Rev Fac Odontol Bauru
2002; 10(4):201-8

The role of biological modulators in endodontic therapy

FUNÇÃO DOS MODULADORES NA TERAPIA ENDODÔNTICA

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Endodontic treatment has significantly evolved in the past 20 years. New equipment and techniques to treat root canal systems are continually being introduced into the market, and researchers are striving to create innovative products. Current studies using stem cell and tissue engineering technologies are making significant progress, and tissue regeneration may shortly become feasible for clinical application. The aim of this paper is to review current treatment modalities that involve the combination of biological modulators (e.g. growth factors), barriers (resorbable or non-resorbable) and restorative materials during different endodontic treatment procedures. These materials can be clinically applied as pulp capping agents during vital pulp therapy; bone graft substitutes (bone fillers) to induce periradicular tissue healing following periradicular surgery; components of artificial apical barriers used during apexification procedures, perforation and resorptive defect repairs. In general, barriers are applied outside the root canal system for obturation and restorative materials to be placed against. The novel aspect of adding biological modulators to barriers is part of a scientific effort to induce tissue regeneration. However, the existence of extraneous factors, such as the interface between restorative materials and the combination of barriers with biological mediators and their interaction with inflamed or contaminated tissue may warrant further analysis. Evidence-based research is required before experimental results can be extrapolated for clinical application.

UNITERMS: Biologic modulators; Tissue regeneration; Endodontic therapy.

INTRODUCTION

A comprehensive understanding of tooth development, etiology, and pathogenesis of pulp and periradicular tissues supports the underlying principles for endodontic treatment. Innovative therapies apply biological modulators that have been identified during tooth and bone embryogenesis and later became cloned for experimental and clinical application. These modulators are intended to improve treatment modalities, and ultimately induce tissue regeneration. Growth factors, cytokines, some of these biological modulators in endodontics as:(i) pulp capping agents during vital pulp therapy[6,7,15,19,24,30], (ii) bone graft substitutes (bone fillers) to induce periradicular tissue healing following periradicular surgery,[8,13,17] (iii) components of artificial apical barriers used during apexification procedures[21], differentiation of cell follicles[18] and tissue engineering[35]. Numerous other studies have assessed the application of a biological approach in cementum[22], tooth ankylosis following trauma[33], bone[17], and periodontal ligaments[10]. This article will review these novel treatment methods which may improve traditional endodontic therapy.

Barriers and Dental Restorative Materials

Modern materials and techniques allow practitioner to select products according to different

clinical procedures. In endodontics, there is a demand for materials that can be applied to supportive structures (i.e. bone and periodontal ligament) during root canal therapy, for correction of: perforation and resorptive and surgical defect repairs, and the treatment of teeth with open and flaring apices. A current method to treat these problems consists of repeatedly filling the canal space with calcium hydroxide (CH), sometimes long-term, until a physiological barrier can be developed and detected clinically and radiographically. These methods have limitations such as patient non-compliance[4], a high prevalence of crown-root fracture[1], interappointment contamination, and bacterial resistance to CH[32].

An alternative treatment currently used by endodontists to manage these above problems, has been the application of barriers into supportive structures, and the placement of restorative materials inside the root canal space. One of the main advantages of applying barriers instead of long-term calcium hydroxide is to decrease the number of patient visits. There are different types of barriers currently used in endodontics (Table 1). They are of two main types: resorbable or non-resorbable. Table 1 does not make a definitive distinction between resorbable and non-resorbable barriers, since the majority of non-resorbable materials, mostly free of organic materials, could release biological modulators (i.e. osteogenic proteins) during wound healing, and therefore should be termed partially resorbable materials.

Innovative techniques suggest the addition of biological modulators to barrier materials. The objective is to identify materials, or their combinations, which can completely perform a desired procedure or biologic tasks. An ideal material should act as an adequate barrier for restorative materials to be condensed against during the procedural phase and later during the biological wound healing, act as an active component during tissue regeneration. Barriers can also be used as mechanical hemostatic agents during periradicular surgery.

