomes extremely mobile it is likely that the pulp has been separated from its blood supply. The tooth may still maintain its gingival attachment and be retained for weeks in the mouth with a necrotic pulp.

A tooth "hanging on by a thread" or one that just fell out on its own is not a candidate for stem cell collection

The eruption pattern of the permanent tooth has a lot to do with the resorption of the remaining root and pulpal tissue. It is common for permanent incisors to erupt lingually to the mandibular deciduous incisors; this however may necessitate a planned extraction of this over retained deciduous tooth.

The anatomy of the first and second deciduous molars make these teeth less than ideal for stem cell collection. These teeth have a greater quantity of pulp when compared to deciduous canines and incisors but because of their resorption pattern they have an unpredictable quantity of pulpal tissue at the time of exfoliation. Most often, the erupting permanent bicuspid will obliterate the deciduous molar's pulpal chamber. The 1st and 2nd molars have a larger circumferential surface area that maintains their gingival attachment for a longer period of time. These teeth have a greater degree of resorption that decreases the amount of pulpal tissue present at the time of exfoliation.

The periodontal ligament serves as a reservoir for many cell types, including fibroblasts, osteoblasts/clasts, cementoblasts/clasts, and odontoclasts. The remarkable characteristic of the periodontal ligament is its ability to regenerate and repair virtually every other tissue type that comprises the periodontium. Undifferentiated mesenchymal cells of the periodontal ligament can differentiate into osteoblasts, chondrocytes and adipocytes. New research has shown that postnatal stem cells are also found in the periodontal ligament. Ligament removed from extracted third molars transplanted into culture yielded multiple rapidly dividing colonies of stem cells that expressed specific proteins characteristic of postnatal stem cells. The replication rate of these stem cells was similar to that of dental pulp stem cells. Following transplantation into mice, these stem cells produced cementum, periodontal ligament and fibrous structures similar to Sharpey's fibers, which anchor cementum to bone.

Developing third molars are an ideal source for dental stem cells. The Mesenchymal stem cells within the pulp and at the apex of these developing teeth are a valuable source of very proliferative mesenchymal stem cells. The follicular tissue overlying these impacted teeth are

also a potential source for stem cells. Researchers are currently investigating these particular stem cells and their specific characteristics.

Pulpectomies on vital pulps is another accessible means to collect viable stem cells.

Other sources of stem cells accessible from the oral cavity during oral surgical procedures
Alveolar bone

Periosteum

The periosteum covers most of the bones in the body. The periosteum is a very dense, tough layer of fibrous tissue intended to act as a covering for bone and provide progenitor cells for bone growth and repair. Periosteum is divided into an outer "fibrous layer" and inner "cambium layer". The fibrous layer contains fibroblasts while the cambium layer contains progenitor cells which develop into osteoblasts. Cultured adult human periosteal stem cells demonstrate mesenchymal multipotency, suggesting that they may be used to repair tissue. Upon enzymatic release and culture expansion, cells harvested from the periosteum can give rise to cartilage and bone. Moreover, the cells differentiated into chondrocyte, osteoblast, adipocyte and myocyte lineages regardless of donor age. In the mouth, the periosteum can be found in most areas where bone is covered with mucosa but not in areas of attached gingiva. If the periosteum is grafted into soft connective tissue, it will grow bone in areas where bone is not found. The hard palate is covered with periosteum, as it is covered with keratinized gingiva but not attached gingiva. Bone covered by periosteum is always cortical bone. In the oral cavity, the periosteum begins at the mucogingival junction and covers the maxilla and mandible. Although more studies are needed to confirm these observations, since harvesting periosteal cells is a relatively easy procedure, the periosteum is a good source of mesenchymal stem cells. The best opportunities to recover periosteum are during a surgical procedure when a partial thickness flap is created. Certain surgical indications to consider: Implant surgery, sinus lift procedures, impacted third molar surgery, periodontal surgery and other surgical procedures that involve exposing cortical bone.

