OPEN ACCESS Freely available online

Annals and Essences of Dentistry

Review Article

Lesion Sterilization and Tissue Repair (LSTR) Technique and its Clinical Application in Primary and Permanent Teeth: A Review

Triveni Mohan Nalawade[1]*, Dhaval Parikh[2] and Rachappa M Mallikarjuna[1]

[1]Department of Paediatric Dentistry, Child Dental Health, Oman Dental College, Muscat, Oman; [2]Department of Pedodontics and Preventive Dentistry, Manubhai Patel Dental College, Vadodara, Gujarat, India

ABSTRACT

Lesion Sterilization and Tissue Repair (LSTR) is also called NIET or Non-Instrumental Endodontic Treatment as it claims to be a "new biologic approach in the treatment of carious lesions with periapical involvement using a mixture of 3 antibiotics (3-Mix)." LSTR involves the use of three antibiotics/antibacterial drugs namely, Metronidazole, Ciprofloxacin and Minocycline. The 3 antibiotics are mixed together with propylene glycol. A fresh creamy consistency is prepared and placed in the pulp chamber which is subsequently sealed with a GIC restoration and stainless steel crown. This concept was developed by the cariology research unit at the Nigata University School of Dentistry in 1988. Several studies done using LSTR on deciduous teeth reported excellent clinical results which may be ascribed to the bactericidal effect of the 3-Mix. Some concerns include the inappropriate use of antibiotics, possible leakage of the antibiotic paste into the oral cavity and its effect on the oral microflora, etc. So this paper attempts to look in depth about the critical impact of LSTR in pulp therapy and also presents the newer concept of a 2-Mix instead of 3-Mix for treatment. Some uses of 3-Mix extend to cases of chronic periapical abscess, failure of pulpectomy in deciduous molars treated with Zinc Oxide Eugenol (ZOE) obturation, revascularization endodontics and reimplantation of avulsed teeth.

Keywords: LSTR; NIET; 3-Mix; 2-Mix; Primary Molars; Revascularization; Pulp Therapy

INTRODUCTION

Pulp therapy includes pulpectomy in primary and permanent teeth and vital pulp therapy techniques. Successful pulp therapy is always challenging to the dentist due to the morphology of the root canal in primary and permanent teeth, delayed presentation of patient for treatment due to financial constraint, dental neglect, lack of co-operation in paediatric patients etc. This delay in availing treatment until the caries progresses further to involve pulp; causing symptoms with excessive external root resorption and excessive peri-radicular bone resorption which makes the prognosis less favourable for conventional endodontic therapy. Many treatment procedures have been proposed such as indirect pulp capping, partial pulpotomy, pulpotomy, pulpectomy and extraction of the primary teeth followed by a space maintainer to treat these issues. In spite of appropriate biomechanical preparation in permanent teeth, irrigation and obturation, the Root Canal Treatment (RCT) fails whereas in young permanent teeth, obturation in teeth with open apices is difficult [1,2].

Early loss of primary teeth can cause a number of problems, such as, drifting of erupted teeth, ectopic eruption, improper eruption sequence, impairment of function, speech alteration and development of oral habits like tongue thrusting [2,3]. Some authors have advocated the extraction of teeth with poor prognosis followed by removable or fixed space maintainers [4]. Yet, these appliances have some inherent disadvantages like patient non-co-operation, frequent breakage, function and oral hygiene. All authors have concluded that the preservation of primary teeth is the best space maintainer for its successor if resolution of the pathological process can be achieved [5]. Thus, it is important that the primary dentition should be maintained in the dental arch provided it can be restored to function and remain disease-free. Also, as the permanent teeth are the last set of teeth in humans, they need to be preserved life-long for mastication and other functions [2].

