Dental Stem Cell Preservation and the Dawning of Regenerative Oral Medicine

by Peter Verlander, PhD

Stem Cell Overview

Stem cells have been defined and discussed in medical journals for more than a century.[1] Simply stated, stem cells are undifferentiated or non-specific cells that not only have the capacity for self-renewal, but also have the ability to produce the differentiated or specific cells needed for a variety of tissue regeneration and therapeutic applications.

A significant amount of the media coverage of stem cells has been devoted to the highly controversial variety of stem cells that are harvested from human embryos. While the potential of embryonic stem cells is enormous, the controversy surrounding the use of these cells has clouded the perception of consumers and health-care professionals to the point that the term "stem cell" is frequently thought to be synonymous with "embryonic stem cell." As a result, the positive developments in stem cell research and preservation using alternative sources that are not linked to a highly personal and oftentimes emotional ethical debate are often lost in the confusion.

Other less controversial sources for stem cells research and clinical application currently in use are bone marrow, peripheral blood stem cells, adipose tissue, and umbilical cord blood cells. Each has its distinct advantages and disadvantages.

Bone marrow stem cells have been primarily used since the 1950s for treatment of leukemia and genetic blood disorders.[2] Today, an estimated 50,000 bone marrow transplants are performed every year,[3] and more than 1,500 clinical trials regarding bone marrow stem cells are either planned, in progress or have been completed for

1. *Ramalho-Santos M, Willenbring H. On the origin of the term "stem cell." Cell Stem Cell. 2007 Jun 7;1(1):35-8.*
2. *Thomas ED, et al. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N Engl J Med. 1957;257:491-496.*
3. *Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med. 2007 Oct 11;357(15):1472-5.*

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therapeutic applications ranging from treating leukemia to spinal cord injuries.[4]

Bone marrow stem cells will continue to grow in importance for regenerative medicine, as these new therapeutic applications come online, but they are not without problems; an invasive surgical procedure is needed to harvest bone marrow stem cells that is somewhat painful and not without risks for the donor. In addition, donor and recipient matching remains a challenge – especially when time is of the essence.

Over the last 20 years, peripheral blood stem cells have become commonly used as a source of hematopoietic stem cells for a bone marrow transplant. Donors are given a growth factor for four or five days to stimulate the release of stem cells into the bloodstream, and peripheral blood stem cells are then collected from the donors' blood by a process called apheresis or leukapheresis. The collection of peripheral blood stem cells is associated with some side effects, but follow-up studies have not yet found any significant increase in long-term health risk for the donor.[5]

Adipose tissue is another source of stem cells, which are commonly obtained from liposuction aspirates or abdominoplasty procedures. They are currently being researched to determine their stem cell viability and potential for a wide range of therapeutic applications, including diabetes, heart failure and arthritis, to name just a few.[6]

In reconstructive surgery, adipose tissue stem cells are being explored for the repair of tissue defects resulting from traumatic injury, tumor resection and congenital defects,[7] as well as from calvarial defects following severe head injury.[8] Potential dental applications include maxillary and mandibular repair.[9]

As with bone marrow stem cells, the biggest downside of adipose tissue stem cells might be the need for an invasive procedure to harvest the stem cells, with the associated pain and risk of adverse events.

A convenient source of stem cells is umbilical cord blood. Cord blood collection is a non-invasive procedure performed at birth, and is easily integrated into the childbirth process, but the window of opportunity is small, as the cord blood must be collected at the time of a child's birth and preserved immediately after.

Since the mid-1980s, the clinical applications for cord blood stem cells have expanded to the point where dozens of diseases can be treated with fresh or cryopreserved cord blood. In fact, more than 9,300 patients have been treated with stem cells derived from umbilical cord blood, with 200,000 units being publically banked.[10]

The rate of consumer adoption of cord blood banking is very impressive, with 550,000 units privately banked to date by parents upon the birth of their children.[11] The growth in private cord blood banking was originally driven by consumer demand, and over time has become recognized as an option that parents need to be fully informed of in order to make an intelligent decision. The need for health professionals to educate their patients led the American College of Obstetrics and Gynecology to issue guidelines for informing parents, noting that some states have passed legislation requiring that parents be informed of their options.[12]

Dental Stem Cells: Cord Blood 2.0?

