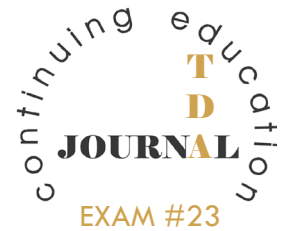


Regenerative Dentistry: Translating Advancements in Basic Science Research to the Dental Practice

Franklin Garcia-Godoy, DDS, MS; Peter Murray, PhD



Introduction

As we enter the 21st century, dentistry has become an exciting and productive area of scientific research. Recent discoveries in genetics, molecular biology and cellular biology will be brought together to develop new dental treatment regimes. Genetic information will increasingly be utilized to screen patients for disease predisposition, and molecular therapies will be used to prevent the occurrence of dental defects before they result in a loss of tissue function or esthetics. The aim being to eradicate caries, periodontal disease, congenital defects and orofacial cancers by replacing the predisposing (faulty) genes before these disorders can develop. It will never be possible to solve all dental disorders at the genetic level, so more effective treatment regimes must be developed to regenerate teeth, bone and soft tissues that are lost or damaged by disease, cancer, chemotherapy, high levels of irradiation or accidental trauma. The close integration of skin, salivary glands, tongue, teeth and periodontal tissues present a complex challenge for the development of new therapies. Any interventions to regenerate a specific tissue must preserve or restore orofacial tissue function and esthetics. Interventions must not interfere with the muscles of speech, respiration, mastication and emotional expression because of the importance of the face and mouth as the projected image of the individual to the world. These requirements make the introduction of new therapies into the dental field a challenging and daunting prospect because they must be proven to be non-hazardous to health, have good cost-benefits ratios and be more effective than existing therapies.

Growth factor therapy

During caries, plaque bacterial acids diffuse through the dental tissues and dissolve the enamel and dentin matrix. This demineralization of the hard tissues may solubilize some non-collagenous

ABSTRACT

Scientific advances in the creation of restorative biomaterials, *in vitro* cell culture technology, tissue engineering, molecular biology and the human genome project provide the basis for the introduction of new technologies into dentistry. This review provides an assessment of how tissue engineering, stem cell, genetic transfer, biomaterial and growth factor therapies can be integrated into clinical dental therapies to restore and regenerate oral tissues. In parallel to the creation of a new field in general medicine called “regenerative medicine,” we call this field “regenerative dentistry.” While the problems of introducing regenerative therapies are substantial, the potential benefits to patients and the profession are equally ground-breaking. In this review, we outline a few areas of interest for the future of oral and dental medicine in which advancements in basic science have already been adapted to fit the goals of 21st century dentistry.

CLINICAL SIGNIFICANCE

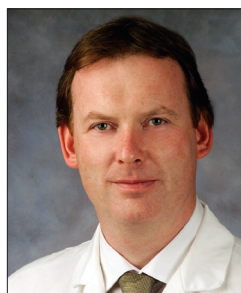
The purpose of this review is to examine how regenerative therapies may complement and possibly replace several established traditional dental treatments.

KEY-WORDS:

cavity preparation, cavity restoration, dental materials, growth factors, inflammation, bacterial leakage



Franklin Garcia-Godoy, DDS, MS is Professor and Senior Executive Associate Dean for Research, Director, Bioscience Research Center, Chair, Department of Bioscience Research, College of Dentistry, University of Tennessee Health Science Center, Memphis, TN.



Peter E. Murray, PhD, Professor, Department of Endodontics, Nova Southeastern University, Ft. Lauderdale, FL.

Neither of the authors reported any conflict of interest disclosures.

Table 1 - Summary of caries lesion progression, tooth remineralization activity, tertiary dentin, pulp response and general therapies.