TABLE 1 - Barriers currently available to be used in bone defects associated with an endodontic treatment complication

Material	Manufacturer	Type
Colla-Cote®	Sultzer Dental Corp., Carlsbad, CA, U.S.A.	Synthetic Collagen
Capset®	Lifecore Biomedical, Chaska, MN, U.S.A.	Calcium Sulfate
Endogain gel®	Biora, Inc., Chicago, IL, U.S.A.	Protein matrix (EMD) [a]
Periglass®	US Biomaterials Corp., Alachua, FL, U.S.A.	Bioceramic
Gen-Ox®	Baumer S.A., Mogi Mirim, Brazil	Lyophilized Inorganic Bovine Bone

[a] EMD: Extracellular Matrix Derivative, from porcine.

TABLE 2 - Restorative/reparative biomaterials currently available, to treat endodontic treatment complications

Material	Manufacturer	Type
ProRoot MTA®	Dentsply/ Tulsa Dental, Tulsa, OK., U.S.A.	Mineral trioxide aggregate
Super-Eba®	Bosworth, Skakie, IL, U.S.A.	Reinforced oxide-zinc eugenol
Diaket®	3M ESPE, St. Paul, MN, U.S.A.	Polyvinil resin
Geristore®	DenMat, St. Maria, CA, U.S.A.	Glass-ionomer
Retroplast®	Retroplast Trading, Rønne, Denmark	Resin

Synthetic collagen membranes (Colla-Cote®, Sultzer Dental Corp., U.S.A.) and medical grade calcium sulfate (Capset®, Lifecore Biomedical, Chaska, MN, U.S.A.) are commercially available and are used clinically because they are easy of handle, are relatively inexpensive, and are biocompatible. Both materials can be used as barriers during endodontic procedures. These materials gradually resorb and provide a scaffold for bone growth. They also act as delivery system for biological modulators (e.g. growth factors) to enhance tissue regeneration. Capset® is presently used as a barrier during surgical endodontic procedures (Figures 1 and 2).

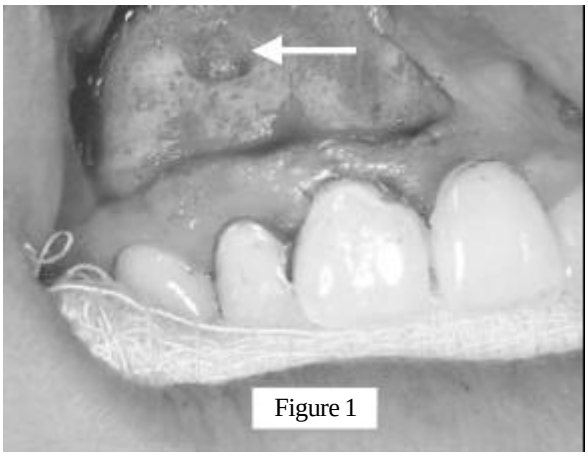

Figure 1

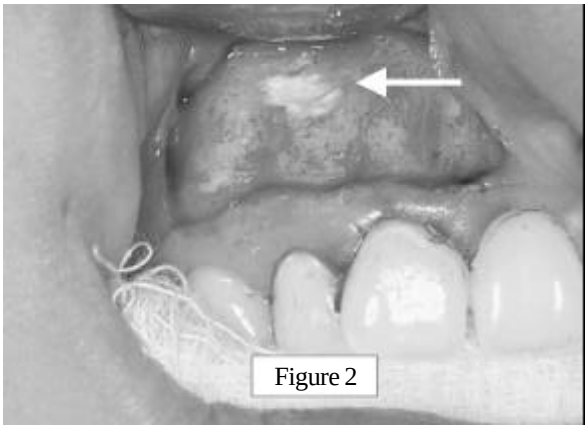

Figure 2

Arrow in Figure1 indicates the original bone defect and arrow in Figure 2 shows Capset® used as a resorbable barrier during a surgical endodontic procedure

Lyophilized freeze-dried bone allograft combined with guided tissue regeneration has been used to repair large periodontal-endodontic defects[2]. Allograft materials can serve as scaffolds for new bone to be deposited against and therefore are classified here as non-resorbable barriers. Other materials such as bioceramics, can also function as scaffolds for physiologic bone deposition[11].

