Odontology (2011) 99:1–7
DOI 10.1007/s10266-010-0154-z

REVIEW ARTICLE

Luciano Casagrande · Mabel M. Cordeiro · Silvia A. Nör
Jacques E. Nör

Dental pulp stem cells in regenerative dentistry

Received: November 9, 2010 / Accepted: November 11, 2010

Abstract Stem cells constitute the source of differentiated cells for the generation of tissues during development, and for regeneration of tissues that are diseased or injured postnatally. In recent years, stem cell research has grown exponentially owing to the recognition that stem cell-based therapies have the potential to improve the life of patients with conditions that span from Alzheimer's disease to cardiac ischemia to bone or tooth loss. Growing evidence demonstrates that stem cells are primarily found in niches and that certain tissues contain more stem cells than others. Among these tissues, the dental pulp is considered a rich source of mesenchymal stem cells that are suitable for tissue engineering applications. It is known that dental pulp stem cells have the potential to differentiate into several cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. The dental pulp stem cells are highly proliferative. This characteristic facilitates ex vivo expansion and enhances the translational potential of these cells. Notably, the dental pulp is arguably the most accessible source of postnatal stem cells. Collectively, the multipotency, high proliferation rates, and accessibility make the dental pulp an attractive source of mesenchymal stem cells for tissue regeneration. This review discusses fundamental concepts of stem cell biology and tissue engineering within the context of regenerative dentistry.

Key words Tissue engineering · Endodontics · Odontoblasts · Endothelial cells · Dentin

L. Casagrande · M.M. Cordeiro · S.A. Nör · J.E. Nör (✉)
Department of Cariology, Restorative Sciences and Endodontics, University of Michigan School of Dentistry, 1011 N. University, Rm. 2309, Ann Arbor, MI 48109-1078, USA
Tel. +1-734-936-9300
e-mail: jenor@umich.edu

J.E. Nör
Department of Biomedical Engineering, University of Michigan College of Engineering, Ann Arbor, MI, USA

J.E. Nör
Department of Otolaryngology, University of Michigan School of Medicine, Ann Arbor, MI, USA

Introduction

The discovery of dental stem cells and recent advances in cellular and molecular biology have led to the development of novel therapeutic strategies that aim at the regeneration of oral tissues that were injured by disease or trauma. Tissue engineering is multidisciplinary by nature, bringing together biology, engineering, and clinical sciences with the goal of generating new tissues and organs.[1] Tissue engineering is a science based on fundamental principles that involves the identification of appropriate cells, the development of conducive scaffolds, and the understanding of the morphogenic signals required to induce cells to regenerate a tissue or organ. Over the last few years, dentistry has begun to explore the potential application of stem cells and tissue engineering towards the repair and regeneration of dental structures. It is becoming increasingly clearer that this conceptual approach to therapy, named "regenerative dentistry," will have its place in the clinical practice of dentistry in the future. This review discusses the state-of-the-science with regard to dental pulp stem cells in tooth tissue engineering, and presents a prospectus for the field of stem cell-based regenerative dentistry.

Stem cells of dental origin

Stem cells are nonspecialized cells that continuously divide, have the ability of self-renewal, and are capable of generating complex tissues and organs.[2] These cells can be classified as either embryonic or postnatal.[3] Embryonic stem cells are found in the inner cell mass of the blastocyst during the early stages of embryo development.[4] Their self-renewal potential and unrestricted ability to generate new tissues and organs (totipotency) make these cells an attractive cellular source for cell-based regenerative therapies. However, the use of embryonic stem cells is controversial. Indeed, legal and ethical issues have significantly impaired the feasibility of their use in the laboratory and in the clinic.[5] Therefore, this review will not discuss the use of embryonic

stem cells in dentistry. Similarly to embryonic stem cells, postnatal stem cells are capable of self-renewal. However, postnatal cells are multipotent; that is, they have a more limited capacity for differentiating into other cell types than the totipotent embryonic stem cells. Notably, postnatal stem cells present the obvious advantage of being a source of cells for autologous transplants, minimizing risks related with immune rejection. And finally, postnatal stem cells can, at least in theory, be obtained from individuals at any stage in life.