Buccal mucosa and Gingiva

The oral cavity contains masticatory mucosa, such as gingiva, and lining mucosa, such as buccal mucosa. Oral mucosal epithelium has drawn attention as a cell source for a variety of tissue-engineered reconstructions, such as oral cavity, epidermis, and especially ocular surface reconstruction and the transplantation of human oral epithelial sheets grown on various substrates can be useful for tissue reconstruction. In the clinical setting, the quality of the cultivated graft is the key to success. This gingival keratinocyte stem cells, have the potential to be useful for studies on stem cell differentiation, for developing gene therapy procedures that target the gingival epithelium, as well as a stable platform for testing oral hygiene products and as potential material for preprosthetic surgery.

Muscle

The developmental origin of the masticatory muscles differ from that of the tongue, limb and trunk muscles. To date only a little is known about adult stem cells in the masticatory and tongue muscles and there may be several different types of stem cells beside the satellite cells that have been identified in skeletal muscles. There are many opportunities to gain access to muscle during oral surgical procedures. At the time of orthognathic surgery the muscle attachments to the mandible are routinely released from the chin and angles. The muscles of mastication are often exposed and in some instances excess muscle is removed and discarded.

Alveolar bone

Bone tissue engineering is a promising approach for bone reconstruction in oral-maxillofacial surgery. Maxillofacial surgeons are already familiar with the regenerative properties of transplanted bone grafts. The bone graft that is being harvested and transplanted contains stem cells in addition cortical and particulate bone. In the future small quantities of patients' bone will be harvested, transported to a lab and expanded to produce larger quantities of bone. This particulate bone graft will be implanted into the donor. Because this is an autologous graft the potential for rejection will be minimal.

Banking Stem Cells from teeth Tooth-derived stem cells are readily accessible, and provide an easy and minimally invasive way to obtain and store stem cells for future use. Banking teeth and tooth-derived stem cells is a reasonable and simple alternative to harvesting stem cells from other tissues requiring invasive surgical procedures, and does not pose the ethical problems associated with embryonic stem cell harvesting. Stem cells can be obtained from loose baby teeth and from extracted permanent teeth. Tooth extractions are common prior to beginning orthodontic treatment or at the time the wisdom teeth are removed. Stem cells can also be obtained from the jawbone during the placement of dental implants and other surgical procedures. Obtaining stem cells from baby teeth is simple and convenient, with little or no trauma. Stem cells from baby teeth are best, as they grow more rapidly than those found in permanent teeth, and have the ability to differentiate into many cells types, including nerves. Stem cells in baby teeth begin to develop during the 6th week in utero thereby capturing these stem cells very early in the differentiation process. These stem cells have gone through fewer cell divisions than other types of adult stem cells which may be the reason for their proliferate nature when grown in vitro. Fortunately, every child loses baby teeth, which creates the perfect opportunity to retrieve and store this convenient source of stem cells. If needed, using your own stem cells (autologous donation) poses fewer risks for rejection following transplantation. Your own cells are less likely to develop into tumors following transplantation than donated tissues. Using your own cells also avoids the risk of contracting diseases from another donor's transplanted tissues. Adults may also preserve their dental stem cells from extracted permanent teeth and from healthy tissues accessed during oral surgery. For example, bone fragments can be obtained during the placement of dental implants, or from the ligaments and supporting structures of extracted teeth for adult orthodontic treatment. Teeth that are diseased or infected are not good sources for stem cell retrieval and storage.

The tooth is transferred into a sample vial containing a buffered saline solution. A recently extracted tooth is at a temperature of 98.6 degrees Fahrenheit. Hypothermia is induced by placing the tooth in the solution at room temperature (74 degrees Fahrenheit). Hypothermia is

the temperature at which cellular metabolism is slowed down and this is a temperature below 92 degrees Fahrenheit.

The specimen vial is placed into an insulated transport vessel. To maintain hypothermia during transport and to prevent spikes in temperature the Sample vial is placed into a thermette. The thermette consists of a phase change material that absorbs heat to maintain the internal ambient temperature of the transport vessel below 90 degrees Fahrenheit. No pre prep or refrigeration of the kit is necessary. The kit should be kept at room temperature and in a dry place.