Given this landscape acknowledging the need for informed consumer choice, we anticipate that dental professionals will play an instrumental role in driving increased consumer acceptance and adoption of dental stem cell preservation.

Leading the charge might be a subset of the dental profession – pediatric dentists. In fact, as early as 2008, the American Academy of Pediatric Dentistry issued the following statement:

The American Academy of Pediatric Dentistry recognizes the emerging field of regenerative med-

4. *http://www.clinicaltrials.gov/ct2/results?term=bone+marrow+transplant. Accessed September 24, 2010.*
5. *Hölig K, et al. Safety and efficacy of hematopoietic stem cell collection from mobilized peripheral blood in unrelated volunteers: 12 years of single-center experience in 3928 donors. Blood. 2009 114:3757-3763.*
6. *http://www.clinicaltrials.gov*
7. *Gomillion CT, Burg KJ. Stem cells and adipose tissue engineering. Biomaterials. 2006 Dec;27(36):6052-63.*
8. *Lendeckel S, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. J Craniomaxillofac Surg. 2004 Dec;32(6):370-3.*
9. *Mesimäki K, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg. 2009 Mar;38(3):201-9.*
10. *http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2801068/table/T2/. Accessed September 23, 2010.*
11. *http://www.cordblood.com and http://www.viacord.com/general-faq.htm. Accessed September 23, 2010.*
12. *Umbilical Cord Blood Banking. ACOG Committee Opinion No. 399. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2008 111:475-7.*

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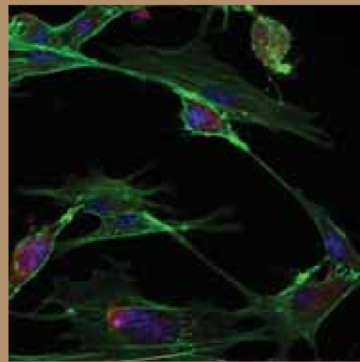
One Dentist's Dental Stem Cell Discovery



Dr. Nicholas Perrotta is a general dentist who practices full-time in Medford, Massachusetts. Dr. Perrotta became an advocate of dental stem cell preservation in his practice after conducting his own research as a consumer and opting to preserve the stem cells of his 13-year-old daughter and 10-year-old son.

According to Dr. Perrotta, cord blood stem cell preservation was not widely discussed when his daughter was born in 1998, but he and his wife had planned on banking his son's cord blood stem cells in 2001. "However, because he was born prematurely, we missed the opportunity, and later regretted it."

In 2004, he read an article published by the National Institutes of Health that explained the potential of preserving dental stem cells, and he recognized this as a possible new opportunity to preserve the stem cells of his children. "I was also very encouraged by other emerging research regarding the use of dental stem cells to repair damaged tissue," explained Dr. Perrotta.

From that point, he conducted his own extensive research into dental stem cells and the subject of biobanking. "I wanted to be sure that the biobanking facility that would be storing my children's dental pulp stem cells was reputable, financially stable and followed best practices when it came to long-term stem cell storage," explained Dr. Perrotta.

He found the company that provided the best overall solution and the one he chose to bank his own children's samples with was Provia Labs, which provides the Store-A-Tooth dental stem cell banking service.

Dr. Perrotta decided to offer his patients the option to bank their own dental stem cells in the very early stages, in 2005. "First of all, I figured that if I chose this option for my own children, why not at least offer the same option to my patients? I also see the dental business model following that of OB/GYN practitioners and cord blood storage: These doctors were on the front lines of patient education and helped explain in simple terms the potential benefits of banking autologous stem cells. I believe dentists like me will serve the same educational role in the increased acceptance of dental stem cell storage," Perrotta said.

The fact that it appears the first applications for dental stem cells will be in the dental field is especially exciting to Dr. Perrotta. "The potential for using dental stem cells to grow teeth, bone and dental pulp is helping dentistry evolve from a restorative to a regenerative discipline. Dental stem cells represent an enormous opportunity for the field of dentistry to provide improved care to our patients. The implications of this I think we still do not fully appreciate yet."