Several, if not all, adult tissues have a subpopulation of stem cells. Examples of such tissues are the bone marrow, brain, skin, muscle, and adipose tissue.[6–9] Stem cells have also been found in several dental tissues. One of the first tooth-related stem cell types was found in the pulp of permanent teeth and was named dental pulp stem cells (DPSCs).[10] In addition, stem cells from human exfoliated deciduous teeth (SHED), stem cells from the apical papilla, dental follicle progenitor cells, and periodontal ligament stem cells have also been characterized.[11–15] Mechanistic studies focused on these cells are certainly improving our understanding of tooth development. In addition, this knowledge has been applied in translational studies that aim at the use of these stem cells in clinical settings where the regeneration of dental and craniofacial tissues is indicated.

The usefulness of stem cells in clinical applications depends on their proliferation rate, differentiation potential, and accessibility. For example, when bone marrow stem cells were compared with DPSCs, DPSCs presented favorable results with regard to odontogenic capability.[16] Stem cells of dental origin can certainly generate dental tissues.[10,11,13,17,18] We have shown that SHED and DPSCs are capable of generating a tissue that has morphological and functional characteristics that closely resemble those of human dental pulp (Fig. 1).[19–22] Other studies have expanded the potential of these cells in the treatment of diseases and conditions such as muscular dystrophies, critical size bone defects, corneal alterations, spinal cord injury, and systemic lupus erythematosus.[23–28] Such studies clearly demonstrate the plasticity and the differentiation potential of stem cells of dental origin. And finally, SHED cells have the unique advantage of being retrievable from naturally exfoliated teeth, which can be considered a "disposable" source of postnatal human tissue. Collectively, these studies suggest that the tooth constitutes an attractive source of stem cells that can potentially be useful in a wide spectrum of clinical scenarios.

Signaling molecules and dental pulp stem cell differentiation

Growth factors and morphogenic factors are proteins that bind to specific membrane receptors and trigger a series of signaling pathways that coordinate all cellular functions. These molecules play a critical role during development, guiding processes that determine the fate of stem cells and regulate the generation of all tissues and organs in the developing embryo. Similarly, these morphogenic molecules play a critical role in physiological processes of tissue regeneration as, for example, wound healing in the skin or dental pulp responses to the progression of dentinal caries. The same growth factors that guide embryogenesis and physiological tissue regeneration can also be used therapeutically to guide stem cell differentiation toward specific cell fates and to coordinate cellular processes that result ultimately in the generation of a new tissue or organ via tissue engineering-based approaches. More specifically, there are many similarities between morphogenic factors regulating dentinogenesis and the factors that regulate reparative dentinogenesis.[29] One rapidly concludes that the field of dental tissue engineering can benefit tremendously from studies focused on the cellular and molecular mechanisms of odontogenesis.

Growth factors have an important role in signaling reparative processes in dentin and pulp.[29,30] Indeed, it is known that factors such as transforming growth factor β, bone morphogenic proteins (BMPs), platelet-derived

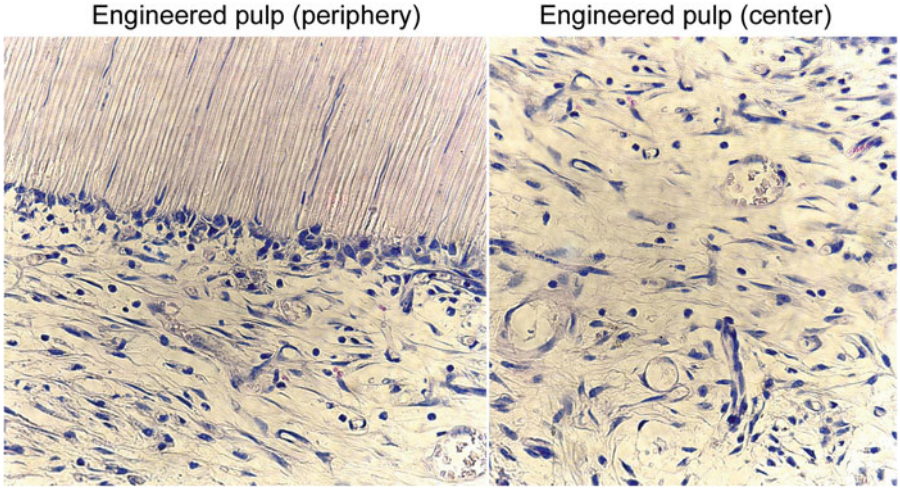

Fig. 1. Dental pulp engineered with stem cells from human exfoliated deciduous teeth (SHED). SHED were seeded into tooth slice/scaffolds and transplanted into the subcutaneous space of immunodeficient mice. After 21 days, the tooth slices were retrieved, fixed, demineralized, and prepared for standard histology. Photomicrographs of hematoxylin/eosin-stained tissue sections (×400) depicting the periphery and the central portion of the dental pulp-like tissue formed in the pulp chamber of these tooth slices

