Comparison of the Antibacterial Effect of Modified 3-mix Paste versus Ultrapex over Anaerobic Microorganisms from Infected Root Canals of Primary Teeth: An *in vitro* Study

Velasco-Loera N * / De Alba-Vazquez Y ** / Garrocho-Rangel A *** / Gonzalez-Amaro AM **** / Flores-Reyes H ***** / Pozos-Guillen AJ ******

Objective: *The aim of this study was to evaluate in vitro the antimicrobial efficacy of a modified 3-mix paste and to compare it with an iodoform paste (Ultrapex) against anaerobic microorganisms isolated from root canals of infected or necrotic primary teeth.* ***Study design:*** *An in vitro experimental assay was performed over isolated and identified anaerobic microorganisms of 21 samples, in order to compare the antimicrobial ability of both root canal filling materials, using a disc-diffusion method. Results: A total of 21 microbial samples (15 polymicrobial and 6 monomicrobial) were obtained, from which 19 different strains were identified. Modified 3-mix paste showed an excellent antimicrobial effect against most of both kinds of microbial samples, although some of them exhibited resistance; on the other hand, Ultrapex showed only minimal antimicrobial ability (null or low categories). Clostridium ramosum exhibited the most resistance to both materials.* ***Conclusion:*** *The bactericidal effect of the modified 3-mix paste was superior to Ultrapex, with a statistically significant difference, against anaerobic microorganisms isolated from infected root canals of primary teeth.*

Keywords: *Modified 3-mix paste, Ultrapex, primary teeth, antimicrobial effect.*
J Clin Pediatr Dent 36(3): 239–244, 2012

INTRODUCTION

If deep carious lesions in the primary dentition are not treated effectively, there can be serious consequences for the physiological and social development of the child.[1] Therefore, one of the most challenging objectives of pediatric dentistry is the preservation of the integrity of the primary arches until they naturally exfoliated.[2] Therefore, it is essential that clinicians select and implement the appropriate treatment for deeply decayed primary teeth.[3] Although the main purpose of pulp treatment in pediatric dentistry is to preserve pulp vitality, often the infectious and inflammatory process is so advanced that it is not possible to carry out conservative pulp management. In these cases, the ideal treatment is pulpectomy. Pulpectomy in primary teeth involves removing the inflamed or infected pulp root tissue, biomechanical cleaning of the canal, and subsequent filling with a resorbable paste.[3,4] The therapeutic purpose of pulpectomy is to reduce or eliminate the microbial population, thus obtaining a clean and healthy pulp canal; this purpose is achieved through an appropriate bio-disinfection of the root canal system by means of mechanical instrumentation and profuse irrigation.[5]

Various materials have been used to fill primary root canals: zinc oxide–eugenol (ZOE; alone or in combination), pure calcium hydroxide, formaldehyde devitalizing pastes, Kri paste, and Guedes-Pinto paste, among others.[6,7] Currently, a material often used in pediatric endodontics is Vitapex or Ultrapex (a mixture of calcium hydroxide, iodoform, and silicon)[7]; its clinical and radiological success rate is very high and, in cases of extrusion beyond the root apex, the material is quickly resorbed.[8-10] In 1996 the lesion sterilization and tissue repair (LSTR) technique was introduced to

* Nelly Velasco-Loera, DDS, Resident, Pediatric Dentistry Posgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
** Yazmin De Alba-Vazquez, DDS, Resident, Pediatric Dentistry Posgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
*** Arturo Garrocho-Rangel, DDS, MS, Associate Professor, Pediatric Dentistry Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
**** Ana Ma. Gonzalez-Amaro, MS, Associate Professor, Endodontics Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
***** Hector Flores-Reyes, DDS, PhD, Associate Professor, Endodontics Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.
****** Amaury J. Pozos-Guillen, DDS, PhD, Associate Professor, Pediatric Dentistry Postgraduate Program and Endodontics Postgraduate Program, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, México.

Send all correspondence to: Amaury de Jesús Pozos Guillén, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava #2, Zona Universitaria, C.P.78290; San Luis Potosí, SLP. Mexico.