The transport vessel is placed into an insulated shipping box. The insulated box adds another layer of temperature protection during the transport of the tissue sample. The patented Transport kit was designed to be used anywhere in the country during any part of the year. Whether it is Arizona at the peak of the summer or Minnesota in the middle of the winter.

A security seal is placed on the front cover of the assembled transport kit. By maintaining the tissue in a hypothermic state the kit needs to be delivered to the processing facility for cryopreservation within 48 hrs of the tooth being extracted.

The sample needs to arrive at the processing facility within 48 hours of being recovered from the individual in order to ensure its viability. At the processing facility the sample is logged into the database. The tooth is removed from the transport container and wiped down with a chemical sterilizer. Care is taken not to apply the sterilizing agent directly to the exposed tissue. The tooth is cracked open and the pulpal tissue is removed and assessed for viability. The tissue is then placed through several washes using an antibiotic solution. After each wash the sample is centrifuged to remove any cellular debris.

Once the tooth arrives at the processing facility the tooth is rinsed several times with an antibiotic solution. The tooth is sterilized with by wiping the external surface of the tooth avoiding any exposed pulpal tissue

The harvesting of the dental stem cells from a tooth is conducted in a sterile environment, following an established protocol.

The pulp is removed from the inside of the tooth using a variety of manual techniques. In most case the tooth is “cracked” open and the pulp is removed from the tooth portions using dental curettes. The tissue is assessed for viability. Only viable tissue goes on to cryopreservation.

The pulpal tissue is washed several times in an antibiotic solution. The pulpal tissue is cryopreserved in its native state. The tissue and stem cells are minimally manipulated and are not enzymatically treated or expanded in cell culture.

The cells go through a final wash and centrifuge to remove any cellular debris prior to cryopreservation

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, such as (typically) -196°C (the boiling point of liquid nitrogen). At these low

temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped. However, when vitrification solutions are not used, the cells being preserved are often damaged due to freezing during the approach to low temperatures or warming to room temperature. The risks of cryopreservation are the phenomena which can cause damage to cells during cryopreservation. These are extracellular ice formation, when water leaves the cells and ice forms between the cells, dehydration when too much water leaves the cells and intracellular ice formation, when ice forms within the cells. To prevent these risks, vitrification provides the benefits of cryopreservation without the damage due to ice crystal formation. In clinical cryopreservation, vitrification usually requires the addition of cryoprotectants prior to cooling. The cryoprotectants act like antifreeze: they lower the freezing temperature. They also increase the viscosity. Instead of crystallizing the syrupy solution turns into an amorphous ice—i.e. it vitrifies. In cryopreservation, the solute such as dimethylsulfoxide a common cryoprotectant must penetrate the cell membrane in order to achieve increased viscosity and depressed freezing temperature inside the cell. The drawback is that the cryoprotectants are often toxic in high concentration.

The liquid nitrogen Storage tanks are connected to automatic liquid nitrogen replenish lines. Each storage tank is monitored separately. The storage facility is required to have emergency backups in place in case of a power outage or other natural emergencies to be certified

The tanks are wired and wirelessly alarmed which allows for 24 / 7 onsite and remote monitoring of the liquid nitrogen storage tanks.

The sample vials are placed in boxes. The boxes are numbered and placed into vertical racks. The numbered racks are submerged in liquid nitrogen. Each box and rack are included in a database, the sample vial can be referenced against this database to identify its location in a particular liquid nitrogen tank.

The liquid nitrogen levels are continuously monitored within the tanks. The tank is prefilled with liquid Nitrogen prior to placement of the racks. Placement of the racks into the tanks causes displacement of the liquid nitrogen. Pre-measuring prior to placement of the racks prevents under filling or overflowing of the liquid nitrogen. Each tank is monitored for temperature fluctuations as well as changes in the liquid nitrogen levels.

