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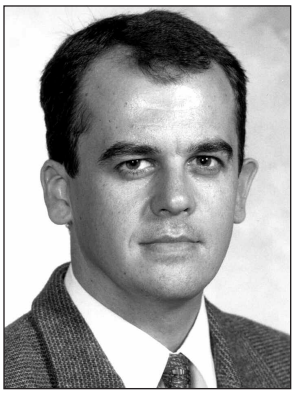
Buonocore Memorial Lecture

Tooth Regeneration in Operative Dentistry

JE Nör

Michael Buonocore



Jacques E Nör

ABSTRACT

For many years, operative dentistry has been using regenerative approaches to treat dental disease. The use of calcium hydroxide to stimulate reparative or reactionary dentin is clearly an example of such a therapeutic strategy. The advent of tissue engineering is allowing dentistry to move forward in the use of regeneration as an underlying principle for the treatment of dental disease. Tissue engineering is a multi-disciplinary science that brings together biology, engineering and clinical sciences with developing new tissues and organs. It is based on fundamental principles that involve the identification of appropriate cells, the development of conducive scaffolds and an understanding of the morphogenic signals required to induce cells to regenerate the tissues that were lost. This review is focused on the presentation and discussion of existing literature that covers the engineering of enamel, dentin and pulp, as well on the engineering of entire teeth. There are clearly major roadblocks to overcome before such strategies move to the clinic and are used regularly to treat patients. However, existing evidence strongly suggests that the engineering of new dental structures to replace tissues lost during the process of caries or trauma will have a place in the future of operative dentistry.

*Jacques E Nör, DDS, MS, PhD, professor of dentistry, Dept of Cariology, Restorative Sciences, Endodontics and professor of Biomedical Engineering, College of Engineering, University of Michigan, Ann Arbor, MI, USA

*Reprint request: 1011 N University, Rm 2309, Ann Arbor, MI 48109-1078, USA; e-mail: jenor@umich.edu

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INTRODUCTION

In the late 1980s, a polymer chemist (Robert Langer) and an organ transplant surgeon (Joseph Vacanti), proposed that it might be possible to generate a tissue or organ by seeding the cells that make this tissue into a biodegradable scaffold.[1] This approach to regenerative medicine was named tissue engineering, which is defined as an interdisciplinary field that applies the principles of engineering and the life sciences toward biological substitutes that restore, maintain or improve tissue function.[2] A seminal publication in the early 1990s describing the fundamentals of tissue engineering[3] and the successful engineering of cartilage in the shape of a human ear in the dorsum of mice[4] brought much attention and visibility to this emerging field. Since then, medical practitioners have increasingly used tissue engineering to treat a variety of conditions. Skin replacements for severely burned pediatric patients[5] and the construction of new bone for patients with severe bone loss[6] are examples of tissue engineering-based strategies that have been used in humans. Over the last few years, dental researchers started to explore the potential of tissue engineering to repair lost

tooth structures and, perhaps, even for complete replacement of an entire tooth. It is projected that strategies involving the use of tissue engineering, nanotechnology and stem cells will have an increasing participation in clinical dentistry over the next 5-20 years.[7] This review aims to provide a broad update on the field of tissue engineering as it applies to the regeneration of tooth structure. It is important to emphasize the fact that most of these technologies are in development and do not currently have approval by the FDA for use in patients. Therefore, this review is written with an eye on the future, exploring the potential impact that tissue engineering may have in the future of clinical dentistry.

Lessons from Molecular Signaling Events During Odontogenesis

The field of tooth tissue engineering uses concepts that originate from early studies on molecular signaling during odontogenesis and also from the study of mutations that lead to tooth-related phenotypes. Morphogenic signals leading to the differentiation and function of odontoblasts and ameloblasts are known to be mediated by specialized molecules. These molecular exchanges start very early during the initial stages of tooth development and progress until the tooth is completely formed.[8-10] Molecular signals flow between odontogenic cells and guide them to position themselves along mineralization fronts, differentiate and start secreting new molecules (proteins). These proteins constitute extra cellular matrices, which will eventually be mineralized into dentin and enamel. Examples of molecular signals are bone morphogenetic proteins (BMPs) and Amelogenin, which will be discussed in greater detail. A hypothetical example of such crosstalk between cells is depicted in Figure 1. Cell "A" secretes several proteins and, among them, one protein (symbolized as a circle) to which cell "B" has a specific receptor. Engagement of this receptor by the protein secreted by cell "A" leads to intracellular signaling in cell "B," which results in synthesis of an mRNA molecule, which will be translated into a new protein that will be secreted to the extracellular environment. A similar phenomenon is observed during tooth development.