Type of caries lesion	Deminerlization activity	Remineralization activity	Tertiary dentin activity and pulp response	Therapies and potential therapies
Caries prevention	Decay is avoided	Normal physiological rate	None	Oral hygiene education
				Fluoride
				Vaccine
Increased; Bacteria, Sugar, Susceptibility				
Slowly progressing caries lesion	Decay of enamel and dentin over 5 years	Mostly peritubular (sclerotic) dentin mineralization	Pulp cell survival and reactionary dentin deposition	Pit and fissure sealant
				Caries monitoring
				Ozone
Increased; Bacteria, Sugar, Susceptibility				
Quickly progressing caries lesion	Decay of enamel and dentin over months	Limited peritubular (sclerotic) dentin mineralization	Pulp cell damage and reparative dentin deposition	Caries removal
				Restoration of tooth
				Resin-modified glass ionomer
Decreased; Bacteria, Sugar, Susceptibility				
Arrested caries lesion	Decay is halted	Some remineralization of caries-affected (hard, stained) dentin, but not soft caries lesions	Regeneration of pulp cells and dentin if caries has not infected the pulp tissue	Growth factors
				Stem cell therapy
				Replacement of tooth substance

proteins and growth factors. Growth factors are protein molecules with potent bioactive effects on a variety of cellular activities including cell survival, cell division, differentiation, migration, etc. They are secreted by a variety of cells including odontoblasts during tooth development, and some are sequestered within the dentin matrix.¹ In an effort to categorize the genes and growth factors expressed in the developing teeth in many species, including humans, researchers in Finland established an online database that is accessible to everyone (<http://bite-it.helsinki.fi>). This website offers a plethora of information on a variety of growth factors. During development, these molecules are responsible for formation of teeth² and for signaling the induction of odontoblast differentiation in the dental papilla.³ Growth factors have also been implicated in the periodontal regeneration of class II furcation defects.⁴ It has been proposed that carious demineralization, cavity preparation, etching, dental materials and loss of tooth dentin can lead to the solubilization of

sequestered growth factors and bioactive molecules from the dentin matrix.^{5,6} Much of the therapeutic effect of calcium hydroxide and mineral trioxide aggregate may be due to their extraction of growth factors from the dentin matrix.⁷ The release of growth factors have a key role in signaling dentin injury and in stimulating dental pulp healing by cell differentiation, proliferation, chemotaxis, extracellular remodeling and the secretion of tertiary dentin matrix.⁸ Growth factors, mainly transforming growth factor beta-1 (TGFβ1), are important for the stimulation of dentin matrix secretion. These growth factors are secreted by odontoblasts and are sequestered by non-collagenous proteins in an active form within dentin matrix.⁹ Damage to tooth structure may release these growth factors into the pulp tissue and play a key role in signaling many of the events of tertiary dentinogenesis.¹⁰ This natural damage signaling activity of teeth may be amplified by the addition of purified dentin matrix proteins,¹¹ bone morphogenic proteins¹² and TGFβ1

following cavity preparation.¹³ These investigations have demonstrated the potential for adding growth factors as part of restorative dentistry to stimulate the reparative activity of teeth. However, it should be noted that growth factors are easily denatured/inactivated and diffuse easily through tissues into the systemic circulation.¹⁴ The dose-response, safety and effectiveness of growth factors in restorative dentistry have not yet been investigated in clinical trials, although growth factors are already used in periodontal therapy¹⁵ and anti-inflammatory mediators are used to treat a variety of medical conditions.¹⁶ A summary of caries lesion progression, tooth remineralization activity, tertiary dentin, pulp response and general therapies is shown in **Table 1**.

Vaccine therapy

Caries lesions or the replacement of restorations that were initially placed to restore teeth following caries removal, account for two-thirds of all restorative treatments.^{17,18} The eradication of caries