growth factor, fibroblast growth factor, and vascular endothelial growth factor (VEGF) are incorporated into the dentin matrix during dentinogenesis and are retained there as "fossilized" molecules.[31–33] Interestingly, when these molecules are released from the dentin, they are bioactive and fully capable of inducing cellular responses, as for example those that lead to the generation of tertiary dentin and to dental pulp repair.[30,34] The tubular arrangement of the dentin facilitates the movement of growth factors released from dentin matrix that has been demineralized by caries, acidic tooth conditioning agents, or pulp capping materials. Interestingly, calcium hydroxide has been shown to solubilize dentin and allow the release of bioactive molecules that can potentially regenerate dentin.[35] Such events involve the recruitment of DPSCs, their differentiation into odontoblasts, and the secretion of mineralizable matrices.[36–38] Collectively, the release of growth factors from the dentin appears to constitute an important mechanism of defense against injuries, allowing for a finite level of dental tissue regeneration.

Studies from the early 1990s demonstrated that BMPs (e.g., BMP-2, BMP-4, BMP-7) trigger signaling events that induce the generation of dentin in animal models.[39,40] However, the ability to induce the formation of dentin is not limited to BMPs. Dentin matrix protein (DMP)-1 has been shown to nucleate apatite crystals and to induce dentin formation.[41,42] Moreover, bone sialoprotein (BSP) can also stimulate the differentiation of pulp cells into cells that are capable of secreting mineralizable matrices in pulp exposure sites.[43,44] Interestingly, different morphologic characteristics are observed when dentin is induced by different factors (e.g., BSP-induced dentin appears to be different from BMP-induced dentin). Such results raise the intriguing possibility that it might be possible to select a specific type of biological inducer of dentin repair according to the patient's dentin needs. Notably, all these morphogenic factors can be found in dentin matrices and are presumptive inducers of DPSC differentiation into odontoblast-like cells.[43,45,46]

Recent studies from our laboratory have added evidence for the important role of the bioactive molecules that are present in dentin as inducers of differentiation of pulp stem cells into odontoblasts.[22] We have observed that SHED seeded in scaffolds surrounded by dentin differentiated into odontoblasts, as demonstrated by the acquisition of markers of differentiation such as DMP-1, dentin sialophosphoprotein, and matrix extracellular phosphoglycoprotein. In contrast, SHED seeded in scaffolds without dentin, or in scaffolds surrounded by dentin that had been previously deproteinized by long-term treatment with sodium hypochlorite, lost their ability to differentiate into odontoblasts. In search of the specific dentin proteins that were mediating the odontoblastic differentiation of SHED, we performed a series of neutralizing antibody experiments. These studies demonstrated that dentin-derived BMP-2, but not BMP-7, is necessary for the differentiation of stem cells into odontoblasts.[22] Such results from cell and molecular biology experiments can provide guidance for translational experiments aimed at the regeneration of dentin via targeted induction of odontoblastic differentiation of DPSCs.

Scaffolds for dental pulp stem cells

Mammalian cells require interactions with their microenvironment to survive, proliferate, and function. In tissue physiology, these three-dimensional (3-D) environments are largely composed of extracellular matrix proteins. In tissue engineering, these 3-D structures are initially provided to the cells through the use of biodegradable and biocompatible scaffolds.[1] They provide an environment that allows for the adhesion of cells and their proliferation, migration, and differentiation until these cells and the host cells begin to secrete and shape their own microenvironment. Therefore, scaffolds are considered a critical component of tissue engineering.[47,48]

Scaffolds made of synthetic polymers allow for the manipulation of their physicochemical properties such as degradation rate, pore size, and mechanical resistance. The most common synthetic polymers in tissue engineering are likely poly-(l-lactic acid) (PLLA), poly-(glycolic acid) (PGA), and the copolymer poly-(lactic-co-glycolic acid) (PLGA). These scaffolds are biodegradable and biocompatible and allow for cell growth and differentiation, making them highly suitable for tissue engineering applications.[17,45,49] The degradation rate can be controlled by the proportion of PLLA/PGA used in the manufacturing of these scaffolds. Notably, it is important for the rate of scaffold degradation to be compatible with the rate of tissue formation. In other words, the scaffold should be designed to provide structural integrity for the cells used in tissue engineering until the newly formed tissue becomes autosustainable.[50] One of the first examples of successful replacement of scaffold by dental tissues was the use of copolymers (PGA/PLLA and PLGA) that allowed for the engineering of complex dental structures with characteristics similar to the crowns of natural teeth.[17]

