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Research Article

PHRMACOLOGICAL BASIS OF REGENERATIVE ENDODONTICS: A DOUBLE EDGED SWORD

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Abstract:

Regenerative endodontics is based on the survival, proliferation and differentiation of stem cells in the pulpal space of non-vital immature permanent tooth with a wide open apex. This option offers an ideal outcome for treatment of open apex cases as they result in the increase in root length and width. These procedures rely heavily on the disinfection of the canal space by antimicrobial agents as no mechanical instrumentation is recommended. The present article discusses the pharmacological basis of regenerative endodontic procedures. It also discusses the dilemma of disinfection vs. regeneration which is faced by the clinician as the antimicrobial agents used for disinfection of the canals will also kill the stem cells which are central to the regenerative procedure. It also discusses the confusion which exists in the literature about the actual concentration of antibiotics which should be used for disinfection.

Key Words

Regenerative endodontics, antimicrobial agents, disinfection, antibiotics

Introduction

Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex [1].These procedures have given the clinician a new ray of hope for treatment of immature non vital infected tooth with open apices which were till now treated by a multiple visit calcium hydroxide therapy[2] or more recently by single visit MTA therapy[3]. Both therapies leave the dentinal walls of the tooth fragile and liable to fracture in function.

Regenerative endodontics is based on three factors, namely stem cells, growth factors and a scaffold. Stem cells are undifferentiated cells which have the capability to produce cells of the same type or more differentiated cells. Many different kinds of stem cells have been identified from in and around the tooth[4,5].A noteworthy property of these stem cells is their survival in chronically inflamed environment and in the presences of necrotic infected tissue and bacteria. These two properties of stem cells allows the disinfected canal to get repopulated with differentiated cells while allowing time for reorganization, angiogenesis and innervation to take place[6]. Growth factors are polypeptides which have the ability to bind to specific receptors on the target cells (in this case the stem cells and other pulpal cells) and modulate or facilitate certain activities like migration, proliferation, differentiation, and apoptosis[7,8]. Growth factors can be supplied from outside or natural growth factors which are present in dentin can be employed. The scaffold is used to provide support for the stem cells to regenerate, proliferate and differentiate. The most commonly used scaffold is the blood clot itself which can be produced by inducing bleeding into the canal. Platelet rich plasma (PRP) is also gaining popularity as a scaffold because it is relatively simple and inexpensive to obtain and provides a better suited scaffold than blood clot.

The fact that disinfection is an essential precondition to regeneration cannot be overstressed. If microorganisms persist in the root canal space, any therapy will be destined to failure because of persistent infection. The aim of the antimicrobial agents is to act against the infection causing bacteria present inside the root canal space and the dentinal walls. It is achieved in a typical regenerative procedure by the use of irrigants which are liquids with antimicrobial properties and with inter appointment intracanal dressing with antimicrobial pastes. Only after the disinfection is confirmed on the subsequent appointment by lack of symptoms and radiographs is the actual regenerative procedure initiated.

The following discussion focuses on the various antimicrobials used in regenerative endodontic procedures and how far they have been successful in fulfilling their role in the procedure and what the future beholds.

Antimicrobial agents Used for Irrigation

Irrigation has always played an important role in the conventional endodontics and it is no different in regenerative endodontics too. Irrigants have the potential to reach places in the complex anatomical system of the root canal where no instrument can reach. Many irrigants like sodium hypochlorite[9], EDTA[10], chlorhexidine[11] have been used for canal debridement invarious published cases of regeneration.

Sodium Hypochlorite:

Sodium hypochlorite (NaOCl) has been used since the time of world war World War I, where it was extensively used for its disinfective properties on wounds. It was known as "Dakin's solution" in honor of Henry Drysdale Dakin, who extensively researched its anti-infective properties and popularized its use for disinfection of wounds[12]. Besides being a broad spectrum antimicrobial, sodium hypochlorite preparations are sporicidal, viricidal and dissolve necrotic soft tissue readily as compared to vital tissue[13]. The same prompted Coolidge to propose the use of sodium hypochlorite as the main irrigant in endodontics in 1919[14]. Many invitro and in vivo studies have proven the effectiveness of sodium hypochlorite against a spectrum of microbes including E. faecalis which is a main culprit found most commonly in failed root canal cases [15],[16],[17]. Sodium hypochlorite is used in the concentration ranging from 0.5% to 6%[18]. The main disadvantage of using sodium hypochlorite is that it is extremely irritating to the periapical tissue if accidentally extrudes and it also has a disagreeable taste[19].