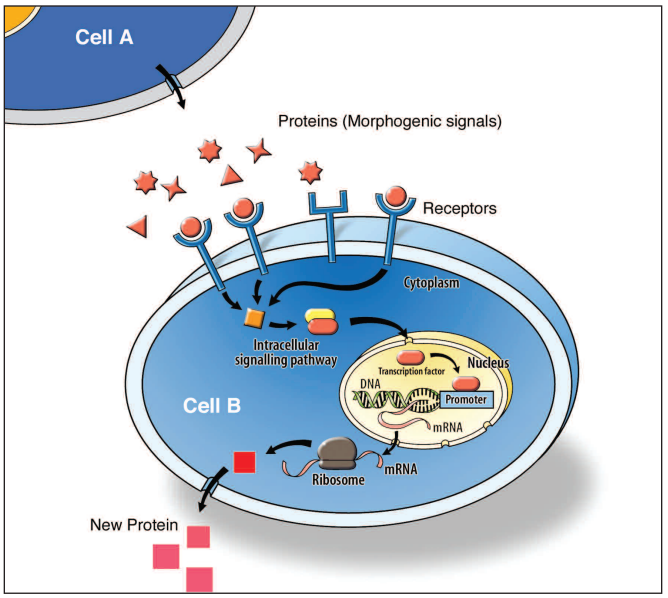

Figure 1. *Diagram depicting the molecular signaling crosstalk between 2 cells.*

Odontoblasts are induced by a process that involves signals from the epithelium for the purpose of synthesizing extracellular matrix proteins required for dentin formation.[11] The cells from the oral epithelium layer (oral ectoderm) secrete BMPs during the early stages of odontogenesis (dental lamina). These first BMP molecules signal a reciprocal response from the cells of the odontogenic mesenchyme and establish an epithelial-mesenchymal crosstalk that is an absolute requirement for tooth morphogenesis.[10-13] This crosstalk is mediated by diffusible molecules (BMPs) that find specific receptors (BMPRs) present in cells that are prepared to respond to the stimulus. Once these receptors are engaged, intracellular signaling occurs, and the odontoblasts begin secreting the extracellular matrix proteins that will eventually be mineralized into dentin. Therefore, these events require a cell that functions as a source of the signaling molecule, one that is responsive to it, one that expresses the specific receptor and possesses the "machinery" required to make a new protein.

From odontogenesis studies, the field of dentistry has learned of morphogenic signals that can induce odontoblasts to make dentin and ameloblasts secrete enamel. This knowledge has been validated by "experiments of nature," in which mutations in certain genes that result in the synthesis of non-functional proteins lead to alterations in the formation of specific tooth structures. For example, dental clinicians know that mutations in the gene Amelogenin lead to enamel hypoplasia.[14] And, that mutations in the gene dentin sialo phosphoprotein (DSPP) lead to the condition called dentinogenesis imperfecta,[14-18] in which dentin is not ideally developed. BMPs are morphogenic signals that play an important role in the development of mineralized tissues in the human body.[19-20] Interestingly, no dental phenotype is caused by mutations of the gene BMP-7.[21-22] This is probably due to the overlapping function of other BMP genes that are redundant and can compensate for the absence of BMP-7. Such studies have provided dental scientists with cues for which molecular signals could be useful to regenerate tooth structure.

Regenerative Dentistry: Making Dentin

Dentists are faced every day with the task of restoring tooth structure lost during the progression of caries

lesions. The development of materials that allow for strong and stable bonding to tooth structure, and the development of esthetic materials that are resistant to wear and degradation in the oral environment, has significantly enhanced the long-term clinical outcomes of restorative procedures. Nevertheless, dental clinicians know that no material available today can mimic all the physical, mechanical and esthetic properties of enamel and dentin. Furthermore, there are specific situations in the clinic were dentists do not have an ideal solution for their patients' needs. One example is the young patient (6-15 years of age) who presents with extensive carious lesions in his or her permanent teeth. At this age, the patient's occlusion is not mature and stable and, therefore, one opts for long-term "temporary" restorations. These restorations frequently constitute large resin composites, perhaps restoring entire cusps as a means to "buy time" until the patient is old enough to receive a ceramic inlay/onlay or a full crown. Unfortunately, these patients return with restoration failures or crown fractures that may lead to the need for tooth extraction. Such patients might benefit from dentin regeneration and the strengthening of tooth structure, if such a therapeutic strategy were available. Much research has been done in the area of biological inducers of dentin mineralization, and the following is a brief summary of the work of many investigators.