Rationale for the LSTR

Endodontic therapy plays an important role in removing bacteria,

Correspondence to: Triveni Mohan Nalawad, Department of Paediatric Dentistry, Child Dental Health, Oman Dental College, Muscat, Oman, E-mail: triveni_nalawade@rediffmail.com

Received: September 25, 2018, **Accepted:** March 11, 2019, **Published:** March 21, 2019

Citation: Triveni MN, Dhaval P, Rachappa MM (2019) Lesion Sterilization and Tissue Repair (LSTR) Technique and its Clinical Application in Primary and Permanent Teeth: A Review. Ann Essence Dent 11:4.

their by-products and their substrates by disrupting and destroying the microbial ecosystem through chemical and mechanical methods. Different drugs and medicaments have also been suggested to accompany these techniques with varying success rates. The use of nonspecific antiseptics and use of steroids application or antibacterial drugs represents one of the methods of eradicating bacteria in the Root Canal Treatment (RCT) [1].

Sterilization of the root canal and peri-radicular region results in good healing of the peri-radicular region [6]. Bacteria which are present mainly in the root canals and superficial layer of infected root canal wall may be easily removed by conventional root canal treatment. Inspite of following proper RCT protocol and good obturation techniques, many RCT failures or flare-ups, are attributed to bacteria which remain in the deep layers of root canal dentine. Application of antibacterial drugs to endodontic lesions can be used to sterilize such lesions [6]. To sterilize such lesions, a single antibacterial drug may not be effective though broad spectrum as the bacterial composition of the infected root canals is complex. In addition, bacteria may also be smeared along the root canal during endodontic treatment. Antibacterial drugs should aim to completely remove all such bacteria [7]. To sterilize the deep layers of infected root dentin, root canal medicaments should penetrate the root canal dentin. The penetrative ability of these drugs was improved by mixing these drugs with propylene glycol and macrogol to form an ointment base as demonstrated [8,9].

History and evolution of lesion sterilization and tissue repair

In recent years, the Cariology Research Unit at Niigata University School of Dentistry has developed the concept of Lesion Sterilization and Tissue Repair (LSTR) therapy that employed a mixture of antibacterial drugs for disinfection. Repair of damaged tissues can be expected if lesions are disinfected [8].

Since the overwhelming majority of bacteria in the deep layers of infected root canal wall dentine consist of obligate anaerobes, metronidazole was selected as the first choice among the antibacterial drugs. Metronidazole, even at high concentrations cannot kill all the bacteria indicating the necessity for other drugs. Thus in addition to Metronidazole, Ciprofloxacin and Minocycline were added to sterilize the infected root dentin [10].

Metronidazole has a wide spectrum of bacterial action against oral obligate anaerobes [8,11]. It has been found that obligate anaerobes form the majority of isolates from carious lesions, infected root dentin, and from non-exposed pulp tissue. Thus, it was observed that a mixture of antibacterial drugs i.e., Ciprofloxacin, Metronidazole and Minocycline can sterilize carious lesions, necrotic pulp and infected root dentine of primary and permanent teeth [7,12,13].

Ratio of the antibiotics and vehicles in 3-Mix: LSTR involves the use of three broad spectrum namely, Ciprofloxacin, Metronidazole, and Minocycline. There is conflicting data on the ratio in which the antibiotics are mixed. Takushige et al. used Ciprofloxacin, Metronidazole and Minocycline in a ratio of 1:3:3. Some researchers endorse a 3:1:1 ratio, whereas Garcia et al. endorse 3:1:3 mix, while others including Professor Hoshino endorsed a ratio of 1:1:1 [13–15]. The rationale behind the different ratio of the mix is unknown, but one can say that a lesser amount of antibiotic usage to attain clinically effectiveness would be preferred. Ferreira et al. found that antibiotics namely Ciprofloxacin, Clindamycin and Metronidazole were cytotoxic to human gingival fibroblasts and lower concentrations of 5-50 mg/L produced viable fibroblasts for a prolonged time [16].