Non-resorbable barriers when placed in bone defects remain partially intact. An example of this type of barrier is mineral trioxide aggregate, commercially available as ProRoot MTA® (Dentsply/ Tulsa Dental, Tulsa, OK,U.S.A.). ProRoot MTA® has been shown to be biocompatible and may be used as both a non-resorbable barrier and a restorative material[28]. Nevertheless, ProRoot MTA® is better referred as reparative material when used on pulp exposures or root perforations.

Reparative materials must meet the characteristics of an ideal dental biomaterial. They must be easy of handle, have low cost, be biocompatible, provide a fluid-tight seal, and be esthetic. Table 2 includes commonly used restorative materials in endodontics.

Care is advised when selecting combination of a barrier and restorative material. For instance, the use of synthetic collagen barriers is contraindicated when certain composite materials are to be used as restoratives, because collagen absorbs moisture and therefore affects the setting of the composite materials. Conversely, Retroplast® (Retroplast Trading, Rønne, Denmark) has been designed to be specifically used as a retro-filling material in the presence of moisture. Moreover, the interface between restorative materials and barriers, and their interaction with inflamed or contaminated tissues warrant further studies before clinical application.

Biological Modulators

Growth factors are biological modulators that are able to promote cell proliferation and differentiation. Naturally occurring osteogenic proteins, such as bone morphogenetic or morphogenic proteins (BMPs), are members of the transforming growth-factor superfamily of bone-matrix polypeptides. These bioactive molecules appear to modulate cartilage and bone deposition and/ or resorption. The osteogenic properties of BMPs were initially demonstrated using demineralized bone matrix[31] or reconstituted extracts of purified solubilized proteins[20] This was followed by molecular cloning and expression of several recombinant human BMPs (rhBMP-2 to rhBMP-6, osteogenic protein-1 [OP-1] and OP-2)[34]. Recombinant human BMP-2, BMP-4, and OP-1 (BMP-7) initiate endochondral bone formation when implanted subcutaneously or intramuscularly simply and when combined with insoluble collagenous bone matrix or which is the inactive residue following dissoative extraction of bone matrix with 4 M guanadine-HCl.

Other osteogenically active growth factors that have been identified are PDGF (platelet-derived growth factor)[13], IGF (insulin-like growth factor[9], and FGF (fibroblast growth factor)[27]. The incorporation of growth factors in a suitable resorbable barrier may ensure accurate delivery of the growth factors, and prolong their presence in the surgical site[14].

Another recently discovered biological modulator is enamel matrix derivative (EMD) (Emdogain®, Biora Inc., Chaska, MN, U.S.A.), obtained from embryonic enamel of amelogenins (90%). EMD was demonstrated *in vitro,* using a wound-healing model, that it is capable of stimulating periodontal ligament cell proliferation at earlier times (i.e., days 1 to 3) compared to gingival fibroblasts and bone cells[5].

Clinical Applications of Biological Modulators Pulp Regenerative Therapies

One of the novel targets of research involving regenerative therapies in endodontics is the preservation of pulp vitality. The widespread opinion among academicians and practitioners is that preserving an intact vigorous pulp is preferable to root canal therapy.

Rutherford *et al.*[19] pioneered the use of human cloned bioactive osteogenic protein-1 (hOP-1) with a carrier matrix of purified bovine type-1 collagen powder (CM), moistened with sterile saline, for inducing reparative dentine formation in monkeys. They reported that substantially more new dentine was present in teeth treated with a contrast of recombinant hOP-1/CM. No such reparative dentine was formed by a collagen barrier or untreated teeth. The appearance of the new tissue suggested that much of the mass of the hOP-1/CM contrast was replaced initially by a pulp-like connective tissue, that subsequently mineralized to form dentine.