may be successful if gene transfer therapy can mediate humoral and cellular immune responses to the pathogenic bacteria involved in these disease processes.¹⁹ This type of gene transfer therapy is called DNA vaccination because DNA containing antigens which can mediate an immune response are delivered in a plasmid to the target bacteria.²⁰ Caries vaccine strategies may use the mucosal immune system in newborn infants, which is functional prior to the appearance of their first teeth, as an effective way to induce immunity against the colonization of teeth by *mutans streptococci* and protection against subsequent dental caries.²¹ A promising alternative approach to eradicate caries may be to replace *mutans streptococci* with genetically altered strains, which have little or no caries-causing potential.²² Few clinical trials have been performed to examine the protective effect of dental caries vaccines containing defined antigens, but the studies that have been performed support the hypothesis that the immunization of pediatric patients with caries vaccines could have a protective effect against caries if *mutans streptococci* are not yet established as part of the permanent dental biofilm.²³ The delivery of DNA requires the use of modified influenza and virus vectors such as Herpes simplex virus.²⁴ These viral gene delivery vectors are highly infectious and generally provide efficient vehicles for the delivery of exogenous genetic material into host cells. However, many unanswered questions remain about the reliability, effectiveness and safety of modified viral vectors or caries vaccines.²⁵ Practical considerations including the optimal timing, route, antigen and delivery vehicles are still to be investigated to help caries vaccines realize their therapeutic potential.

Another area that shows great promise for therapeutic vaccination is in oral cancers. Oral cancer is the sixth most common type of cancer in the world. In the US alone, about 35,000 individuals will be diagnosed with oral cancer this year, and approximately 8,000 of those individuals will die as a result.^{26,27} The most common oral cancer is the squamous cell carcinoma that originates in the epithelium that lines the oral cavity and lips. Patients diagnosed with oral cancer

have a particularly low 5-year survival rate due to the compounding factors of late detection and the lack of a truly effective therapy.²⁸ There seems to be a strong link between human papilloma virus (HPV) infection and some oral cancers.²⁹⁻³¹ Interestingly, oral cancers that are HPV-positive have a better prognosis than HPV-negative oral cancers.²⁶ HPV strains that are responsible for almost 70% of cervical cancers have been proposed to cause oral cancer.²⁹⁻³¹ Pharmaceutical company Merck has developed an FDA-approved recombinant vaccine (Gardasil®) against cervical cancer by helping to prevent HPV strains 6, 11, 16, and 18 infections. HPV-positive oral cancers can be considered a sexually transmitted disease, and thus vaccination in sexually active young men and women would not only prevent cervical cancers but possibly the HPV-positive oral cancers. The preventive vaccination in both male and female population will serve as a tool in better combating cervical cancers and the HPV-positive fraction of the oral cancers.

Gene therapy

The scope of human genetic information is immense. The human genome contains approximately 3 billion nucleotides, making up about 100,000 alleles, which in turn are contained on 46 chromosomes. Transcription of these chromosomes releases the information necessary to synthesize some 6,000 proteins. These proteins make up the trillion cells giving rise to the nearly 4,000 anatomical structures that constitute human-kind. Mutation, the accidental alteration of the genome, may result in heritable conditions or syndromes affecting any aspect of growth and development. Congenital defects occur in one in every 700 live births in the United States. Three-quarters of these include craniofacial defects.³² Five thousand genetic syndromes are known; over 700 of these have craniofacial defects and over 250 have associated clefting. Many different classes of genes or proteins when mutated cause craniofacial defects, and these include transcription factors, growth factors and their receptors, hormone receptors, cell adhesion molecules, G proteins, enzymes, transporters and collagens.

The chromosomal map location is known for over 50 inherited orofacial disorders, and the genes are known for more than 60 orofacial abnormalities. One of the most common orofacial disorders is a cleft of the lip and/or palate,³³ resulting from the failure of tissue to fuse or merge early in prenatal development.³⁴ Many other syndromes associated with orofacial defects such as Apert³⁵ and Crouzon³⁶ have a significant impact on the quality of life. The estimated lifetime cost per orofacial cleft is \$100,000 per child amounting to \$750 million for all affected children born every year.³⁷ The cost of providing treatment for these inherited disorders is compounded by non-inherited orofacial disorders that are created by material exposure to teratogens,³⁸ environmental factors, or that lead to fetal alcohol syndrome.³⁹ Finally, some 10 million Americans each year are afflicted with some type of acquired orofacial malformation resulting from acute traumatic injuries in accidents.⁴⁰