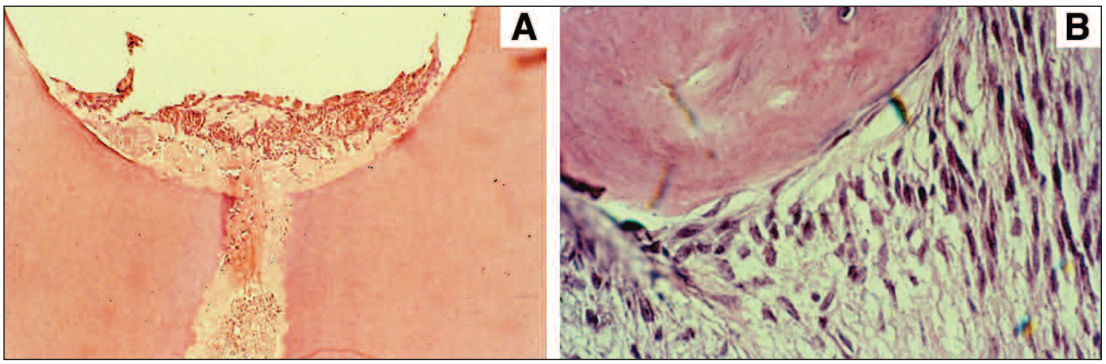

Figure 2. *Reparative dentin induced by BMP-7/OP-1 in monkeys. (A) Reparative dentin induced by OP-1 in the pulp exposure site and in the floor of the cavity preparation. Reparative dentin presents cellular inclusions, 3 weeks after treatment. (B) Reparative dentin induced by BMP-7/OP-1 (high magnification). Reparative dentin is dense and does not present cellular inclusions, 6 months after treatment. Note the absence of signs of inflammatory response in the pulp tissue. These images are courtesy of Dr Bruce Rutherford.*

The concept of inducing reparative dentin to treat loss of this tissue due to the progression of caries is not new. Early work on the biological induction of dentin was inspired by a seminal paper by Urist,[23] which demonstrated, for the first time, that demineralized bone powder had inductive potential and led to ectopic bone formation. Like bone, demineralized dentin powder also has an intrinsic capability to induce mineralization.[24-26] When applied directly to areas of pulp exposure, demineralized dentin induces the local formation of mineralized tissues.[27-28] An understanding of which components of the dentin powder had the inductive capability began in the early 1990s, when it was discovered that specific fractions of dentin, which presumably contained bone morphogenetic protein (BMP) activity,[29] induce reparative dentin formation.[30-31] These studies correlated well with the work of developmental biologists who evaluated the role of BMPs in the processes leading to the differentiation of odontoblasts and synthesis of the dentin matrix[10,32-35] and suggested the potential application of BMPs for dentin regeneration.[19,36]

The ability of pulp cells from (erupted) permanent teeth to respond to the inductive stimuli mediated by BMPs is dependent on the presence of the specific receptors for these proteins. Indeed, BMP receptors (BMPR-IA, -IB and II) were found to be expressed by pulp cells retrieved from human adult teeth.[37] Early attempts to use recombinant human proteins to induce dentin regeneration involved the use of BMP-7,[38-40] BMP-2 and BMP-4.[41-42] The application of these recombinant proteins was performed using collagen-based matrices and resulted in the induction of reparative dentin at the sites of pulp exposure within a period of 2 to 4 months. The general mechanism underlying this response is that reparative dentin replaces the stimulating agents that were placed in direct contact with the dental pulp.[36] A demonstration of this phenomenon can be seen in Figure 2A, where the reparative dentin induced by BMP-7 is not limited to the area of pulp exposure but extends laterally at the floor of the cavity preparation. This concept is strengthened by the observation that the area of reparative dentin induced by BMP-7 is directly proportional to the amount of BMP-7 that is applied.[38] Taken together, these data suggest that one can potentially induce a pre-determined amount of dentin. In this case, the clinician would have the opportunity to strengthen the coronal structure of teeth that present extensive caries (Figure 3). Notably, after mineralization of the reparative dentin (6 months after treatment), the cells lining the pulp side appear non-polarized and plain (Figure 2B). These are the morphological characteristics of cells that are no longer in the active process of secreting extracellular matrices.