How can dentists successfully introduce dental stem cell preservation into their practices? "They have to be believers," says Dr. Perrotta. "Then they have to carefully educate the rest of the staff – especially the hygienists." Dr. Perrotta conducted a series of lunch-and-learns with his staff, armed with educational materials that he downloaded from www.store-a-tooth.com.

"Our hygienists are the ones who introduce and explain the Store-A-Tooth program to our patients, especially those with children. They give them a brochure, have them watch a video and also encourage them to do their own online research to determine whether or not dental stem cell research is right for their families. There is no hard sell, just alerting them of the existing option of dental stem cell preservation. The hygienists in my practice appreciate being able to offer patients this service option."

If a patient is interested, the process is simple. They request a Store-A-Tooth collection kit from Provia Labs, bring it to Dr. Perrotta's office for the extraction, and the tooth is sent overnight by 10 a.m. the next day to Provia Labs' biobanking facility. "It's very simple, non-invasive and affordable, with consumer interest in dental stem cell preservation growing every day, thanks to increasing news and scientific coverage of dental stem cells. I can offer access to expanded services without carrying any extra inventory.

"To those who think it is too early to bank dental stem cells," Dr. Perrotta says, "science and technology will continue to evolve quickly over the next few years. We can help the patients save these tissues today. Researchers are looking at amazing treatments in human and animals such as treating spinal cord injury. This gives patients an opportunity to store dental stem cells from a tooth that otherwise would be discarded. In the end, our obligation as dentists is to provide our patients with the access to the best dental technology and services possible. Store-A-Tooth puts us in the forefront of dentistry and regenerative medicine."

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icine and encourages dentists to follow future evidence-based literature in order to educate parents about the collection, storage, viability and use of dental stem cells with respect to autologous regenerative therapies.

By following this recommendation, pediatric dentists, general practitioners and virtually any dental specialist can help propel the growth of regenerative medicine, an emerging field identified by the U.S. Department of Health and Human Services[13] that is already making us rethink the current roles of healthcare professionals. Regenerative medicine might enable the treatment of diseases that are incurable at the present time, which in turn will create a new industry with potentially strong economic impact.[14]

"Ongoing research suggests that these stem cells will be used first for dental applications, such as replacing bone, periodontal ligaments, or dental pulp, and to treat periodontal disease."

Moreover, the increased acceptance of dental stem cell preservation will put the dental professional at the forefront of an interdisciplinary and more integrated healthcare industry working towards the common goal of repairing, replacing, or enhancing biological function compromised due to congenital deformities, injury, disease and aging.

Why Dental Stem Cells? Why Now?

Dental stem cell research originating from the National Institute of Dental and Craniofacial Research and replicated worldwide has proven that odontogenic tissues are a viable source of mesenchymal stem cells (MSCs), especially the dental pulp of third molars[15] and exfoliating deciduous teeth.[16]

What's more, the supply of these odontontic tissue resources is enormous and generally untapped at the present time. For example, it is estimated that each year in the United States, there are 80 million exfoliating deciduous teeth, 1.5 million extracted pre-molars and 1.3 million extracted third molars.[17]

Most of these exfoliated and extracted teeth are merely discarded, when they can be easily collected, processed and cryopreserved in a quality biobanking facility in preparation for future regenerative applications.

The future of medical applications for dental stem cells currently being studied is very exciting, with the potential of helping countless number of people afflicted with a range of conditions, from type 1 diabetes, muscular dystrophy, stroke, Parkinson's disease, spinal cord injuries and corneal injuries. Also on the horizon are prospective regenerative applications for cranial defects, bioengineered teeth, liver disease, myocardial infarction and more.

While the potential medical applications of dental stem cells are encouraging, several dental and oral health applications have already been evaluated, positioning the dental professional to leapfrog other health-care providers to be the first to introduce the benefits of regenerative medicine to their patients. Here are two examples of what is on the horizon:

- Alveolar bone regeneration (seeding bone cavities with dental stem cells in a collagen matrix)
- Periodontal tissue repair (replacing diseased gingival tissue with stem cells derived from periodontal ligaments)

In a less than a decade from their discovery, dental stem cells had been used in two human studies, and new research and clinical developments continue to help the potential evolve into reality.