Nakashima[15] found that the bone morphogenic proteins BMP-2 and BMP–4 were capable of inducing dentin formation in amputated canine pulp. The amputated pulps were capped with BMP-2 and BMP-4 or with inactive dentin matrix. At two months the pulps were covered with osteodentin at the wound side and tubular dentin at the pulp tissue side in the group treated with recombinant BMP-2 and BMP-4. The amount of dentin formed was markedly diminished when dentin matrix alone was implanted. The authors noted that BMP-2 and BMP–4 induce differentiation of adult pulp cells into odontoblasts, and concluded that BMPs may have a role in dentistry as bioactive pulp-capping agents for dentin formation.

Jepsen *et al.*[7] placed recombinant human osteogenic protein-1 (hOP-1) in 16 teeth of miniature pigs with artificially exposed dental pulps. They evaluated three different barriers associated with hOP-1: collagen barrier (2.5 µg/mg), collagen barrier alone or calcium hydroxide paste. Teeth were removed in block sections after a healing period of 5 weeks. Decalcified sections were processed for light microscopy and histomorphometric analysis. In hOP-1 treated teeth substantial amounts of hard tissue formation (osteodentin and tubular dentin) consistently resulted in a complete bridging of the defects. Less dentin formation was seen after calcium hydroxide application, and that control defects collagen barrier alone failed to form complete dentin bridges. These authors concluded that hOP-1 in a collagen barrier appeared to be suitable as a bioactive capping agent for surgically exposed dental pulps.

Hu *et al.*[6] applied several growth factors (i.e. epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor II, platelet-derived growth factor-BB, and transforming growth factor-beta-1) onto a synthetic collagen barrier. These growth factors with collagen barriers were used separately as pulpal medicaments in rat molars. Dycal, unimpregnated collagen barrier, and no medication were used as controls. Eight samples from each group were collected 2 and 3 weeks after surgery. Pulps treated with TGF-beta-1 showed significantly improved soft and hard tissue healing at week 3, compared with the procedural controls. TGF-beta-1, when used as a pulp-capping medication enhanced reparative dentin formation in rat molars.

Tziafas *et al.*[30] wrote an extensive review of new strategies in vital pulp therapy. The highlights of the review were the involvement of growth factors and extracellular matrix molecules in signaling and regulating dentinogenic events during tooth development. They recommend the application of exogenous signaling factors for regenerative therapies, although a number of delivery considerations must be addressed before these can be introduced into clinical practice.

More recently, Six *et al.*[24] assessed the effect of bone morphogenic protein-7 (BMP-7) in inducing reparative dentinogenesis in the exposed pulps of rat molars. To determine if the effect was dose-dependent, collagen pellets containing 1, 3 or 10 µg

of recombinant BMP-7 were inserted into intentionally perforated pulps (10-12 pulps per group) in the deepest part of half-moon class V-like cavities cut on the mesial aspect of upper first molars. As controls, a collagen barrier alone and calcium hydroxide were used as capping agent. Half of the animals were euthanized after 8 days and the other half after 28 days. The molars were processed for histological evaluation by light microscopy. No difference in effect could be detected between the three concentrations of BMP-7 groups at either time interval. In the calcium-treated pulps, the initial formation of thick reparative osteodentine left some unmineralized areas at the exposure site and some interglobular unmineralized areas containing pulp remnants. In most BMP-7-treated specimens, the initial inflammation resolved within 8 days and at 28 days heterogeneous mineralization or osteodentine filled the mesial coronal pulp. Complete filling of the radicular pulp by homogeneous mineralization occurred in the mesial root. This effect was observed in 11 teeth of the BMP-7 group, in one tooth of the collagen barrier group and in none of the calcium hydroxide group. The authors recommended the application of BMP-7 as an alternative conventional endodontic treatment. However, the complete filling of the root pulp with calcified tissue is not a reparative response as the authors suggest it. Rather, it may be considered pulp calcific degeneration since the entire root canal is replaced by mineralized tissue. Calcium hydroxide may induce the same response when not properly applied and, for this reason, its use has been criticized.