The work described above was performed in healthy, non-carious teeth. Scientists knew that, to become a clinical reality, the strategy, based on the application of recombinant proteins directly at the site of pulp exposure, would also have to be effective in carious teeth. The first attempts to induce reparative dentin in teeth with reversible pulpitis were unsuccessful. The application of BMP-7 to inflamed dental pulps did not consistently result in the induction of reparative dentin.[43] The authors concluded that the amount of active recombinant protein might not have been sufficient to induce reparative dentin in inflamed dental pulps. It is also possible that the observed lack of inductive response of the recombinant BMP was due to its relatively short half-life and faster degradation rates of the protein in the environment of an inflamed pulp. Gene therapy with BMPs was proposed in an attempt to overcome the limitation observed with the use of recombinant protein. Indeed, the *ex-vivo* gene transfer of BMP-7 with an adenoviral vector was shown to be a more effective method for inducing reparative dentin in teeth that were experimentally inflamed.[44]

The ability to induce dentin is not limited to BMP-2, -4 and -7. Growth/differentiation factor 11 (Gdf11) is capable of inducing reparative dentin when delivered to pulp cells by a gene transfer strategy.[45] Recent investigations have demonstrated that bone sialoprotein (BSP) stimulates the differentiation of dental pulp cells into cells that secrete an extracellular matrix that is eventually mineralized into reparative dentin at the site of pulp exposure.[46-47] Interestingly, the authors observed different morphological characteristics of the reparative dentin that was induced by BSP or 2 forms of Amelogenin, when compared to BMP-7.[48-49] These results suggest the intriguing possibility that the clinician may one day be able to select the ideal type of biological inducer of reparative dentin according to the patient's needs.

The study of biological inducers of reparative dentin is complex. Perhaps, one the most difficult challenges ahead will be to overcome the intrinsic difficulties of treating a tooth that presents with an inflamed pulp. These teeth may need a therapeutic agent that will help to control the inflammatory response in addition to inducing mineralization. Another important challenge is to further develop suitable carriers for the biological inducer of mineralization to be used in the site of pulp exposure and, perhaps, in a portion of the cavity preparation. These carriers should be biocompatible and also have physical and mechanical properties that are compatible to their use in restorative dentistry. And, finally, a well-sealed restoration will be of fundamental importance to preventing microleakage and subsequent contamination of the pulp exposure site before mineralization of the reparative dentin.

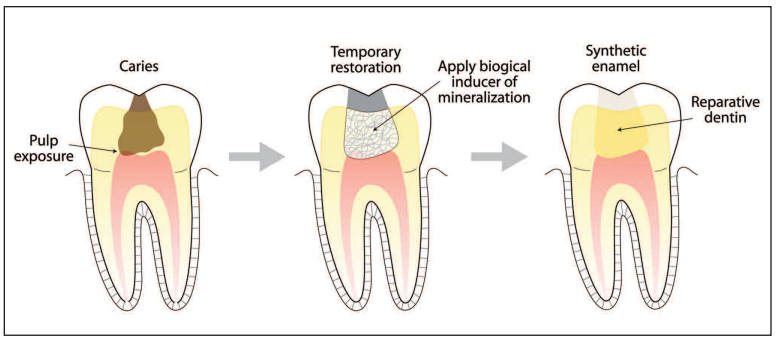

Figure 3. *Diagram depicting the prospect of using a biological inducer of mineralization for dentin regeneration, and using synthetic enamel for final restoration of the cavity preparation.*

To date, the use of a biological inducer for the regeneration of dentin does not have approval of the US Food and Drug Administration (FDA, Rockville, MD, USA). However, the FDA has approved the use of recombinant human BMPs for medical use, for example, for the acceleration of bone fusion in the treatment of slow healing bony fractures. Such approval suggests that biological inducers of reparative dentin may find their way into the dental clinic once the effectiveness and safety of such procedures are tested in preclinical models and in well-designed clinical trials.