We have used PLLA scaffolds extensively in dental pulp tissue engineering.[19–22] The PLLA scaffolds are cast inside the tooth slices prepared in the cervical area of extracted sound human third molars (Fig. 2). Stem cells are seeded in the tooth slice/scaffolds and transplanted into the subcutaneous space of immunodeficient mice. We have named this experimental approach the tooth slice/scaffold model of dental pulp tissue engineering.[19–22,51]

Our studies have demonstrated that 21–28 days after transplantation, DPSCs seeded in tooth slice/scaffolds and transplanted into mice generate a tissue with morphological characteristics similar to those of human dental pulp (Fig. 1).[19–22] We have also investigated the effect of the method for the creation of pores in the scaffolds on the differentiation of DPSCs into odontoblasts and on the generation of pulp-like tissues. These experiments revealed that scaffolds generated with gelatin or salt porogens resulted in similar proliferation of DPSCs, but the expression of odontoblastic markers (e.g., DMP-1) was higher in gelatin-based scaffolds in vitro.[21] These initial experiments demonstrated that the structural characteristics of the scaffold may play a significant role in the differentiation of DPSCs.

From a translational standpoint, it would be beneficial for scaffolds designed for dental pulp tissue engineering

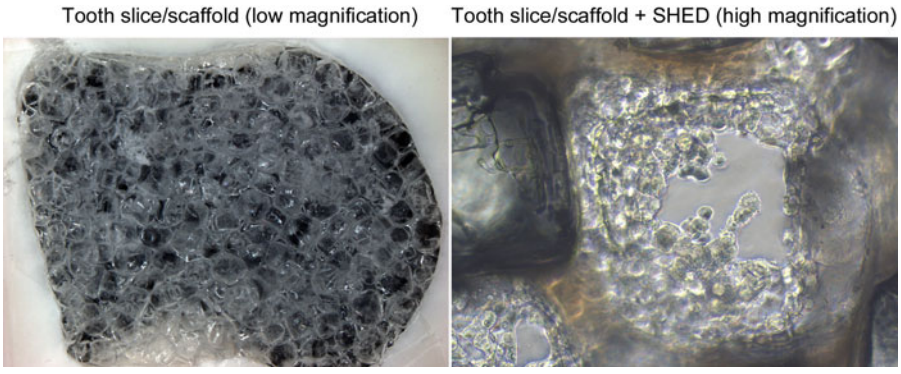

Fig. 2. Tooth slice/scaffold model of dental pulp tissue engineering. Highly porous biodegradable poly-l-lactic acid scaffolds were cast in the pulp chamber of 1.5-mm-thick human tooth slices. The porogen used here was NaCl particles. The higher magnification image depicts a pore containing multiple SHED cells

purposes to be made of injectable materials. The goal of these injectable scaffolds is to allow for stem cell transplantation throughout the full extent of the root canal and pulp chamber. An excellent example of such an approach was recently described by Galler and colleagues.[52] In this case, self-assembling multidomain peptide hydrogels were generated and characterized as highly biocompatible and injectable. Interestingly, the addition of a matrix metalloprotease 2 specific cleavage site and a cell adhesion motif (i.e., RGD) enhanced cell survival and induced cell motility within these hydrogels. In summary, scaffold development and characterization is quickly becoming a critically important new area in the field of dental materials. We firmly believe that this is an emerging area that will play a critical role in the translation of laboratory findings to the application of stem cell-based tissue engineering approaches in the dental clinic.