The hypothetical advantage of somatic gene therapy is that it may be able to correct the cellular cause of the disorder rather than the side effects. This may be accomplished in the following ways: the regeneration of missing or the repair of defective orofacial tissues, such as craniofacial bone repair;⁴¹ the creation of systemic gene products, such as interferon, insulin and growth hormones;⁴² the delivery of genetic therapeutics to treat orofacial infections⁴³ and cancer lesions.⁴⁴ Vaccines may also be genetically engineered against diseases with significant oral complications such as herpes simplex virus, human papilloma virus and human immunodeficiency virus. Gene therapy may also have a role in providing effective analgesia for severe chronic pain.⁴⁵ The theory is that treating genetic, environmental and accidental malformations at the genomic level will save large amounts of money spent on remedial treatment and save much patient pain and suffering. The reality of the situation is that gene replacement therapies suffer from technical problems, are difficult to control and are impossible to reverse. Currently gene therapy is limited to terminally-ill patients with no alternative treatment options; clearly decades of evaluation and safety research

Table 2 - Comparison of traditional restorative therapies and regenerative therapies to treat dental problems.

Dental problem	Traditional Restorative therapy	Regenerative therapy
Erosion, wear, abfraction, loss of enamel and dentin	Restore tooth structure with filling materials	Stimulate enamel and dentin formation with growth factor therapy
Pulpitis, hypersensitivity or loss of vitality	Pulpectomy or endodontic therapy	Stem cell regeneration of pulp tissue
Early caries	Seal pit and fissures with adhesive sealant	Growth factor therapy to remineralize lesions
Active caries in partially decayed teeth	Excavate caries and apply restorative materials	Transplant new vital tooth tissues
Decayed, fractured, severely injured or avulsed tooth	Place implant or prosthetic teeth	Harvest bioengineered teeth for implantation

lie ahead to realize the full potential of gene therapy.

Tissue engineering of biomimetic materials

The regeneration of decayed or lost tooth tissue is problematic. No restorative material exists that can be placed into a tooth that provides better protection for pulp tissue than dentin.⁴⁶ Any injury to the dentin-pulp complex can trigger inflammatory cell activity.⁴⁷ These inflammatory reactions can injure the pulpal cell populations and lead to complications. Severe inflammatory activity, if unchecked, can often progress to total pulp necrosis and lesion development, followed by local bone destruction.⁴⁸ Observations of the low success of some types of restorative materials and how they contribute to postoperative complications such as hypersensitivity, pulp inflammation⁴⁹ and treatment failure⁵⁰ has stimulated some interest in the transplantation of natural tooth substance, which has been grown using *in vitro* culture. Few restorative materials share the same physical or chemical characteristics of the natural tooth and this may explain why a high proportion of cavity restorations fail mechanically. Resin modified glass ionomer is a common restorative material; however, surveys show that more than 50% of the time these restorations require replacement due to mechanical failure.^{51,52}

In addition, few restorative materials share the appearance of tooth esthetics, although resin composite materials can be color matched.⁵³ This contrasts with the use of tooth tissue, which shares the same chemical, physical and esthetics of natural teeth. These properties are ideal for restoring damaged tooth surfaces because abrasion, erosion, attrition and tooth wear remain prevalent.⁵⁴ Porcelain veneers are often placed for esthetic purposes, but the low success and longevity of this treatment⁵⁵ would ideally make the immediate replacement of lost tooth surfaces with synthetically mineralized tooth tissues a welcomed advance in restorative dentistry. However, adhesive bonding may still be required to attach biomimetic tissues to teeth. This suggests that complications associated with marginal fracture and bacterial microleakage may still persist in the future. A comparison of traditional restorative therapies and regenerative therapies to treat dental problems is shown in **Table 2**.