Whenever a rack needs to be removed from a liquid nitrogen storage tank, strict protocols are followed that prevents temperature changes to the cryopreserved tissues. The rack is removed from the storage tank and transferred to a vat filled with liquid nitrogen. This vat can be moved to a bench top from where the samples are added to or removed from the rack. Performing this stepped transfer also prevents the cover of the storage tank from being open for any extended period of time and avoids the loss of liquid nitrogen by evaporation.

Stem Cell thawing and expansion

Cells that have been cryopreserved for 35 years have been thawed and were still viable and able to proliferate in vitro. In 1949 sperm was cryopreserved for the first time by a team of scientists led by Christopher Polge. Cell suspensions (like blood and semen) and thin tissue sections can sometimes be stored almost indefinitely at liquid nitrogen temperature (cryopreservation).

Human sperm, eggs and embryos are routinely stored in fertility research and treatments. In the early 2000s a baby was born from a cryopreserved egg fertilized by a cryopreserved sperm.

In order for stem cells that have been cryopreserved to be used in regenerative therapies they must be thawed and woken up. In the case of dental stem cells, the tissue was cryopreserved in its native state. This tissue contains in addition to mesenchymal stem cells, other surrounding cells which is included in the recovered tissue. The thawing process begins when the tissue sample is taken from the liquid nitrogen storage and then placed in a warm water bath at 37C. The warm water is maintained at this temperature while the bath is agitated. It is important to prevent refreezing of the cells during the thawing processes. The cells thawing on the exterior could be cooled below freezing by the frozen cells in the interior. Maintaining an agitated bath at 37 degrees Celsius would minimize the risk for this happening. After thawing the tissue is enzymatically treated to release the stem cells from the tissue. The cells are sorted and then placed in a cell culture medium and allowed to expand. The cells are allowed to grow over several passages and the culture medium and the environmental factors are maintained to prevent the stem cells from differentiating and losing their stemness. In many cases, creation of functional tissues and biological structures *in vitro* requires extensive culturing to promote survival, growth and inducement of functionality. The basic requirements of cells must be maintained in culture, which include oxygen, pH, humidity, temperature, nutrients and osmotic pressure maintenance. The optimal way to grow cells is to employ a bioreactor. A Bioreactor may refer to any device or system that supports a biologically active environment. Bioreactors allow for precise and continuous control of culture conditions and also allow for introduction of different stimuli to tissue cultures.

Stem cells will proliferate in a Petri dish in only two dimensions. Researchers are developing three-dimensional scaffolds that will direct the shape and size of tissue growth. The scaffolds that are presently under development are absorbable. The Scaffolds are lined with active stem cells that are then implanted into animals or placed in culture medium and allowed to grow. Over a period of time the scaffolds are replaced with normal tissue and become vascularized. One day these scaffolds will be used to direct stem cells to grow into organs that can be implanted in humans to replace diseased or injured organs.

Potential Uses for Stem cells Researchers are approaching this emerging field of stem cells from many different angles. Presently all of the current therapies involve adult stem cells and these are **cell-based therapies**. Transplanting or “grafting” tissue to combat shortages of donated organs seems to be high on the list of priorities. Specifically treating diseases such as Alzheimer’s, Parkinson’s, spinal cord injury, stroke, burns heart disease, arthritis and diabetes. Currently the longest therapy using multipotent stem cells has been bone stem cell transplants. But other uses for stem cell will prove to have an even more powerful role near term. Stem Cells are valuable for **testing new drugs**. Drugs are being applied directly to human cells and this will provide more relevant data than drug testing on animals. Testing medications for safety on differentiated cells from pluripotent cell lines, as anti-cancer drugs are now tested on cancer stem cell lines. **Understanding cell division and development.** Understanding the process of

turning on and off to control division and differentiation of stem cells. They will also help to better understand how birth defects develop and how cancers react based upon the characteristics of abnormal cell division and differentiation. Stem cells are also providing a means to better understand a particular disease. We can **understand particular genetic diseases** through studying cells with those mutations. Cell lines have already been made from stem cells carrying the mutation from certain orphan disease i.e. muscular dystrophy.