Blood vessels and tooth tissue regeneration

Vasculogenesis is defined as de novo formation of blood vessels. Temporal and spatial regulation of vasculogenesis is required for normal embryogenesis. Indeed, loss of a single allele of the *VEGF* gene causes early embryonic lethality.[53,54] There is solid evidence for a functional link between vasculogenesis and bone development.[55,56] It is also well known that the teratogenic effect of thalidomide is caused by aberrant vasculogenesis leading to impaired long bone development and limb truncation.[57] We have recently observed that SHED have the potential to differentiate into functional vascular endothelial cells via a process that closely resembles that of vasculogenesis,[19,20] as depicted graphically (Fig. 3). We have reported that DPSCs differentiate themselves into endothelial cells that make functional, blood-carrying blood vessels.[19,20] These findings raise the intriguing possibility that stem cells of dental pulp origin might be useful in the treatment of severe ischemic conditions of the heart, brain, or limbs. Furthermore, it is unquestionable that the success of tissue engineering relies heavily upon the rapid establishment of local microvascular networks to provide blood and nutrients for cells that are engaged in tissue regeneration processes. More specifically, one of the critical challenges of dental pulp tissue engineering is the generation of a functional vascular network, considering the anatomical constraints imposed by the fact that all vascularization must access the root canal through the apical foramen. Therefore, much research is needed in the area of induction of vasculogenesis accompanying efforts of dental pulp tissue engineering. Notably, the use of stem cells as a single cellular source for blood vessels and for "tissue making" is obviously very attractive from a translational standpoint.

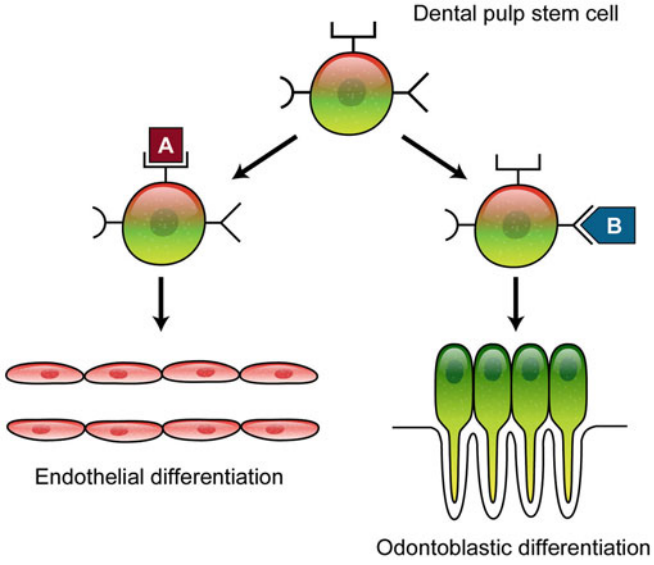

Fig. 3. Schematic representation of the multipotency of dental pulp stem cells. In this hypothetical example, factor *A* induces vasculogenic differentiation of the dental pulp stem cells, while factor *B* induces odontoblastic differentiation

Angiogenesis is the process of new blood vessel formation from preexisting vasculature. Therefore, it is fundamentally different from the process of vasculogenesis. While vasculogenesis is critically important in the early stages of embryonic development, angiogenesis allows for the remodeling of the vascular networks later in embryonic development and plays a major role in postnatal physiological responses (e.g., wound healing). In the context of the dental pulp, it is well known that conservative pulp treatments such as direct pulp capping trigger wound healing events that are orchestrated by an exquisitely regulated angiogenic

response. During physiological wound healing, cells from wounded sites release chemotactic factors that contribute to the organization of a transient inflammatory process.[58–60] Notably, local cells release angiogenic factors that quickly organize a robust proangiogenic response that allows for the influx of inflammatory cells and provides the oxygen and nutrients that are required to maintain the high metabolic demands of cells actively engaged in tissue repair.[60] In the dental pulp, elegant work has recently shown that endothelial cell injury is involved in the recruitment of odontoblastic-like cells.[61]

VEGF is considered the most important regulator of vasculogenesis and angiogenesis in physiological as well pathological conditions.[62,63] VEGF induces endothelial cells to form capillary structures when seeded in 3-D collagen gels.[64] In vivo, VEGF enhances permeability and induces potent proangiogenic responses.[65,66] Notably, VEGF plays a critical role in the regulation of angiogenesis by enhancing endothelial cell survival.[64] We have observed that VEGF induces angiogenesis and enhances the survival of dental pulp cells from human tooth slices transplanted in the subcutaneous space of immunodeficient mice.[67] We have also demonstrated that VEGF induces the differentiation of DPSCs (i.e., SHED) into endothelial cells.[20] The Nakashima group has elegantly shown that porcine pulp stem cells enhance local blood flow in experimental ischemic sites by secreting angiogenic factors (e.g., VEGF) and inducing an angiogenic response by host endothelial cells.[68] Collectively, these data suggest that a local increase in VEGF availability is highly beneficial for stem cell-mediated regeneration of dentin and pulp. Notably, dentin matrices contain VEGF,[33] which likely contributes to the angiogenic responses mediated by dentin extracts.[69] We have recently begun to explore the possibility of incorporating VEGF in the scaffolds that are used for transplantation of stem cells for dental pulp tissue engineering purposes.