Stem cell regeneration of orofacial tissues

While the first bone marrow transplantations were performed in 1955, the first successful case was performed in 1969, fourteen years after the initial six human transplant recipients had died. Bone marrow transplantation, since that time, has been one of the

main approaches for curing blood-borne diseases. Progenitor and adult stem cells have been isolated from many tissues, including all dental /oral related tissues. Embryonic stem cell research and the new induced pluripotent stem cell field all together provide for a promising future for regenerative medicine.⁵⁶

Stem cell therapy is one of the most promising areas of tissue engineering because the transplantation of scaffold-materials, containing pulp stem cells grown in the laboratory provides an excellent inductive means to regenerate new orofacial tissues.⁵⁷ The transplantation of stem cells to accomplish orofacial tissue regeneration removes the problems of delivering growth factors and genes into host target cells and waiting for the target cells to differentiate, proliferate and migrate to sites of injury before the tissue reparative activity can commence. Orofacial stem cell therapy is in the early evaluation stages for the regeneration of pulp,⁵⁸ gingiva,⁵⁹ cementum,⁶⁰ mucosal tissues,⁶¹ periodontium⁶² and bone.⁶³⁻⁶⁵ However, the sourcing of stem cells from fetal tissues is subject to legal and ethical restrictions. This has focused some attention on the xenotransplantation of animal tissues into humans. Recently some researchers have called for a moratorium on research using xenographs⁶⁶ because of the health hazards this therapy presents to humans. These problems have caused

Table 3 - Summary of advantages and limitations of regenerative dental therapies.

Type of Therapy	Advantages	Limitations
Growth factors	Stimulate host cells to proliferate and differentiate to create new tissues.	Requires existing cells to be capable of proliferation and differentiation
	Stimulates the mineralization and functional activity of cells	Anchoring and delivery of molecules in an active state is problematic
	Growth factor stimulus can be dose-dependent to provide a high degree of therapy controllability	Stimulates inflammatory cell activity and has a high potential for creating side effects in other tissues
Stem cells	Creates entirely new tissues and doesn't rely on existing cells to proliferate	Sourcing stem cells from fetus is problematic
	Cells can be precisely positioned and have a potential for fast tissue and organ renewal	Stem cells have proliferation and differentiate sequences that may be difficult to control and predict
Gene therapy	Alter oral bacterial DNA to arrest and prevent subsequent caries lesion development	Gene vectors commonly use modified viruses and these have side effects
	Cures cells and tissue defects instead of side effects to correct congenital defects, and a possible cure for cancer	Gene therapy complications may be impossible to reverse
Tissue engineering	Create new tissues <i>in vitro</i> culture and transplant them to restore orofacial function	Creation of new tissues is complex and expensive
	Grow all types of tissues for transplantation: teeth, salivary glands, epithelium, periodontal tissues, gingiva	Obtaining stem cells is a problem. The transplants may stimulate inflammatory cell reactions

some investigators to search for natural adult stem cells,⁶⁷ but this has been the subject of controversy. It appears that the implantation of stem cells is generally not sufficient in isolation to accomplish orofacial regeneration. These cells must be subject to tissue engineering using *in vitro* gene therapy, growth factors and tissue scaffolds to effectively form multi-layer cell structures.⁶⁷ These cell-polymer constructs must be transplanted into suitable host animals to provide a vascular blood supply to support the growth and development of higher-ordered tissue structures.⁶⁸ This latter step involving the implantation of tissues into host animals is necessary to accomplish some functionality and to produce more mature synthetic tissue structures. However, this latter step is also problematic as it creates a high potential for stimulating tissue-antigenic inflammatory and rejection responses when implanted into human recipients. These problems must be overcome, and no simple solutions appear to be available to allow these treatments

to be introduced clinically in the near future.