Regenerative Dentistry: Making Enamel

Odontoblasts, the specialized cells that make dentin, are found in the dental pulp of erupted teeth. In their absence, undifferentiated pulp cells or dental pulp stem cells can be differentiated into odontoblasts that will replace lost cells and restore the capability of the dental pulp to synthesize reparative dentin. In contrast, the cells that specialize in the making of enamel (ameloblasts) are no longer present in teeth with complete crown development. Therefore, an *in situ* cell-based strategy to regenerate enamel is not feasible. However, researchers' creativity and ingenuity has recently allowed for the development of synthetic enamel that is fundamentally based on the use of the principles of tissue engineering and nanotechnology. These scientists have gone even further. Proof-of-principle experiments have demonstrated that it is possible to engineer synthetic enamel containing fluoride, as described below.

Non-carious enamel is a highly organized structure made of enamel prisms consisting of bundles of nanorod-like calcium hydroxyapaptite crystals arranged roughly parallel to each other.[50-51] The prisms can be considered micro-architectural units of enamel. Surfactants, which may mimic the biological action of enamel proteins in enamel development, can be used as reverse micelles or microemulsions to synthesize

nanoscale structures that may self-assemble into "one-dimensional building blocks."[51] The resulting hydroxyapatite nanorods are similar in size and composition to natural enamel crystals. These nanorods have a strong potential to serve as a platform for the development of flowable restorative materials designed for the restoration of lost enamel. More recently, the Clarkson group used the basic knowledge of the biological processes involved in amelogenesis, together with recent advances in nanotechnology, to create ordered enamel prism-like structures consisting of fluorapatite nanorods under mild hydrothermal conditions without the help of surfactants, proteins or cells (Figure 4).[52]

Further advances in the development of synthetic enamel will make use of this knowledge about the role of enamel-making proteins, such as Amelogenin. Mutations in the Amelogenin gene have led to enamel hypoplasias, which can range from fairly mild to severe.[14,53-55] The mineralization process of enamel is compromised when mutations of the Amelogenin gene lead to alterations in the ability of the Amelogenin protein to self-assemble in the enamel organic matrix.[56-57] Such alterations may ultimately affect the enamel architecture and structural organization[57-58] and its mechanical properties.[59] Such studies have led to the conclusion that Amelogenin is fundamental for the assembly of enamel crystals into prisms and the assembly of prisms into the enamel covering of the dental crown.

This knowledge was used by a group of investigators who studied the role of Amelogenin in the control of apatite crystal growth. These investigators observed that Amelogenin allowed for the synthesis of elongated crystals.[60] A further development made by this group was to incorporate fluoride into the process of Amelogenin-driven apatite crystal growth. The authors observed that the combination of Amelogenin and fluoride allowed for the formation of rod-like apatite crystals with dimensions that resemble the ones observed in natural enamel.[61-62] This work clearly has the potential to contribute to the engineering of an enamel-based biomaterial that may have the added benefit of having fluorapatite intrinsically incorporated into its composition

Regenerative Dentistry: Making Dental Pulp

The maintenance of dental pulp vitality is an underlying goal of most restorative procedures. However, dental clinicians frequently face circumstances in which this is no longer possible. Perhaps one of these situations is when trauma leads to coronal fracture and pulp necrosis in young, immature anterior teeth, as depicted in Figure 5. These teeth have incomplete apex formation, which makes endodontic procedures quite challenging. In addition, these teeth present very large pulp chambers and incomplete lateral formation of the root structure. In other words, these teeth are fragile, since lack of a vital pulp stops the process of dentinogenesis and leads to the maintenance of thin lateral dentin walls along the root. The current treatment for these teeth is induction of the apical closure followed by conventional endodontic treatment. The long-term prognosis of these teeth is questionable. They are subject to additional trauma, which frequently results in root fractures and an eventual need for tooth extraction. A tissue engineering-based approach that results in new pulp tissue could potentially allow for the completion of vertical and lateral root development and, perhaps, prevent the premature loss of these teeth (Figure 5).