A major challenge for stem cell-based tissue regeneration is to ensure rapid establishment of efficient blood vessel networks that allow for the survival of transplanted cells and provide the influx of oxygen and nutrients required to maintain the high metabolic demands of cells participating in tissue regeneration. Our laboratory has made the novel and unexpected observation that SHED have the potential to differentiate into functional blood vessels in vivo.[19,20] This raises the exciting possibility that dental pulp stem cells may constitute a single cell source for regenerating the tissue in question (e.g., dental pulp, dentin), while providing at the same time the required vascular network that will support the newly formed tissue. Notably, during embryonic development the molecular cues required for timely differentiation of stem cells are inherently regulated. However, the same is not necessarily true when stem cells are used therapeutically in foreign microenvironments. Therefore, studies focused on the cellular and molecular signaling events regulating the determination of stem cell fate will be critically important for the translation of laboratory findings to the clinic. These studies should provide the knowledge to determine the nature of biological modifiers that can guide stem cells toward the desired differentiation paths.

Prospectus for stem cell-based dental tissue engineering

Stem cell-based regenerative therapies certainly hold much potential in the treatment of medical and dental conditions. Indeed, many patients around the world have already benefited from such therapies. However, the decision to incorporate stem cell-based therapies into routine clinical dental practice requires careful analysis of the risks and benefits associated with the procedure. For example, while the potential benefits of stem cell transplantation for patients with hematological cancer tend to outweigh the risks, the same may not be necessarily true for their use in dental procedures. It is unquestionable that the processes of storage and expansion of stem cells in laboratory settings, as well as the transplantation of these cells back to the patient, carry certain risks. There is a risk of transformation of the stem cells, and there is also a risk of unwanted contamination of these cells with pathogens during these procedures.[70] While these risks are relatively small, they exist and cannot be ignored. Indeed, it is certainly imperative that patients undergoing such procedures with stem cells in investigative or clinical settings are made fully aware of such risks.

Based on the analysis of the existing literature, and our own clinical and research judgment, it is becoming increasingly clear that dentistry will embrace new concepts of tissue regeneration. It is likely that such approaches will involve the use of stem cell-based therapies combined (or not) with biomimetic approaches. While the use of stem cells brings many new therapeutic opportunities, and perhaps will allow for the treatment of dental conditions that are untreatable with today's materials and procedures, one must proceed with caution. It is imperative for clinical procedures with stem cells to be supported by solid basic and translational research. It will be only through rigorous research that the full extent of the potential risks involved in the use of these cells will be understood, and the means to prevent (or overcome) them will be discovered. It will also be only through research that the biology of tooth-related stem cells and the therapeutic potential of these cells will be better understood.

In conclusion, stem cell-based dental tissue regeneration is a new and exciting field that has the potential to transform the way that we practice dentistry. Its future will depend on the understanding of the biology of the cells that will be used to regenerate tissues, and its boundaries will be demarcated by an in-depth knowledge of the potential risks and likely benefits associated with each regenerative procedure. The field of stem cell-based regenerative dentistry is complex and multidisciplinary by nature. Progress will depend on the collaboration between clinicians and researchers from diverse fields (e.g., biomaterials, stem cell biology, endodontics) working together toward the goal of developing biological approaches to regenerate dental and craniofacial tissues.

Acknowledgments The authors would like to thank Chris Jung for his contribution with the schematic drawing presented here, and the

members of the Angiogenesis Research Laboratory at the University of Michigan School of Dentistry for their invaluable input and technical help during the execution of these studies. We also would like to acknowledge the support received from CAPES (Brazilian Government) for the pursuit of the studies presented in this review.