Vehicles used: Cruz et al. demonstrated the penetrative effect of propylene glycol into root dentin; to rapidly and effectively deliver intracanal medicaments. Takushige et al. used a combination of Macrogol and Propylene glycol (MP) to mix the 3 antibiotics (3-Mix). Hence their antibiotic medicament was also referred to as 3-Mix-MP. Propylene Glycol and Macrogol, are found to be excellent vehicles to carry the 3-Mix into the entire dentin and through the dentinal tubules and kill all the bacteria in the lesions. Pinky et al. and Burrus et al. have used only Propylene glycol as the vehicle or carrier for the 3-Mix [17,18]. Nalawade et al. compared only the vehicles for their antimicrobial activity and concluded that Propylene glycol had the maximum antimicrobial properties and provided the most ideal consistency for the antibiotic paste.

Different combinations of antibiotics in the 3-Mix: Sato et al. carried out a series of *in-vitro* experiments using the 3-Mix with and without Rifampicin. Later they combined Ciprofloxacin and Metronidazole along with a third antibiotic, namely Amoxicillin, Cefaclor, Cefroxadine, Fosfomycin or Rokitamycin [11,19]. Sato et al. and Hoshino et al. confirmed the 3-Mix to be bactericidal and most effective. Ruparel et al. in an *in-vitro* study substituted Minocycline with Cefaclor and referred to it as the modified Triple Antibiotic Paste (TAP) and tried another combination referred to as the Double Antibiotic Paste (DAP) [20]. The DAP can be referred to as the 2-Mix, because it consisted only of Ciprofloxacin and Metronidazole. They also tried Augmentin on its own and its effect on the survival of stem cells of apical papilla [20]. Later, Pinky et al. and Nanda et al., eliminated Metronidazole from the 3-Mix and tried Ornidazole instead [17,21]. The main concern was Minocycline, which when used for revascularization procedures caused discoloration of the young permanent teeth [22]. Another concern was its possible effects on the developing successor when used in an indicated deciduous tooth for LSTR.

Algarni et al. used antibiotic gels containing Clindamycin modified 3-Mix and compared it to the 2-Mix. Park et al. reported a case using an Amoxycillin modified 3-Mix for revascularization of necrotic immature permanent tooth and overcame discoloration aspect after one year clinical follow-up [23].

Many researchers started the quest for a suitable alternative for overcoming the drawbacks of 3-Mix but also equally effective as the 3-Mix had been.

The journey from 3-Mix to 2-Mix: Ruparel et al. had already paved the way for 2-Mix consisting of Ciprofloxacin and Metronidazole but its bactericidal efficacy was not tested in their *in-vitro* study [20]. The 2-Mix consisted of Ciprofloxacin and Metronidazole while Minocycline was removed. Sabrah et al. compared the antimicrobial activity of the 3-Mix and, 2-Mix with Calcium hydroxide against *E. fecalis* and *P. gingivalis*. They found out that calcium hydroxide lacked effective bactericidal properties and that the 2-Mix has comparable antibacterial activity to the 3-mix, so it can replace the 3-Mix [24]. Algarni et al. too confirmed similar findings of the 2-Mix having a similar bio-film inhibition as the modified 3-Mix consisting of Clindamycin. Later they continued *in-vitro* studies assessing the biocompatibility of the 3-Mix and 2-Mix and found the 2-Mix to be more biocompatible with Dental Pulp Stem Cells (DPSCs). Lastly they concluded that the 2-Mix had longer residual antibacterial effect as compared to the same concentration of the 3-Mix [25]. Kim et al. confirmed that a low concentration of

1 mg/mL of the 2-Mix had fewer negative effects on attachment and proliferation of DPSCs to dentin [26]. Hence these *in-vitro* studies suggested that the 2-Mix could be used where 3-Mix was indicated.