Irrigation with sodium hypochlorite is the first step in canal debridement to wash out necrotic infective tissue from the canal. According to a well-structured meta-analysis all reported cases of regeneration have used sodium hypochlorite. The same meta-analysis further states that 63% of the reported cases were irrigated with 3% hypochlorite while 36% cases were irrigated with full strength hypochlorite (5-6%) and only 1% of the cases were irrigated with 1% hypochlorite which was the lowest reported percentage used The immature nonvital tooth where the root formation is incomplete, the apical opening is large and chances of sodium hypochlorite reaching the periapical area are more. Therefore it has been recommended that the irrigating needle be kept atleast 1mm short of the working length while irrigating[20].

It has also been reported that the use of 6% sodium hypochlorite has an adverse effect on the survival of the stem cells of apical papilla (SCAP) which are of paramount importance in the regenerative procedure[21,22]. Therefore it has been recommended that a concentration of 1.5% sodium hypochlorite be used for irrigation in the cases of regeneration as it has been proven to have minimal adverse effect on the SCAP[21].

Ethylenediaminetetra acetic acid (EDTA)

Ethylenediaminetetra acetic acid is a water soluble colourless polycarboxylic acid which is widely used in the chemical industry because of its chelating properties. It has been used in the field of medicine to treat heavy metal poisoning[23] and as an anticoagulant.

The use of chelation therapy in endodontics was suggested as early as 1957 by Nygaard- Ostby[24]. Its use for the removal of the inorganic component of the smear layer which form on instrumented tooth surfaces is well documented[25,26,27]. EDTA is used as gel to provide lubrication while instrumentation or as a 17% liquid for irrigation. Because of its chelating properties, its action is self-limiting.

In regenerative endodontics, where the aim is not only to disinfect the canal space but also to improve the survival rate of the stem cells, EDTA plays a pivotal role. It has been proven from many studies that a final rinse of EDTA promotes the survival and differentiation of stem cells [21,22]. It has been postulated that EDTA while causing the demineralization of dentin also increases the bioavailability of important growth factors like TGF-β which are embedded in the dentin[28,29].

Chlorhexidine

Chlorhexidine is a synthetic cationic bis-guanide that alters the cell's osmotic equilibrium which increases permeability of the cell wall and allows chlorhexidine to enter the bacterial cell. It is bacteriostatic at low concentration (0.2%) while at higher concentration (2%) which is recommended for use in endodonticschlorhexidine is bacteriocidal[30].

Chlorhexidine also has the property of substantivity, which means that the antibacterial effect of chlorhexidine remains for some time even after its actual removal[31].

Though chlorhexidine has been successfully used in conventional endodontics along with sodium hypochlorite and EDTA, but in regenerative endodontics there are conflicting reports regarding its use and its effect on stem cells. One school of thoughts advocates the use of chlorhexidine because of its low cytotoxicity in contact with the periapical tissues[32,33]and various reports of successful revascularization have found place in published literature[34,35,36,37].

Contrary to these there is reports which claims that chlorhexidine is highly cytotoxic to stem cells[22,38,39]. Further research is warranted on the usage of chlorhexidine in regenerative endodontics.

Antimicrobial Agents For Inter Appointment Dressing

The inter appointment dressing is given with the aim of eliminating the microbial flora of the infected root canal before inducing bleeding into the canal.

Triple antibiotic paste and its modifications have been used in majority of reported cases of successful regeneration. Calcium hydroxide has also been used in some cases.