Some of the intrinsic difficulties of engineering dental pulps are related to the anatomy and location of this tissue. The fact that, all the vascular and neural supply to pulp tissue is provided through a foramen located at

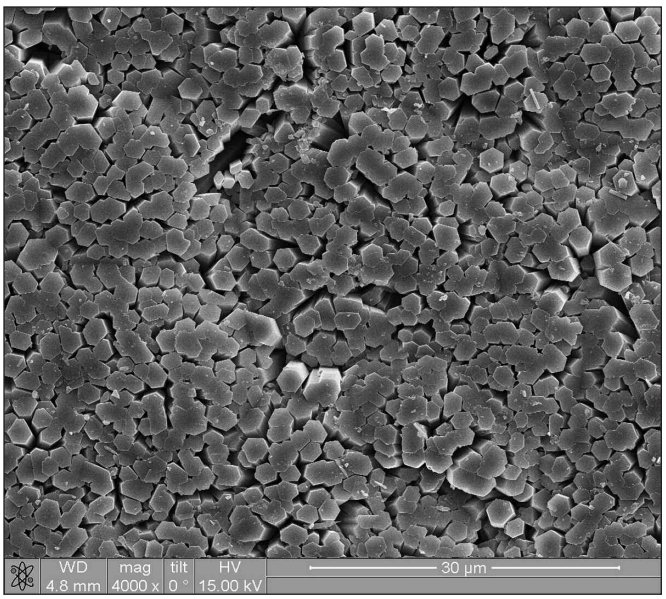

Figure 4. *Scanning electron micrograph showing an ordered, fluorapatite grown on an iron substrate under mild hydrothermal conditions. This image is courtesy of Dr Brian Clarkson and Dr Haifeng Chen.*

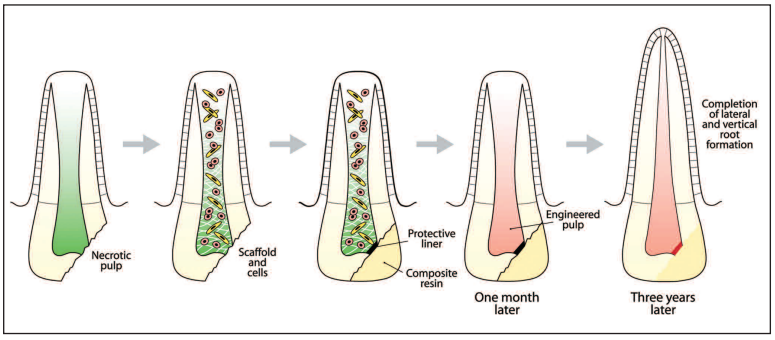

Figure 5. *Diagram depicting the concept of using a tissue engineering approach to create new pulp tissue and allow for completion of the vertical and lateral root formation in a young tooth that had pulp necrosis induced by trauma.*

one extremity, poses a challenge for tissue engineering. The engineering of dental pulp in young, single rooted, permanent teeth seems more feasible than engineering pulps for teeth with multiple roots or in older patients where the pulp chamber and root canals may be quite small or inaccessible. Another potential challenge is that dental clinicians still do not know which are the required signals for the differentiation of cells that compose the dental pulp, nor do dentists know how such signals should be spatially distributed and delivered in a timely fashion.

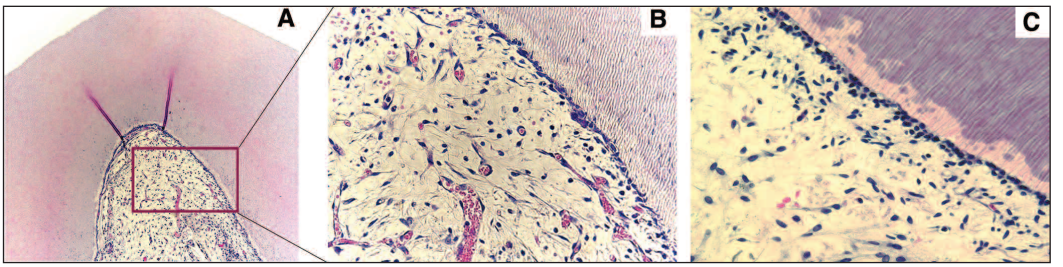

Figure 6. *Engineering of dental pulp tissue. (A) Low magnification (100x) and (B) high magnification (400x) of a representative pulp that was generated by tissue engineering. This pulp was engineered by seeding human dental pulp stem cells and human dermal microvascular endothelial cells in a biodegradable scaffold prepared inside the pulp chamber of a human third molar. The scaffold containing cells were implanted in the subcutaneous tissue of an immunodeficient mouse, and retrieved after 14 days for histological evaluation. (C) Histology of a dental pulp of a human third molar (control tooth).*