Advantages of 2-Mix with Propylene glycol: The major advantage of the usage of 2-Mix over 3-Mix was its comparable antibacterial efficacy, longer residual antibacterial effect and fewer detrimental effects on DPSCs. The main concern of localized Minocycline staining of the permanent tooth bud could be overcome, with the use of the 2-Mix. It also had similar antibacterial efficacy. Disadvantages of Macrogol i.e. Polyethylene glycol like, respiratory tract irritation, skin irritation, eye irritation, central nervous system depression; whereas long term exposure could cause liver damage, reproductive and mutagenic effects; could be overcome too [27].

Mechanism of action of 2-Mix: The mode of action for 2-Mix is same as for 3-Mix, i.e, it sterilizes the infected tissues; both pulpal and periapical.

Concerns and issues against the use of 2-Mix are similar to that of 3-Mix for LSTR which are mentioned in the following text but reduced number and lower concentrations of antibiotics should overcome significant side-effects [16,25].

Preparation of the 3- Mix

For the Ciprofloxacin tablet, the enteric coating is removed with a scalpel. The tablet is pulverized using mortar and pestle. For the Metronidazole and Minocycline which come in tablet form and capsules respectively, the powder is segregated. Antibiotics are prepared freshly before every use. Once mixed with the vehicle, the 3-Mix remains are discarded but the powdered tablets can be stored, sealed in air tight containers under refrigeration. The 3 antibiotics are mixed together with propylene glycol in a ratio of 1:1:1. A creamy consistency is prepared. Then this mix is rolled into a small ball of 1 mm diameter approximately and placed on pulpal floor on the root canal orifices. Initially Takushige et al., prepared a medication cavity, also followed by Prabhakar et al. and Pinky et al., [17,28] but overtime many authors including Takushige in 2008 have simply placed the 3-Mix paste near the root canal orifices and observed it's diffusion due to the presence of many accessory canals in the furcation area of the pulpal floor of deciduous teeth [13,29,30].

Proposed mechanism of action of LSTR

Sato et al. and Hoshino et al. confirmed through series of *in-vitro* studies that the 3-Mix is bactericidal to aerobic bacteria and resistant obligate anaerobes. Hosino et al. identified obligate anaerobes from carious dentine, infected pulp, and infected root canal dentine predominating at 80%, 92% and 80% respectively. Obligate anaerobes are sensitive to Metronidozole. Metronidazole with its wide spectrum bacterial action against anaerobes makes it the first choice to eliminate obligate anaerobes. However, since all bacteria cannot be eliminated by Metronidazole alone, other drugs may be necessary to sterilize the infected dentin. Hence, Ciprofloxacin and Minocycline were added to the antibiotic paste for LSTR. In the absence of Minocycline in the market, Cephalexin was substituted [10,12,19,31].

The contention behind the 3-Mix is that it sterilizes the infected and softened dentin and the pulpal lesions, intentionally left during cavity preparation. Also, the 3-Mix can eliminate bacteria from infected dental tissues of both deciduous and permanent teeth [8,10]. The assumption is that if you eliminate bacteria, infection can be eliminated, thereby reducing inflammation, and consequently, pain both acute and chronic. According to Hoshino, 94% of the cases that experienced pain were relieved within 24 hrs after LSTR using the 3-Mix regardless of the nature and severity of the pain [30].

Success of LSTR:

Several studies done with LSTR on deciduous teeth reported the following;

- Excellent clinical results like healing of draining sinuses, gingival abscesses and periradicular lesions may be ascribed to the bactericidal action of the 3-Mix [8].
- Recalcification of softened dentin immediately following sterilization of the lesion [30].
- LSTR was also proven to be effective in cases of deciduous teeth with resorbed roots and draining fistulae [8].

Studies also claimed that:

- Metronidazole was reported to be clinically safe when used according to recommended dosage guidelines.
- Although side effects with Ciprofloxacin have been reported when used systematically in young patients, it has been proven safe in children 3 to 16 years old when used in recommended doses.
- Minocycline may not discolour the entire tooth because only a small amount was used.