Stem Cell Timeline: 1956 First successful bone marrow transplant, 1981 Embryonic stem Cells are isolated from a rat blastocysts, 1998 The first human embryonic stem cells are isolated, 2000 Mesenchymal Stem Cells discovered in teeth by researchers at the NIH.

Stem cell therapies rely on several key advances in biomedical research. For example, studies of stem cell therapies would not have been possible without the completion of human genome project. With the completion of human genome project, we are at an unprecedented position to explore the full potential of stem cells.

In 1988, the National Institutes of Health and National Science Foundation published this definition of tissues engineering. Tissue engineering is positioned to capture the fruit of stem cells, polymer chemistry, materials science, molecular biology and genetics, nanomaterials and with the input of clinicians, towards the regeneration of tissues and organs.

Twenty years later: *Translational Approaches in Tissue Engineering and Regenerative Medicine*. Mao, Vunjak-Novakovic, Mikos, Atala. The first-ever book to focus on the translational aspects of tissue engineering and regenerative medicine. It offers a unique approach that helps bridge the gap between laboratory discovery and clinical applications. It presents comprehensive coverage of the technological, regulatory and funding aspects of tissue engineering and regenerative medicine. 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. Strategies for education, training, research, development, and commercialization and practice models need to be formulated and implemented.

Stem cells in dentistry Stem Cells and stem cell therapies will emerge to become an important aspect in the everyday practice of Dental professionals. It is important for all Dentists, Dental Specialist, Hygienist and their auxiliary staff to educate themselves on the basics of stem cell science. 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 two of the highly prevalent disorders involving the mankind. Whereas native tissue is missing in congenital anomalies, diseases such as caries or tumor resection result in tissue defects. 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 host. (Mao, 2008)

Adult stem cells may be used to regenerate bone and correct oral and craniofacial defects. Both in vitro studies and in vivo research in animal models has shown that tooth-derived adult stem

cells can be used to regrow tooth roots in the presence of proper growth factors and a biologically compatible “scaffold.” Regenerative therapy is less invasive than surgical implantation, and early animal studies suggest comparable results in strength and function of the biological implant as compared to a traditional dental implant. Stem cells extracted from the dental pulp of a third molar could be harvested, then directly implanted into the pulp chamber of a severely injured tooth. The goal is to regenerate the pulp inside the damaged tooth, preventing the need for endodontic treatment. Stem cells derived from the periodontal ligament may offer promise for regenerating the periodontal ligament and other supporting structures of the periodontium that have been destroyed by gingival disease, with an alternative approach to traditional clinical therapies. Tissue-engineered bone grafts will be useful for practitioners in all of the dental specialties. Future tissues may also include engineered TMJ joints and cranial sutures, which would be especially helpful to craniofacial and oral maxillofacial surgeons.

When current therapies including autologous tissue grafts, xenogenic grafts, allogeneic tissue grafts, synthetic materials, are compared with stem cell based therapies, it is equivocal that stem cell based therapies come out as a winner. For example, autologous tissue grafts are harvested with donor site trauma and morbidity. In contrast, stem cells can be isolated by aspiration, or in the case of dental stem cells, from extracted teeth for otherwise medically needed procedures, with little trauma. Autologous tissue grafts are subjected to limited supply. Foreign materials are subjected to immunorejection. In comparison, a small number of stem cells can be readily expanded to sufficient amount for healing relatively large defects. Pathogen transmission can be associated with allogeneic and xenogenic transplants, but is not an issue with autologous stem cells. Most synthetic materials are subjected to wear and tear, whereas stem cell based therapies are anticipated to integrate with the patient.

Mesenchymal stem cells have been shown to differentiate into bone, cartilage, fibrous, adipose and muscle tissues. These are only a few examples of the potential use of MSCs, including dental stem cells that are likely specialized MSCs, in medical and dental treatments. MSCs are being explored for their potential in therapies in cardiac infarcts, immune disorders, Parkinson’s and liver disease.