Engineering dental pulp will require application of the basic principles of tissue engineering.[3,19,63] Key elements for dental pulp engineering are: A) Molecular signals which induce the differentiation of cells that constitute dental pulps; B) Cells that will respond to the signals and C) Scaffolds that will either carry or attract these cells and provide an environment where they can proliferate, differentiate and develop a tissue with the characteristics and function of normal pulp.

The signals required to induce differentiation of dental pulp stem cells or progenitor cells into odontoblasts have been studied in considerable detail.[64-66] Clinicians also know that, in response to stimulation with recombinant BMPs, dental pulp cells differentiate into dentin-forming odontoblasts.[38-39,41-42] In addition, dental clinicians know that specific pro-angiogenic factors, such as Vascular Endothelial Growth Factor (VEGF), are potent inducers of endothelial cell survival and differentiation of new blood vessels, and they can be used therapeutically to induce tissue neovascularization.[67-68] However, clinicians still do not understand what is the ideal combination of signals required to engineer all the cellular components of a fully functional dental pulp. The minimum set of cells required to engineer a fully functional dental pulp is also not known. One speculates that dental pulp stem cells may have the potential to differentiate into most cells of dental pulp, but this has not yet been unequivocally demonstrated. In addition, it might be necessary to provide (or attract) endothelial cells to speed up the process of tissue vascularization, since it is evident that any engineered tissue (with the exception of cartilage) will not last without a functional blood vessel network.

The development of scaffolds, which are adequate for the engineering of the dental pulp, started several years ago. Initial studies involved the seeding of dental pulp fibroblasts onto synthetic matrices fabricated from fibers of polyglycolic acid (PGA). These cells were capable of adhering to the fibers, proliferating and developing a tissue with similar cellularity as compared to normal pulps.[69] When compared to a type I collagen hydrogel and an alginate, PGA proved to be a more conducive scaffold for dental pulp cell proliferation.[70] A recent study has compared the behavior of human dental pulp stem cells that were cultured in 3 different scaffolds: a spongeous collagen, a porous ceramic and a fibrous titanium mesh.[71] The authors concluded that the 3 scaffolds tested allowed for the attachment, growth and differentiation of human stem cells *in vitro*. When implanted *in vivo*, the cells organized into a tissue that expressed DSPP and was well vascularized, with limited mineralization of the extracellular matrix observed only with the ceramic scaffolds.[71] Biodegradable porous calcium phosphate scaffolds have also been proposed for dental pulp tissue engineering.[72] *In vitro* studies have indicated that calcium phosphate scaffolds were not toxic to the dental pulp cells. These scaffolds also allowed for the adherence and spreading of dental pulp cells that presented fine processes and matrix secretory granules.[72]

The authors have recently performed proof-of-principle experiments in which poly-L(lactic) acid (PLLA) scaffolds were manufactured in the root canal of tooth slices sectioned from teeth that had their pulps previously extirpated. Dental pulp stem cells (DPSC)[64] or stem cells from exfoliated deciduous teeth (SHED)[66] were seeded in the scaffolds (generous gift from Dr Songtao Shi). Together with the stem cells, the human dermal microvascular endothelial cells were seeded to engineer functional blood vessels, as described.[73] These tooth slices, containing stem cells and endothelial cells, were then implanted in the subcutaneous tissue of immunodeficient mice. After 14 to 28 days, the authors observed that the engineered dental pulp tissue (Figure 6A, 6B) presented morphological characteristics similar to that of a normal dental pulp (Figure 6C). These studies suggest the possibility of engineering a dental pulp with morphological characteristics similar to normal pulps. However, several challenges will have to be over-

come before functional dental pulps can effectively and safely be engineered in patients: A) a clinically feasible way of delivering the scaffold (with or without cells) needs to be found, along with the morphogenic signals to the root canal and pulp chamber; B) a strategy for inducing angiogenesis will need to be developed and characterized. The engineered dental pulp tissue will have to be vascularized to allow for initial survival of the cells and sustainability of the pulp tissue over time and C) the correct morphogenic signals, as well as the timing and sequence of their use, will have to be identified. Ideally, cells will differentiate into matrix-producing odontoblasts to allow for the continuing synthesis of dentin, which is observed along the walls of the pulp chamber and root canal throughout life.