Concerns and issues against the use of 3- Mix for LSTR

A. The inappropriate use of antibiotics: Pallasch in the Journal of the California Dental Association identified six effects when antibiotics are employed. He states that only the first reason cited below is beneficial.

The later five possibilities are all negative effects and may occur with the inappropriate use of antibiotics. It may occur with the use of the LSTR employing the 3-Mix [32,33].

- The antibiotics may aid the immune system to gain control of the infection.
- Toxicity or allergy may occur.
- Already resistant microbes may be selected for and a super infection may result.
- The antimicrobial may promote microbial chromosomal mutations.
- Gene transfer may be encouraged from resistant to nonresistant microbes; and
- Latent resistance genes may be expressed [32,33].

B. The use of triple antibiotic paste for sterilization and disinfecting a pulpally involved canal presents several questions:

- The inappropriate use of antibiotics and the ecologic potential for selective pressure. The rise in the number of bacterial microorganisms that are resistant to currently available antibiotics is well documented. The WHO collaborating center in Nijmegen has clearly expressed this concern in their Basic Package of Oral Care and have emphasized the need for judicious and prudent use of antibiotics. They also reported that many

studies from various countries on antibiotics use in dentistry have revealed that antibiotics were prescribed unnecessarily in 22 to 74 percent of cases [34].

- The use of the 3-Mix is clearly intended to decrease the possibility of microbial resistance. However with every case selection, the length of time that the mix will stay inside the pulp or pulp chamber remains unclear. It raises the possibility of selective pressure to the three antibiotics. Selective pressure is the phenomenon in which the more susceptible bacteria are destroyed but the more resistant bacteria survives. Dosage regimens that do not destroy the target bacterial population create selective pressure resulting in a population of resistant bacteria. This population may not cause disease in the initial human host but may be spread to others [35].
- The rule of thumb when using antibiotics is to use it correctly and only when indicated. It's inappropriate use can lead to several serious consequences. The question with the use of the 3-Mix paste is whether it is truly indicated considering the possible consequences that may take place with its use. One must weigh the options and alternatives prior to its usage. When is the LSTR approach indicated and when do the benefits outweigh the risks? Definitely, there may be clinical instances when the approach may be suitable, but it cannot be an alternative public health measure. The risks outweigh its "expected" benefits.
- The possible leakage of the antibiotic paste into the oral cavity and its effect on the oral micro-flora. If a paste of such kind is placed in the pulp chamber or in the cavity and sealed with a glass ionomer cement or a composite onlay, over an uncertain period of time, what is the possibility of antibiotic seepage out of the tooth and into the oral cavity? What is the possibility that the minute seepage and presence of small amount of antibiotics, may affect the oral micro-flora? What evidence can be shown that there is no such seepage and the normal micro-flora is not affected?
- If the paste is placed in a pulp chamber that is not hermetically sealed, what are the possibilities that it would be absorbed into the system? The approach likewise violates the rule that systematic antibiotics should not be used for topical application. The antibiotics used for the 3-Mix are very potent antibiotics that can cause very serious side effects [36].

The prolonged use of Ciprofloxacin can induce arthropathy and fluoroquinolone-related tendinopathy. It is also more effective against gram-negative bacteria rather than gram- positive ones. The antibiotic can easily be distributed in blood tissues and fluids.

Minocyline (Tetracycline group of antibiotics) was widely used in the 1950s and 60s and has resulted in a large number of resistance strains that now limits the use of the antibiotic. It is known to depress bone growth in the fetus and children. Minocycline is also known to cause serious adverse events including a Hypersensitivity Syndrome Reaction (HSR), Serum Sickness Like Reaction (SSLR) and drug-induced lupus [37].

Metronidazole is widely prescribed by dentists. The emergence of resistance to this drug may be slower if it were used alone. In order to target both aerobic and anaerobic organisms, Metronidazole is used empirically in combinations with one or more antibiotics. Resistance to the drug may be associated with mobile genetic elements, aiding its spread [38].