There are many opportunities for collection of dental stem cells during childhood: they can be collected ancillary to routine dental procedures, and they can be preserved in long-term storage at a relatively affordable cost. They are suited for autologous use, meaning the adult stem cells can be collected from and used on the same person, so there are no issues of immunological or genetic incompatibility.

13. *Regenerative Medicine. Department of Health and Human Services. August 2006. http://stemcells.nih.gov/info/2006report/*
14. *Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000 Dec 5;97(25):13625-30.*
15. *Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003 May 13;100(10):5807-12.*
16. *American Dental Association. 2005-06 survey of dental services rendered. Chicago (Ill.): ADA; 2007.*
17. *Perry BC, et al. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. Tissue Engineering. 2008 14(2):149-156.*

Taking it to the (Bio)Bank

Here is how dental stem cell collection and storage typically works:

Once the dentist or hygienist collects the tooth, it is transported from the office to the laboratory. The tooth should always be transported via express courier in a sterile, isotonic solution, and shipped chilled to reduce the growth of contaminating microbes. It should be noted that although stem cells can be successfully recovered from teeth several days post-extraction, the yield of viable cells can decline significantly over time.[18]

Dental professionals should be certain that the laboratory they use for dental stem cell storage has validated processes with appropriate quality control metrics in place, in order to verify its ability to remove contaminating oral flora from the tooth and to recover viable tissue.

Ideally, the laboratory should also have the ability to grow these cells in culture and verify that the cultured cells exhibit the baseline set of cell surface markers required for mesenchymal stem cells.

The cryopreservation of stem cells typically involves equilibrating the cells with a cryoprotectant solution – a solvent that protects the cells from the formation of ice crystals and also helps preserve the integrity of cell membranes during the freezing and thawing processes.

The temperature is typically slowly brought down to freezing using programmable controlled-rate freezers. Frozen stem cells are then transferred to vapor-phase liquid nitrogen freezers for long-term storage at ultra-low, stable temperatures at -150 degrees Celcius or lower.

The clinical use of cryopreserved tissues is currently regulated at the state and federal levels. Laboratories storing or expanding human cells for future clinical use must be registered with the FDA[19] and often must be licensed by the department of health of the state in which the laboratory operates. A good sign of quality is if the laboratory is accredited by the American Association of Blood Banks and/or the American Association of Tissue Banks.

It is extremely important that dentists inquire about the tooth transport system and the biobanking facility used by any dental stem cell service provider they are considering working with.

Summary

Teeth are clinically proven to be a source of viable stem cells that may be used for the regenerative treatment of a wide variety of medical and dental diseases.

Therefore, dentists are well positioned to become one of the key providers of stem cells and foster closer collaboration and convergence with the medical field.

The high-quality dental stem cell preservation process begins with the dentist or oral surgeon, who can be involved in the extraction, collection and storage of the stem cells from their patients' teeth. Ongoing research suggests that these stem cells will be used first for dental applications, such as replacing bone, periodontal ligaments, or dental pulp, and to treat periodontal disease.

In order for dentists to fully participate in this new role, which includes responsibility for providing their patients with information regarding the option of dental stem cell banking, they should utilize existing information, including emerging research, to become knowledgeable and conversant regarding the applications, clinical use and banking of dental stem cells. Ultimately, dental stem cell banking is an option every eligible patient should know about. ■

18. *Dominici, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006 8(4):315-17.*
19. *http://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/QuestionsaboutTissues/ucm136323.htm. Accessed April 13, 2012.14(2):149-156.*

Author's Bio

Dr. Peter Verlander is a founder and chief scientific officer for Provia Labs, Inc., the company that provides the Store-A-Tooth dental stem cell preservation program. He was formerly the Associate Director for Strategic Development of the Laboratory for Molecular Medicine (LMM) within the Harvard Medical School – Partners Healthcare Center for Genetics and Genomics. Dr. Verlander is a molecular geneticist with nearly 15 years of experience in various aspects of human genetics in both academia and industry, including research at the Rockefeller University in New York on the molecular genetics of Fanconi anemia (FA). In 2000, he came to Boston to join Millennium Predictive Medicine's Patient Management effort. Dr. Verlander received his PhD in Microbiology from Duke University.