References

1. Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
2. van der Kooy D, Weiss S. Why stem cells. Science 2000;287: 1439–41.
3. Fortier LA. Stem cells: classifications, controversies, and clinical applications. Vet Surg 2005;34:415–23.
4. Pera MF, Reubinoff B, Trounson A. Human embryonic stem cells. J Cell Sci 2000;113:5–10.
5. Gardner RL. Stem cells: potency, plasticity and public perception. J Anat 2002; 200:277–82.
6. Bosch P, Musgrave DS, Lee JY, Cummins J, Shuler T, Ghivizzani TC, Evans T, Robbins TD, Huard. Osteoprogenitor cells within skeletal muscle. J Orthop Res 2000;18:933–44.
7. Gage FH. Mammalian neural stem cells. Science 2000;287:1433–8.
8. Gimble J, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy 2003;5: 362–9.
9. Jones PH, Watt FM. Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. Cell 1993;73:713–24.
10. 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 2000:97:13625–30.
11. 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;100:5807–12.
12. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149–55.
13. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Shi S, Wang S. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 2006;1:e79.
14. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008;34:166–71.
15. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009;88:792–806.
16. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, Jin Y. Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell 2007;99:465–74.
17. Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J Dent Res 2002;81:695–700.
18. Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. J Dent Res 2004;83:518–22.
19. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nör JE. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008;34:962–9.
20. Sakai VT, Zhang Z, Dong Z, Neiva K, Machado M, Shi S, Santos C, Nör JE. SHED differentiate into functional odontoblasts and endothelium. J Dent Res 2010;89:791–6.
21. Demarco FF, Casagrande L, Zhang Z, Dong Z, Tarquinio SB, Zeitlin BD, Shi S, Smith AJ, Nör JE. Effects of morphogen and scaffold porogen on the differentiation of dental pulp stem cells. J Endod 2010;36:1805–11.
22. Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nör JE. Dentin-derived BMP-2 and odontoblast differentiation. J Dent Res 2010;89:603–8.
23. Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca SA, Cabral RM, Maranduba, CM, Gaiad, TP, Morini AC, Vieira NM, Brolio, MP, Sant'anna OA, Miglino, MA, Zatz, M. Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? J Transl Med 2008;3:35.
24. Seo, BM, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K, Lee JS, Shi S. SHED repair critical-size calvarial defects in mice. Oral Dis 2008;4:428–34.
25. Monteiro BG, Serafim RC, Melo GB, Silva MC, Lizier NF, Maranduba CM, Smith RL, Kerkis A, Cerruti H, Gomes JA, Kerkis I. Human immature dental pulp stem cells share key characteristic features with limbal stem cells. Cell Prolif 2009;42:587–94.
26. Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, Haapasalo M. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod 2010;36:469–74.
27. Nosrat IV, Widenfalk J, Olson L, Nosrat CA. Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. Dev Biol 2001;238:120–32.
28. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, Wang S, Shi S. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther 2010;1:5.
29. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med 2001;12:425–37.
30. Smith AJ, Murray PE, Sloan AJ, Matthews JB, Zhao S. Transdentinal stimulation of tertiary dentinogenesis. Adv Dent Res 2001;15: 51–4.
31. Finkelman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ. Quantitation of growth factors IGF-I, SGF/IGF-II, and TGF-beta in human dentin. J Bone Miner Res 1990;5:717–23.
32. Ruch JV, Lesot H, Bègue-Kirn C. Odontoblast differentiation. Int J Dev Biol 1995;39:51–68.
33. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. Arch Oral Biol 2000;45:1013–6.
34. Tziafas D. Basic mechanisms of cytodifferentiation and dentinogenesis during dental pulp repair. Int J Dev Biol 1995;39:281–90.
35. Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ. The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. Biomaterials 2006;27:2865–73.
36. Fitzgerald M, Chiego DJ Jr, Heys DR. Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. Arch Oral Biol 1990;35:707–15.
37. Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot H. Reactionary dentinogenesis. Int J Dev Biol 1995;39:273–80.
38. Murray PE, Smith AJ. Saving pulps: a biological basis. An overview. Prim Dent Care 2002;9:21–6.
39. Nakashima M. Induction of dentine in amputated pulp of dogs by recombinant human bone morphogenetic proteins-2 and -4 with collagen matrix. Arch Oral Biol 1994;39:1085–9.
40. Rutherford RB, Wahle J, Tucker M, Rueger D, Charette M. Induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. Arch Oral Biol 1993;38:571–6.
41. He G, Dahl T, Veis A, George A. Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein 1. Nat Mater 2003;2: 552–8.
42. He G, Gajjeraman S, Schultz D, Cookson D, Qin C, Butler WT, Hao J, George A. Spatially and temporally controlled biomineralization is facilitated by interaction between self-assembled dentin matrix protein 1 and calcium phosphate nuclei in solution. Biochemistry. 2005;44:16140–8.
43. Decup F, Six N, Palmier B, Buch D, Lasfargues JJ, Salih E, Goldberg M. Bone sialoprotein-induced reparative dentinogenesis in the pulp of rat's molar. Clin Oral Investig 2000;4:110–9.
44. Six N, Decup F, Lasfargues JJ, Salih E, Goldberg M. Osteogenic proteins (bone sialoprotein and bone morphogenetic protein-7) and dental pulp mineralization. J Mater Sci Mater Med 2002;13:225–32.
45. Nakashima M. Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. Cytokine Growth Factor Rev 2005;16:369–76.