Tel: 52 (444) 8262357 X 114
Fax: 52 (444) 8139743

E-mail: apozos@uaslp.mx

disinfect primary dentinal, pulpal, and periapical lesions. It employs a 3-mix paste of antibacterial drugs: metronidazole, ciprofloxacin, and minocycline; the aim is to remove the bacterial constituents harbored in the root canals and deepest dentin layers.[10-13]

More than 700 bacterial species or phylotypes, normally present in the oral cavity and dental ecological niches, have been identified and isolated, but over 50% have not yet been cultivated.[14,15] Pulp canal infections are usually of polymicrobial nature, involving a variable number of bacterial species, mainly obligate anaerobes, facultative anaerobes, and aerobes.[16-18] According to Brook,[19] endodontic lesions in deciduous teeth are primarily or secondarily caused by infectious bacteria, and the bacteria most commonly identified in irreversibly inflamed pulps, pulp necrosis, and dentoalveolar abscesses are *Prevotella, Porphyromonas, Fusobacterium, and Peptostreptococcus spp*. Likewise, Ledezma *et al*[20] studied 21 primary teeth with at least one necrotic pulp canal, isolating and identifying predominantly anaerobic species (facultative and moderate anaerobes) in all their samples. It is well-established that bacteria are the primary cause of endodontic infectious processes and periradicular diseases; some are asymptomatic and others are associated with serious symptoms.[21,22]

In this context, the purpose of this study was to evaluate *in vitro* the antimicrobial effect of the 3-mix paste, modified by the authors, and to compare this effect with that of an iodoform paste (Ultrapex) against anaerobic bacteria isolated from root canals of infected primary teeth.

MATERIALS AND METHODS

An *in vitro* experimental assay was carried out in the postgraduate programs of Pediatric Dentistry and Endodontics, Facultad de Estomatología, San Luis Potosí University, Mexico. The study was reviewed and approved by the Institutional Ethics Committee. Two materials: 1. Modified 3-mix paste and 2. Ultrapex, were evaluated by sensitivity tests (standard agar diffusion method), using isolated strictly facultative anaerobic bacteria previously isolated from necrotic primary pulp canals.

Microbial specimens were collected from 21 primary molars with at least 1 necrotic canal, abscess, or sinus tract, following the standardized technique previously described.[20] Prereduced thioglycolate tubes supplemented with hemin (5 mg L^{-1}) and menadione (1 mg L^{-1}) were used as a transport medium. Each treated tooth was cleaned with pumice and isolated with a rubber dam. To disinfect the operative field, we followed the protocol previously described.[18] The carious tissue was removed with a sterile round bur cooled with sterile saline solution. A sterile cotton pellet was placed on the floor of the chamber to prevent penetration of disinfectants into the canals, and, with another sterile bur, the root canal was accessed. After estimating the canal length with the preoperative periapical radiograph, 3 sterile absorbent paper points of size compatible with root canal diameter, were sequentially placed for 30 seconds. The retrieved paper points were immediately placed into the tube with thioglycolate. In the laboratory, samples were immediately processed inside an anaerobic chamber (85% N_2, 10% H_2, 5%CO_2). Each sample was inoculated on a 5% sheep-blood CDC anaerobic agar plate with vitamin K and hemin, incubated at 37°C, and observed every 24 hours until growth was present. Stains isolated were Gram-stained and classified by colony morphology, oxygen tolerance, and biochemical test. All microorganisms were characterized using identification kit API 20A. API Lab software (Biomeriéux) was used to ascertain strain identification on the basis of the numeric code generated.

Development and modification of the 3-mix paste

In order to be manipulated properly and to add the radiopaque property, the paste's original formula[11] was modified, adding an inert material such as zinc oxide, but keeping the minimal inhibitory and bactericidal concentration of each constituent antibiotic of the 3-mix paste (metronidazole, ciprofloxacin, and minocycline)—these two concentrations were determined previously as 0.5 mg for each drug in a ratio (potency) 1:1:1. To create a paste with the proper thickness to impregnate the sensi-discs, the 3 drugs were mixed with 2 g of zinc oxide powder and 1 ml of polyethylene glycol as vehicles.