The American Academy for Pediatric Dentistry as well as the European Association of Pediatric Dentistry recognized the increasing prevalence of microbial resistance and have both issued guidelines on antibiotic therapy among pediatric patients. Both advocate for the prudent and conservative use of antibiotics to minimize the risk of developing resistance to current antibiotic regimens.

From the above review, the following clinical applications of LSTR using the 2-Mix can be listed:

- In Primary teeth [39,40].

1. Primary teeth with pain and tender on percussion.
2. Primary teeth with Grade I and II mobility as a natural space maintainer for a short period until the permanent tooth is about to erupt.
3. Primary teeth with presence of an abscess or draining sinus.
4. Radiolucency in the furcation area of multirooted primary teeth.
5. In hemophiliac patients to save necrotic primary teeth instead of extraction.
6. Immature primary teeth with a non-vital pulp.
7. Endodontically failed primary tooth without the removal of previous Zinc-Oxide Eugenol obturation.
8. As an intra-canal medicament in chronic infected primary teeth and later followed by conventional obturation.
9. Unco-operative children instead of using general anaesthesia.
10. Un-negotiable root canals in primary teeth.

- In Permanent teeth [41–43]

1. Intra-canal medicament in weeping canals and symptomatic teeth including those obturated with gutta-percha [44].
2. Treatment of endo-perio lesions [45].
3. Inter-appoinment dressing to prevent flare-up during endodontic treatment in diabetic patients [46].
4. To heal large peri-radicular lesions around permanent teeth with closed apices and treat without periapical surgery [6].
5. To endodontically re-treat using the 3-Mix-MP without removal of previous root canal obturation [47].
6. Revascularization endodontics [48].

CONCLUSION

The following conclusions were drawn from this review of LSTR:

It is a simple, time-saving and cost-effective method for relief of symptoms in community based dental programmes especially in underdeveloped regions of service provision.

Endodontic treatment using an antibacterial mix (a combination of Ciprofloxacin, and Metronidazole mixed with propylene glycol) in primary teeth has shown good clinical success.

Few cases were radiographically unsuccessful with continuation of internal resorption but clinically asymptomatic.

Lastly, due to the concerns regarding usage of antibiotics, 2-Mix can be placed as an intermediate intra-canal medicament in low concentrations for 2 weeks followed by conventional obturation,

once the tooth is asymptomatic.

ACKNOWLEDGEMENT

We would like to thank Dr Asha Selvaraj (BDS) Lecturer (English Language) for her intellectual help during the proof reading and English language check of the manuscript.