Systemic Diseases that can be treated with dental mesenchymal Stem Cells,

Diabetes

Muscular dystrophy

Parkinson’s

Cardiac infarcts

Arthritis

Soft tissue reconstruction

Liver Disease and More

Stem cells used in medicine today Applications of regenerative medicine technology may offer new therapies for patients with injuries, end-stage organ failure, or other clinical problems. Currently, patients suffering from diseased and injured organs can be treated with transplanted organs. However, there is a shortage of donor organs that is worsening yearly as the population ages and new cases of organ failure increase. Scientists in the field of regenerative medicine and

tissue engineering are now applying the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. The stem cell field is a rapidly advancing aspect of regenerative medicine as well, and new discoveries here create new options for this type of therapy.

Stem cell-based therapies are being investigated for the treatment of many conditions, including neurodegenerative conditions such as Parkinson's disease and multiple sclerosis, liver disease, diabetes, cardiovascular disease, autoimmune diseases, musculoskeletal disorders, and for nerve regeneration following brain or spinal cord injury. Currently, patients are being treated using mesenchymal stem cells for bone fractures, cancer (bone marrow transplants) and spinal fusion surgery. The Mesenchymal stem cells found in teeth may be beneficial for the treatment of neurodegenerative diseases and the repair of motor nerves following stroke or injury. Mesenchymal stem cells from wisdom teeth release chemicals that may allow the remaining nerves to survive the injury. Future research will investigate if using tooth-derived stem cells can be used to regenerate neurons following spinal cord injury. New research has found that dental stem cells can differentiate into cardiac muscle and skeletal muscle cells. This exciting research will lead to future treatment options that allow muscles to repair themselves following injury, such as the muscle damage that occurs after a heart attack, or the structural damage that occurs following a knee injury. New stem cell therapies are already under review or have been approved by the U.S. Food and Drug Administration (FDA). Many other therapies are in various stages of product development. As the number of people affected by degenerative diseases continues to increase, there will be a greater need for new treatment options for the ever-growing aging population. Harvesting and storing stem cells now will ensure their availability in the future when they will be needed most.

Summary: Since 2000, over 1000 research studies from institutions around the world have been published that make reference to dental stem cells. In 2007 alone there were over 1000 publications. The current research on Dental stem cells is expanding at an unprecedented rate. Additionally, over 60 clinical investigations with animals and human volunteers have been published seeking to identify potential new medical treatments from mesenchymal stem cells. Stem cell-based therapies are being investigated and are going to clinical trials for the treatment of many conditions, including neurodegenerative conditions, liver disease, diabetes, cardiovascular disease, autoimmune diseases, musculoskeletal disorders, and for nerve regeneration following brain or spinal cord injury. As these clinical studies continue to advance in the years ahead, it is widely expected that to avoid autoimmune rejection from donor tissues and to maximize therapeutic efficacy, stem cells will be used to generate a specific treatment for a specific patient. The emerging field of "Personalized Medicine" is a popular topic in the media, which generally refers to new medical technologies derived from a patient's own stem cells and the use of genomic diagnostics. Recent findings and scientific research supports the use of these very powerful mesenchymal stem cells found within teeth and other accessible tissue harvested from the oral cavity for use in a multitude of regenerative therapies. A young and healthy patient is a better candidate for mesenchymal stem cell collection and storage. At a younger age most individuals are free from chronic diseases and their stem cells have undergone fewer cell divisions and there is a lower likelihood of somatic mutations of these stem cells. In addition, the mesenchymal stem cells found in exfoliating and developing teeth proliferate at a more vigorous rate when compare to other sources of mesenchymal cells presently used in available

clinical therapies. While we can see the promise of human stem cell therapies for the future, dentists know that it is important to act now to harvest and store these mesenchymal stem cells from deciduous teeth, extracted permanent teeth and other accessible living tissues from the oral cavity and making these opportunities available to their child, adolescent and adult patients for future regenerative therapies.