The same group[3] evaluated bone sialoprotein (BSP), bone morphogenetic protein–7 (BMP-7, also termed OP-1) and chondrogenic inducing agents (CIA) A+4 and A-4 implanted in the pulp of the first molars. Pulp tissue was evaluated for different levels of mineralization. They reported that these agents caused the formation of a reparative dentinal bridge closing the pulpal wound (CIA A+4), or filled the mesial part of the coronal pulp (BSP), or filled totally the pulp located in the root canal (BMP-7 and CIA A-4), and concluded that these molecules have great potential for clinical application in the near future.

Periradicular Regenerative Therapies

The literature on bioactive modulators and their respective function in periodontal disease is extensive. The application of specific growth factors has been shown to promote periodontal regeneration[25], and combinations of factors (e.g. competence growth factors with progression growth factors) have been shown to act synergistically[26].

The use of biological mediators and their potential to engineer a regenerative response subsequent to endodontic procedures have not been extensively studied. A group from Loma Linda University[13] evaluated the effects of resorbable membrane placement and human osteogenic protein-1 (hOP-1) on hard tissue healing after periradicular surgery in maxillary canines of cats. Subsequent to periradicular surgery, and before reapproximation of the surgical flaps, eight of the osteotomies were covered with a resorbable membrane and eight filled with hOP-1 on a collagen barrier. The remaining eight sites received no further treatment and served as controls. The animals were euthanized in 12 weeks and specimens examined histomorphometrically for the presence or absence of inflammation, osseous regeneration, and cementum formation on the root ends. The results showed that the sites treated with membrane exhibited significantly more inflammation adjacent to the resected root ends, and that the use of the membrane had no statistically significant effect on osseous healing or new cementum formation. The use of hOP-1 was associated with a significant decrease in the thickness of new cementum formed on the resected root ends, but had no statistically significant effect on osseous healing or degree of inflammation. Based on the results of this study, either the use of hOP-1 nor membranes have a positive effect on periradicular tissue healing in endodontic surgery.

A group from Baylor University[17] evaluated the healing of the periradicular tissues when exogenous growth factors (e.g. PDGF-BB, IGF, and FGF) were delivered to the respected root end after periradicular surgery. The healing response was compared with that when Diaket® (3M ESPE, St. Paul, MN, U.S.A.), a polyvinyl resin, was used as control. Non-surgical endodontic treatment was performed on mandibular teeth of mongrel dogs. The surgical treatment included root-end resection and root-end cavity preparation. IGF in combination with PDGF-BB, or FGF alone, were placed in the root-end preparations on a polygalactic acid carrier (Atrisorb®, CollaGenex Pharmaceutical Inc., U.S.A.) with or without incorporation of the carrier tetracalcium sulfate. The healing was evaluated at 60 days with regard to presence of inflammatory response, bone regeneration, periodontal ligament formation and

cementum formation. Osseous regeneration in the excisional wound and periodontal formations were significantly greater when Diaket was used as the root-end filling material. Likewise, cementum deposition occurred significantly more frequently in the Diaket group. The polygalactic carrier Atrisorb remained in the surgical sites for the duration of the study. They concluded that the use of the growth factors FGF and a combination of IGF/PDGF, delivered to the prepared root end in a collagen carrier did not initiate the desired periradicular tissue response of regeneration However, Diaket as used in this study did stimulate a periradicular tissue response compatible with regeneration.

Kim *et al.*[8] have conducted a prospective qualitative analysis of the effect on healing of periradicular perforation using the growth factors PDGF-BB and IGF-I with calcium hydroxide in dogs. Fifty-one periradicular lesions were induced after the root apices were intentionally overinstrumented with a #4 profile (Profile Co., Tulsa, OK, U.S.A.) of 0.06 taper. The teeth were divided into three groups. In group 1 (n=17) the apical perforation sites were not sealed. In group 2 (n=17) the apical perforation sites were sealed with calcium hydroxide. In group 3, calcium hydroxide and 4µg of PDGF-BB and IGF-I in cellulose gel were used. Canals in all groups were sealed by the lateral compaction technique. Animals were euthanized after 12 weeks. Tissue was harvested, stained with hematoxylin and eosin and immunostained for osteonectin. The amount of inflammation was evaluated histomorphometrically. The results indicated that sections in group 3 showed no inflammatory reaction of apical tissue, and the connective tissue adjacent to the newly formed hard tissue was strongly immunostained for osteonectin. Most group 1 sections showed no apical healing. Moderate healing was found in group 2. The authors concluded that the combination of PDGF-BB and IGF-I with calcium hydroxide improved healing of apical perforations in dogs.