REFERENCES

1. Cohen. Pathways of the Pulp. 10th edition. Vasa. 2011.
2. Fuks AB. Pulp therapy for the primary and young permanent dentitions. Dent Clin North Am. 2000;44:571-596.
3. Fabris AS, Nakano V, Avila-Campos MJ. Bacteriological analysis of necrotic pulp and fistulae in primary teeth. J Appl Oral Sci. 2014;22:118-124.
4. Fuks AB, Peretz B. Current concepts in pulp therapy for primary and young permanent teeth. Pediatric Endodontics. 2016.
5. Holan G, Fuks AB. A comparison of pulpectomies using ZOE and KRI paste in primary molars : a retrospective study. Pediatr Dent. 1993;15:403-407.
6. Taneja S, Kumari M. Use of triple antibiotic paste in the treatment of large periradicular lesions. J Investig Clin Dent. 2012;3:72-76.
7. Hoshino E, Sato I, Uematsim H, Sato M, Kota K, Iwaku M, et al. *In-vitro* antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J. 1996;29:125-130.
8. Takushige T, Cruz E V, Asgor Moral A, Hoshino E. Endodontic treatment of primary teeth using a combination of antibacterial drugs. Int Endod J. 2004;37:132-138.
9. Cruz EV, Kota K, Huque J, Iwaku M, Hoshino E. Penetration of propylene glycol into dentine. Int Endod J. 2002;35:330-336.
10. Hoshino E, Iwaku M, Sato M, Ando N, Kota K. Bactericidal efficacy of metronidazole against bacteria of human carious dentin *in vivo*. Caries Res. 1989; 22:280-282.
11. Sato T, Hoshino E, Uematsu H, Kota K, Iwaku M, Noda T. Bactericidal efficacy of a mixture of ciprofloxacin, metronidazole, minocycline and rifampicin against bacteria of carious and endodontic lesions of human deciduous teeth *in vitro*. Microb Ecol Health Dis. 1992;5:171-177.
12. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. Int Endod J. 1996;29:18-24.
13. Nakornchai S, Banditsing P, Visetratana N. Clinical evaluation of 3Mix and Vitapex as treatment options for pulpally involved primary molars. Int J Paediatr Dent. 2010;20:214-221.
14. Trairatvorakul C, Sastararuji T. Indirect pulp treatment vs antibiotic sterilization of deep caries in mandibular primary molars. Int J Paediatr Dent. 2014;22:217-222.
15. Nalawade T, Sogi SP, Bhat K. Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms. J Int Soc Prev Community Dent. 2015;5:114-119.
16. Ferreira MB, Myiagi S, Nogales CG, Campos MS, Lage-Marques JL. Time- and concentration-dependent cytotoxicity of antibiotics used in endodontic therapy. J Appl Oral Sci. 2010;18:259-263.
17. Pinky C, Subbareddy V, Shashibhushan K. Endodontic treatment of necrosed primary teeth using two different combinations of antibacterial drugs: An *in vivo* study. J Indian Soc Pedod Prev Dent. 2011;29:121-127.
18. Burrus D, Barbeau L, Hodgson B. Treatment of abscessed primary molars utilizing lesion sterilization and tissue repair: literature review and report of three cases. Pediatr Dent. 2014;36:240-244.
19. Sato T, Hoshino E, Uematsu H, Noda T. *In vitro* antimicrobial susceptibility to combinations of drugs of bacteria from carious and endodontic lesions of human deciduous teeth. Oral Microbiol Immunol. 1993; 8:172-176.
20. Ruparel NNB, Teixeira FBF, Ferraz CCCR, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod. 2012;38:1372-1375.
21. Nanda R, Koul M, Srivastava S, Upadhyay V, Dwivedi R. Clinical evaluation of 3 Mix and Other Mix in non-instrumental endodontic treatment of necrosed primary teeth. J Oral Biol Craniofacial Res. 2014;4:114-119.
22. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: A case report. J Endod. 2010; 36:1086-1091.
23. Park H-B, Lee B-N, Hwang Y-C, Hwang I-N, Oh W-M, Chang H-S. Treatment of non-vital immature teeth with amoxicillin-containing triple antibiotic paste resulting in apexification. Restor Dent Endod. 2015; 40:322-327.
24. Sabrah AH, Yassen GH GR. Effectiveness of antibiotic medicaments against biofilm formation of *Enterococcus faecalis* and *Porphyromonas gingivalis*. J Endod. 2013; 39:1385-1389.
25. Algarni AH, Yassen GL, Gregory R. Inhibitory effect of gels loaded with a low concentration of antibiotics against biofilm formation by *Enterococcus faecalis* and *Porphyromonas gingivalis*. J Oral Sci. 2015; 57:213-218.
26. Kim KW, Yassen GH, Ehrlich Y, Spolnik K, Platt JA, Windsor LJ. The effects of radicular dentine treated with double antibiotic paste and ethylenediaminetetraacetic acid on the attachment and proliferation of dental pulp stem cells. Dent Traumatol. 2015;31:374-379.
27. Rowe RC, Sheskey PJ QM. Handbook of Pharmaceutical Excipients. 6th edition. Pharmaceutical Press and American Pharmacists Association and RPS publishing. 2009.
28. Prabhakar AR, Sridevi E, Raju OS, Satish V. Endodontic treatment of primary teeth using combination of antibacterial drugs: an *in vivo* study. J Indian Soc Pedod Prev Dent. 2008; 26:5-10.
29. Agarwal M, Das UM, Vishwanath D. A Comparative Evaluation of Noninstrumentation Endodontic Techniques with Conventional ZOE Pulpectomy in Deciduous Molars: An *in vivo* Study. World J Dent. 2011;2:187-192.
30. Takushige T, Venzon Cruz E, Ali Asgor Moral M, Hoshino E. Non-surgical treatment of pulpitis, including those with history of spontaneous pain, using a combination of antibacterial drugs. J LSTR Ther (International WEB version) 2008;7:1-5.
31. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. *In-vitro* antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J. 1996; 29:125-130.
32. Pallasch TJ. Global antibiotic resistance and its impact on the dental community. J Calif Dent Assoc. 2000; 28:215-233.
33. Pallasch TJ. How to use antibiotics effectively. J Calif Dent Assoc. 1993;21:46-50.
34. Frencken JE . WHO Basic Package of Oral Care. 2002;1:1-17
35. Cobos-Trigueros N, Solé M, Castro P, Torres JL, Rinaudo M, De Lazzari E, et al. Evaluation of a Mixing versus a Cycling Strategy of