Manufacture of sensi-discs.

Ultrapex and 3-mix sensi-discs were prepared with filter paper cut into circular pieces (6 mm in diameter) and placed on concave containers, then each sensi-disc was impregnated with 0.5 mg of the respective paste and finally covered with another disc, like a sandwich. Both sensi-discs (experimental and control) were placed together on sterile glass plates.

Disc-diffusion method

This method was used in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA) under a controlled atmosphere (85% N_2, 10% H_2, and 5% CO_2). First, an inoculum was prepared extracting 100 µL from the tube with the means of transport, containing the previously collected bacteria from the necrotic pulp canals, and deposited on 8 mL of fresh culture medium (thioglycolate enriched with hemine and vitamin K). Then the inoculum was incubated for 48 hours in the chamber, thus obtaining a young culture (in the logarithmic phase of anaerobic growth) with a turbidity corresponding to McFarland's scale of 5, which was employed for the sensibility tests; 100 µL were taken from the former culture with an automatic pipette and uniformly distributed on 5% sheep-blood CDC anaerobic agar plates with hemine and vitamin K. The sensi-discs were placed on the agar surface 3 cm apart, and finally the plates were sealed with parafilm. The cultures were incubated at 35°C for 48 hours in the anaerobic chamber. The sensibility tests were performed in triplicate.

Measurement of inhibitory halos

After the incubation period, the diameters of the inhibi-

tion halos were measured in mm with a vernier, in triplicate. Each measurement was done by a precalibrated and independent observer and corroborated under a stereoscopic microscope. In addition, a classification of the inhibition halos' diameters was developed, based on a previous study,[23] consisting in 4 different-diameter categories (or ranks)—null: 0 mm, low: 0.1–13.4 mm, medium: 13.5–26.8 mm, and high: 26.9–45 mm. Finally, to identify potential resistant strains, Gram staining was carried out and an additional subculture was done for each of these strains.

Statistical analysis

Means and standard deviations were calculated for the measured diameters of the inhibition halos; because the sample did not show a normal distribution, a Wilcoxon signed rank, nonparametric test was performed. For the halos' diameter classification (null, low, medium, and high), frequencies in each category were obtained for each single microorganism and for each polymicrobial sample and analyzed using Fisher's exact test. The significance value of α for both tests was established at 0.05.

RESULTS

In all, 21 micobiological samples were obtained from necrotic primary pulp canals; 15 consisted of polymicrobial cultures and 6 were monomicrobial; 19 different anaerobic microorganisms could be isolated and identified. The most-frequently found microorganisms were *Bifidobacterium spp2* (23)*, Streptococcus intermedium* (6), *Actinomyces israelii* (4), and *Bifidobacterium 1* (4).

Means and standard deviations were calculated from the measurements in triplicate of the inhibition halos, for each identified microorganism (Table 1). Microorganisms exhibiting the largest mean diameters of inhibition halos when they were exposed to the 3-mix paste were: *Propionibacterium acnes* (43 mm ± 1.25), *Gemella morbillorum* (36 mm ± 0)*, Prevotella melaninogenica* (35.33 mm ± 1.15), and *Bacteroides ovatus thetaiotaomicron* (30.25 mm ± 1.75). For Ultrapex, the microorganisms showing the largest mean diameters were *P melaninogenica* (9.66 mm ± 0.57), *Actinomyces naeslundii* (4 mm ± 6.9), and *Bifidobacterium spp2* (3 mm ± 4.7) and *spp1* (2.6 mm ± 4.6). When the results of both study groups were statistically compared, it was determined that a significant difference existed between the means of the inhibition halos of the 2 materials investigated ($p < 0.05$) in favor of the modified 3-mix paste group, for all the single microorganisms except *Clostridium ramosum* and *Candida albicans*; both microorganisms evidenced resistance (no inhibition halo) against the 2 pastes.

Regarding the classification of the inhibition halo diameters in 4 different ranks, the Ultrapex group exhibited low inhibition against *P melaninogenica oralis, Bifidobacterium spp2, Bifidobacterium spp1, A naeslundii* and *S intermedium*; all other inhibition halos belonged to the null category, while there were no cases of this group found in the medium and high categories. In the modified 3-mix paste

Table 1. Inhibition halos in mm (diameter) of each isolated microorganism (data expressed in means, standard deviations, and ranks).