Regenerative Dentistry: Making the Entire Tooth

In theory, the ideal replacement for a tooth lost due to caries or trauma is to generate a new tooth locally using autologous cells. This therapeutic strategy was unthinkable until few years ago. However, recent developments in the fields of tissue engineering, developmental and stem cell biology have made tooth regeneration a more achievable goal. Clearly, there are many obstacles to overcome before one can engineer a complete tooth to treat a patient. However, the following body of work represents, collectively, the evidence that the way one thinks about restorative dentistry in the future might be quite different from what one is accustomed to today.

In 2002, a seminal paper published by the Yelick research group from the Forsyth Institute demonstrated that, for the first time, enamel, dentin, pulp and a structure that resembled a developing root can be engineered using cells from the dental pulps of unerupted molars and a scaffold.[74] Development of the original approach for tooth engineering was demonstrated in a second publication from the same group, in which the cells were collected from the donors, expanded in culture for a number of days, then seeded onto scaffolds.[75] This important discovery raised the possibility that cells required for the engineering of teeth may be obtained from small biopsies from the same patient. Interestingly, the sequence of events leading to the development of engineered teeth seems to mimic the process of natural odontogenesis.[76]

Because stem cells are capable of differentiating into several different cell types, they are attractive for the tissue engineering of complex organs or structures. The concept of using stem cells for dental tissue engineering was explored by Paul Sharpe and his research group.[77] They demonstrated that it is possible to engineer teeth of normal size and structure using stem cells.[78] The same group has also demonstrated that adult stem cells of non-dental origin can be used to engineer teeth.[79]

The discovery of dental stem cells in the pulp tissue of permanent teeth[64] and also in primary teeth[66] raises the exciting possibility of retrieving these cells, expanding them in culture and seeding them in biodegradable scaffolds for tooth engineering. These cells have 2 characteristics that make them attractive for dental tissue engineering: A) Dental pulps can be easily obtained from the patient who needs tooth replacement. Stem cells can be retrieved and isolated from pulp with relatively minimal morbidity. This is especially true for the pulp of exfoliated primary teeth, which were shown to be a feasible source of stem cells (SHED)[66] that can be frozen and used later in life. And, B) dental pulp stem cells are pluripotent cells, that is, they have the capability of differentiating into most, if not all, cells that give rise to tooth structures.

There are certainly many challenges that still need to be addressed in the area of tooth engineering before this strategy can be used in the clinic: A) Scaffold design will require significant work before it fulfills its role of providing a conducive environment for tooth development; B) It will be fundamental for the clinician to have control over the size and shape of the tooth to be formed and for the results of the procedure to be predictable. An important step in this direction was reported recently in a publication which demonstrates that, by adjusting the number of mesenchymal cells associated with the epithelial cells in the implants, one can control the shape of the crown and generate teeth with pre-defined morphologies.[80] And, C) the procedure for tooth engineering should be relatively simple, controlled and feasible in the clinical setting.

CONCLUSIONS

Dr Buonocore stated that "one can expect little progress from narrow, stereotyped thinking that fails to intelligently appraise and utilize the potential of improved treatment methods. Fortunately, the dental profession has never been handicapped by such thinking."[81] Dr Buonocore challenged existing paradigms and was faced with some criticism, as can be noted when he stated that "the advent of the successful use of adhesives was of course attended by queries and controversies."[81] However, Dr Buonocore was clearly a man ahead of his time, and his contributions to dentistry are still felt today by dental practitioners and patients around the world.

The field of tooth tissue engineering is certainly one in which there are more questions than answers. Regenerating tooth structures is a complex proposition. Essentially, dentists are trying to reproduce odontogenic processes that were perfected by nature. From a conceptual standpoint, there is little doubt that the best material to replace tooth structure is tooth structure. The question the field faces is: Can we do it in a way that is predictable, clinically feasible and practical? High quality research and effective collaborations between basic scientists and clinicians is the way to

move this field toward its ultimate goal of regenerating either individual tooth structures or the entire tooth as a means to treat the consequences of tooth-related diseases.

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