Antibiotic Use in Critically-Ill Medical Patients: Impact on Acquisition of Resistant Microorganisms and Clinical Outcomes. PLoS One. 2016;11:e0150274.

36. Mombelli A, Samaranayake LP. Topical and systemic antibiotics in the management of periodontal diseases. International Dental Journal. 2004; 54:3-14.

37. Ritter J, Lewis L, Mant T, Ferro A. A Textbook of Clinical Pharmacology and Therapeutics, 5 Edition. Hodder Arnold. 2012.

38. Casavant MJ. Goodman and Gilman's The Pharmacological Basis of Therapeutics. J Am Med Assoc. 2002.

39. Kayalvizhi G, Subramaniyan B, Suganya G. Topical application of antibiotics in primary teeth: an overview. J Dent Child (Chic). 2013;80:1-9.

40. Goswami S. Lesion sterilization and Tissue repair in pediatric dentistry. SRM J Res Dent Sci 2018;9:79-82.

41. Shreya S, Kanthaswamy C. Lesion sterilization and tissue repair-A review. Research Journal of Pharmacy and Technology. 2017;10:1539-1542.

42. Şimşek N, Özan U, Er K. Lesion sterilization and tissue repair. Cumhur Dent J. 2014; 31:898-900.

43. Vijayaraghavan R, Mathian V, Sundaram A, Karunakaran R, Vinodh S. Triple antibiotic paste in root canal therapy. J Pharm Bioallied Sci. 2012;4:230-233.

44. Turk T, Ozisik B, Aydin B. Time-dependent effectiveness of the intracanal medicaments used for pulp revascularization on the dislocation resistance of MTA. BMC Oral Health. 2015;15:130.

45. Saleh AA, Eid HA, Abdelaziz KM. Lesion Sterilization Tissue Repair as Adjunct to Conventional Root Canal Treatment of Combined Periodontic-Endodontic Cases. World J Dent. 2014;5:47-52.

46. Vivekananda Pai A, Pai S, Thomas M, Bhat V. Effect of calcium hydroxide and triple antibiotic paste as intracanal medicaments on the incidence of inter-appointment flare-up in diabetic patients: An *in vivo* study. J Conserv Dent. 2014;17:208-11.

47. Takushige T, Hataoka H, Ando M, Hoshino E. Endodontic Retreatment using 3Mix-MP without Removal of Previous Root Canal Obturation. J LSTR Ther. 2009;8:3-7.

48. Mohammadi Z, Jafarzadeh H, Kinoshita J. A review on triple antibiotic paste as a suitable material used in regenerative endodontics. Iran Endod J. 2018;13(1):1-6.