46. Prescott RS, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J, John AS, George A. In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J Endod 2008;34:421–6.
47. Nakashima M, Reddi AH. The application of bone morphogenetic proteins to dental tissue engineering. Nat Biotechnol 2003;21: 1025–32.
48. Nakashima M, Akamine A. The application of tissue engineering to regeneration of pulp and dentin in endodontics. J Endod 2005;31:711–8.
49. Bohl KS, Shon J, Rutherford B, Mooney DJ. Role of synthetic extracellular matrix in development of engineered dental pulp. J Biomater Sci Polym Ed 1998;9:749–64.
50. Freed LE, Vunjak-Novakovic G, Biron RJ, Eagles DB, Lesnoy DC, Barlow SK, Langer R. Biodegradable polymer scaffolds for tissue engineering. Biotechnology 1994;12:689–93.
51. Gonçalves SB, Dong Z, Bramante CM, Holland GR, Smith AJ, Nör JE. Tooth slice-based models for the study of human dental pulp angiogenesis. J Endod 2007;33:811–4.
52. Galler KM, Aulisa L, Regan KR, D'Souza RN, Hartgerink JD. Self-assembling multidomain peptide hydrogels: designed susceptibility to enzymatic cleavage allows enhanced cell migration and spreading. J Am Chem Soc 2010;132:3217–23.
53. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435–9.
54. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996;380:439–42.
55. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 1999;5: 623–8.
56. Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, Bouxsein ML, Faugere MC, Guldberg RE, Gerstenfeld LC, Haase VH, Johnson RS, Schipani E, Clemens TL. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J Clin Invest 2007;117:1616–26.
57. Stephens TD, Bunde CJ, Fillmore BJ. Mechanism of action in thalidomide teratogenesis. Biochem Pharmacol 2000;59:1489–99.
58. Martin P. Wound healing; aiming for perfect skin regeneration. Science 1997;276:75–81.
59. Tedder TF, Steeber DA, Chen A, Engel P. The selectins; vascular adhesion molecules. FASEB J 1995;9:866–73.
60. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res 2010;89:219–29.
61. Mathieu S, Battari AE, Dejou J, About I. Role of injured endothelial cells in the recruitment of human pulp cells. Arch Oral Biol 2005;50:109–113.
62. Carmeliet P, Collen D. Molecular analysis of blood vessel formation and disease. Am J Physiol 1997;273:H2091–104.
63. Ferrara N. Vascular endothelial growth factor. Arterioscler Thromb Vasc Biol 2009;29:789–91.
64. Nör JE, Christensen J, Mooney DJ, Polverini PJ. Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. Am J Pathol 1999;154:375–84.
65. Ferrara N, Gerber HP, Lecouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
66. Ferrara N. Molecular and biological properties of vascular endothelial growth factor. J Mol Med 1999;77:527–43.
67. Mullane EM, Dong Z, Sedgley CM, Hu JC, Botero TM, Holland GR, Nör JE. Effects of VEGF and FGF2 on the revascularization of severed human dental pulps. J Dent Res 2008;87: 1144–8.
68. Iohara K, Zheng L, Wake H, Ito M, Nabekura J, Wakita H, Nakamura H, Into T, Matsushita K, Nakashima M. A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem Cells. 2008;26:2408–18.
69. Zhang R, Cooper PR, Smith G, Nör JE, Smith AJ. Angiogenic activity of dentin matrix components. J Endod 2011;37:26–30.
70. Vats A, Bielby RC, Tolley NS, Nerem R, Polak JM. Stem cells. Lancet 2005;366:592–602.