Triple antibiotic paste (TAP)

Triple antibiotic paste is a mixture of metronidazole, ciprofloxacin and minocycline. In the metanalysis by Diogenes et al. 51% of the total published reports used TAP[20].The use of TAP in endodontics was first recommended by Sato et al. in 1996[40]. Its usage in endodontics is justified by the fact that microbial flora in the root canal is of mixed nature, with a predominance of obligate anaerobes, but it was observed that even with a very high concentration of metronidazole (which is primarily effective against anaerobes) some microorganisms still survived. To solve this problem it was suggested that a group of antibiotics be used together. This mixture of antibiotics eliminated all the microbes from the root canals in various studies. Also a mixture of antibiotic prevents antibiotic resistance[41].

Metronidazole is a nitroimidazole antibiotic which is primarily effective against anaerobic bacteria and protozoa. Ciprofloxacin belongs to the fluoroquinolone group of antibiotics which are active against a wide variety of gram negative and gram positive organisms. Minocycline is an antibiotic of the tetracycline group which have are classified as broad spectrum bacteriostatic antibiotics. Together it has been proven that these three antibiotics have been able to eliminate the entire microbial flora from the root canals[41,42].

Many variations of TAP have been tried but none has been as successful as the original formulation. The variations are a mixture of four antibiotics with the addition of rifampacin to the original TAP[42], double antibiotic paste consisting of metronidazole and ciprofloxacin[42], modified triple antibiotic paste consisting of metronidazole, ciprofloxacin and cefeclor[43]and a combination of amoxicillin and clavulonic acid[44]. As can be observed in the modifications, minocycline is the one which has been mostly commonly replaced or eliminated. This can be attributed to the discoloration of the crown caused by minocycline.

It is noteworthy that considerable confusion exists in the literature about the concentration of the antibiotics which should be used in the preparation of TAP. According to Hoshino et al. who proposed the use of TAP for the first time a minimal concentration of antibiotic which will eliminate all the microbes of the root canal should be used[45], which was determined by them to be 100 µg/ ml of each antibiotic making it a total of 300 µg/ ml. This makes the concentration of all the antibiotics in a ratio of 1:1:1. In another study by Takushige et al[46] the recommended ratio is 1:3:3 of ciprofloxacin, metronidazole and minocycline. This mixture of antibiotic was made by removal of the enteric coating from the tablets and grinding them to powder with a mortar and pestle. It should be noted that none of the above mentioned antibiotics are

commercially available in the same concentration: ciprofloxacin is available as a 250 mg or 500 mg tablet, metronidazole is available as a 200 mg or 400 mg tablet and minocycline is available as a 50 mg and 100 mg tablet. Therefore taking one tablet of each will never give a ratio of 1:1:1 as far as the actual concentration of the mix is concerned. To complicate the matter further little attention is given to the actual concentration of TAP. The powder of antibiotics is mixed with the vehicle (which can be saline or water or macrogol/ propyl gycol MP) till the physical consistency of the mixture is deemed fit for use by the clinician. It was found that such mixtures contain a very high concentration of antibiotics (1g/ml). According to in vitro studies such high concentration of antibiotics has been found to be detrimental to the stem cells[47]. Therefore it is recommended that the triple antibiotic paste used in regenerative endodontics be made with the minimal effective concentration of antibiotics (about 100µg/ml of each antibiotic) rather than depending on the physical consistency of the paste. It is also a matter of further research to standardize a minimal effective concentration of antibiotics and the procedure for obtaining the same from commercially available antibiotics tablets or capsules.

Conclusion

As has been observed, in regenerative endodontic procedures, it is a competition between disinfection vs.regeneration which is unlike conventional endodontics. In conventional endodontics disinfection is the primary target of antimicrobial agents and no consideration for survival of any cells exist. But as regenerative endodontics is based on the survival, proliferation and differentiation of stem cells, the use of strong antimicrobial agents can prove to be a self-defeating prophecy. Therefore future research will concentrate on the use of minimal concentration of antimicrobial agents which will be established by well controlled studies and also on development of antimicrobial agents which will be selective towards microbes while sparing the stem cells.

Regenerative endodontics also indicates a paradigm shift for the dentist for whom till now “stronger was better” when it came to the use of antimicrobial agents in conventional root canal therapy.

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