Shabahangs *et al.*[21] reported that ProRoot MTA® promoted apical hard tissue formation with significantly greater consistency than calcium hydroxide, including the use of collagen matrix saturated with the bone morphogenic protein BMP-7. Although the use of ProRoot MTA® as a periradicular barrier in endodontically related teeth has shown good sealing ability[31] and biocompatibility[29], comprehensive studies on the therapeutic efficacy of bone-like tissue and cementum induction by a combination of bone morphogenetic proteins and different matrices is still missing.

DISCUSSION AND CONCLUSIONS

An increased understanding of embryonic development and wound healing at the cellular and molecular level has led to the development of novel regenerative therapies. Most of the reviewed articles support the use of biological modulators in regenerative endodontic treatment modalities. Questions concerning the application of these biological modulators during endodontic treatment include:

1. Development and classification of biological modulators.
2. Lack of information about the interaction among artificial barriers, biological modulators, and restorative materials.
3. Differential effects of the above-mentioned materials related to the status of the tissue to be treated (i.e. normal, inflamed or infected). Most of the research articles only used vital, non-inflamed tissues as a model.
4. The effect of aging on pulpal and periradicular response to the above-mentioned materials.

Contamination by microorganisms or their byproducts remains an enormous threat to any type of endodontic procedure. Despite innovative technologies and improvement of new materials, cases will fail if pathogenic microorganisms are not completely eliminated. Regenerative therapies have wide and essential applications in Dentistry and Medicine. Several studies have proved that the incorporation of bonelike inorganic fillers, such as DFDBA and hydroxiapatite and biological mediators for the development of composites that present a degradable behavior, an interesting mechanical behavior that is associated with a clear bioactive character[24].

Further research is desired to provide continuance towards tissue regeneration and its clinical applicability.

RESUMO

O tratamento endodôntico evoluiu muito nos últimos 20 anos. Equipamentos e técnicas inovadoras utilizadas no tratamento de canal são constantemente

introduzidas no mercado, como consequência do esforço dos pesquisadores. Atuais estudos utilizando tecnologia de engenharia de tecidos e células tronco estão demostrando um progresso expressivo, e talvez estejam disponíveis em breve para aplicação clínica. O objetivo deste artigo é revisar as modalidades de tratamentos que involvem a combinação de moduladores biológicos (e.g. fatores de crescimento), barreiras (reabsorvíveis e não-reabsorvíveis) e materiais restauradores durante diferentes procedimentos do tratamento endodôntico. Estes materias podem ser clinicamente aplicados como agentes de proteção pulpar durante terapias de polpa viva; substitutos de enxertos ósseos (preenchimento ósseo) para induzir cicatrização do tecido perirradicular após cirurgia paraendodôntica; componentes de barreiras apicais artificiais usadas durante processos de apexificação; reparo de defeitos perfurativos ou decorrentes de reabsorções. Em geral, as barreiras devem ser inseridas fora do canal radicular com o intúito de facilitar a obturação do canal radicular, ou de servir como suporte para que materiais restaurados sejam adequadamente aplicados contra estas. O aspecto inovador em se adicionar moduladores biológicos à barreiras é parte de um esforço científico de induzir regeneração tecidual. Entretanto, a existência de fatores de variabilidade, tais como a interface entre materiais restauradores e a combinação de barreiras com moduladores biológicos e respectiva interação com tecidos inflamados ou contaminados, exigem análises futuras. Pesquisas fundadas em evidências são necessárias antes que resultados experimentais sejam extrapolados para aplicação clínica.

UNITERMOS: Moduladores biológicos; Regeneração tecidual; Terapia endodôntica.

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