In addition to the traditional oral related tissue engineering approaches proposed for dental pulp stem/progenitor cells, dental pulp cells have shown promise in basic science research for use in debilitating disorders of the nervous system. Dental pulp cells produce an array of growth factors, *in vivo* and *in vitro*,⁶⁹⁻⁷² that are crucial for the maintenance of the developing and adult nervous system. These same growth factors have been shown to protect and regenerate affected nerve cells and alleviate symptoms in diseases or injuries in the nervous system.⁷³ Dental pulp proper cells are a neural crest-derived easily accessible source of cells for transplantation therapies in the nervous system. They produce neurotrophic growth factors and contain a subpopulation of stem cells that might replace lost or dying cells. We have also shown that dental pulp cells support a wide variety of neuronal cells (sensory, motor and dopaminergic neurons) and can

be grafted into the brain and the spinal cord in animal models (Table 3).^{73, 74}

Summary of the advantages and limitations of new regenerative therapies

The advantages of growth factors are that they can stimulate host cells to proliferate and differentiate to create new tissue. These factors can also stimulate the mineralization and functional activity of cells and have the advantage of being dose-dependent to give a high degree of therapy controllability (Table 3). The limitations of growth factors are that they require the target existing cells to be capable of proliferation and differentiation. Growth factors diffuse easily in the systemic circulation and through tissues, therefore, anchoring them to a fixed locus in an active state may be problematic. This creates the potential for creating inflammatory side effects in other tissues (Table 3). The advantages of stem cells are that they do not need existing cells to proliferate

and regenerate orofacial tissues. This can speed up orofacial regenerative activity. Stem cells can be implanted or injected, and this allows precise site-specific positioning (Table 3). A limitation of pluripotent stem cells is that they must generally be sourced from fetal tissues. Furthermore, the pattern of proliferation and differentiation *in vivo* may be difficult to predict because their activity is prone to change with differentiation/maturation (Table 3). Gene therapy may be useful for altering caries-causing bacterial DNA to prevent or arrest caries lesion development. Gene therapy provides a cure for damaged cells, rather than the side effects of defective tissue activity (Table 3). The limitations of gene therapy are that gene vectors use modified virus vectors, and these may have side effects. Because gene therapy alters the host genome DNA, the therapy may be irreversible if complications occur (Table 3). The advantage of tissue engineering is that it brings together gene, growth factor and stem cell therapies to create new tissues *in vitro* for transplantation to restore orofacial tissue function. It may be possible to create almost any type of tissue including teeth, salivary glands, mucosa, epithelium, periodontal and gingival tissues. The limitation of this therapy is that it is likely to be complex and expensive. The sourcing of appropriate stem cells and the avoidance of creating host immune rejection-inflammatory cell reactions without any complications may be problematic (Table 3).

Conclusion

The advent of regenerative dentistry will herald a new era of unparalleled advances in treatments that are set to take place with the availability of artificial teeth and oral tissues, as well as the ability to stimulate dental repair, regenerate lost tissues, produce vaccinations against viruses and genetically alter disease pathogens to help eradicate caries and periodontitis. Advances in tissue-engineering science are allowing new craniofacial tissues to be created by using a combination of growth factors and gene therapy to modify stem cell proliferation and differentiation on biodegradable polymer scaffolds. In the future, many dental problems may be solved by the

regeneration or replacement of oral tissues affected by disease, trauma and inherited disorders.

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Questions for Continuing Education Article - CE Exam #23

1. Regeneration of oral tissues is, in part, due to advances in:
 - a. tissue engineering
 - b. molecular biology
 - c. the human genome project
 - d. all the above
2. In order to become successful, the regeneration regime must:
 - a. be non-hazardous to health
 - b. have good cost-benefits
 - c. be more effective than existing therapies
 - d. all the above
3. What has been implicated in the periodontal regeneration of class II furcation defects?
 - a. growth factors
 - b. glass ionomers
 - c. Factor VIII
 - d. hemoglobin
4. The human genome contains:
 - a. approximately 3 billion nucleotides
 - b. approximately 100,000 alleles
 - c. 49 chromosomes
 - d. a and b
5. HPV-positive oral cancer can be considered:
 - a. a sexually transmitted disease
 - b. 100% curable
 - c. sequelae of smoking
 - d. all the above

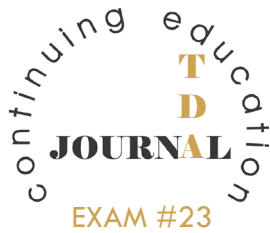
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2.	a	b	c	d
3.	a	b	c	d
4.	a	b	c	d
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