Identified microorganisms	ULTRAPEX			MODIFIED 3-MIX PASTE		
	N	Rank	Mean ± SD	Rank	Mean ± SD	*p*
Lactobacillus acidophylus	1	0	0 ± 0	24 – 25	24.6 ± 0.8	< .05
Prevotella melaninogenica oralis	1	9-10	9.6 ± 0.6	34 – 36	35.3 ± 1.1	< .05
Bifidobacterium spp2	20	0-13.8	3 ± 4.7	12.5 – 35	28.7 ± 6.7	< .05
Bifidobacterium spp1	4	0-14	2.6 ± 4.6	14 – 39	20.3 ± 4.2	< .05
Actinomyces naeslundii	2	0-12	4 ± 6.9	20 – 27	23.8 ± 4.1	< .05
Actinomyces israelii	4	0	0 ± 0	24 – 37	27.7 ± 6.9	< .05
Streptococcus intermedium	6	0-9	0.3 ± 2.3	16 – 39	27.2 ± 7.6	< .05
Propionibacterium propinicus	1	0	0 ± 0	28	28 ± 0	< .05
Propionibacterium acnes	1	0	0 ± 0	42.5 – 45	43.6 ± 1.2	< .05
Clostridium beijerinckii / butyricum	2	0	0 ± 0	29 – 35	30.8 ± 2.5	< .05
Clostridium spp	2	0	0 ± 0	19 – 30	24.7 ± 5.7	< .05
Clostridium tentium	1	0	0 ± 0	15 – 28	21.5 ± 6.7	< .05
Clostridium ramosum	1	0	0 ± 0	0	0 ± 0	1.0
Gemella morbillorum	1	0	0	36	36 ± 0	< .05
Collinsella aerofaciens	1	0	0	18.5 – 26	22.5 ± 3.7	< .05
Veillonella parvula	1	0	0	20	20 ± 0	< .05
Veillonella atipica	1	0	0	21	21 ± 0	< .05
Candida albicans	3	0	0	0	0 ± 0	1.0
Bacteroides ovatus theralotaomicron	1	0	0	28.5 – 32	30 ± 1.7	< .05

group only *C ramosum* and *C albicans* exhibited null inhibition halos, no cases showed low inhibition, and the remaining 16 were located in the medium and high categories. For the polymicrobial samples, the same classification was employed, and when frequencies were counted in each category, it was observed that in the Ultrapex group, 15 of these strains belonged to the null category and 6 to the low category; no cases were present in the medium or high categories. In the modified 3-mix paste, there were 2 strains located in the null category, 1 in the low category, 11 in the medium category, and 7 in the high category. Statistical comparison between the inhibition halos in both study groups showed a statistically significant difference in favor of the modified 3-mix paste ($p < 0.05$).

DISCUSSION

Pulpectomy in primary teeth is still considered a controversial procedure due to, among other things, the complex canal system characteristic of these teeth, specially in primary molars; however, there has been a growing tendency to conserve and maintain primary teeth as functional anatomic units in their dental arches until the time of their natural exfoliation; this treatment is therefore considered a special procedure in pediatric dentistry.[1,5,24]

Success in endodontic treatment depends largely on the eradication of bacteria through proper instrumentation and debridement of the pulp canals, irrigation with antiseptic solutions,[25-27] and employment of root canal filling materials having antibacterial properties.[1,28-30] Cox *et al*[2] studied the bactericidal and bacteriostatic effects of several endodontic compounds (ZOE and 8 other materials added to ZOE, Sargenti's N-2 paste, and a control); based on their results, they concluded that ZOE with zinc acetate inhibits gram positive and gram negative bacteria from growing, except *E coli, S aureus,* and *S viridans.* Furthermore, they suggest that addition of such highly cytotoxic chemicals as formocresol or paraformaldehyde is not necessary to ensure treatment success. Tchaou *et al*[24] compared the inhibitory effectiveness of 10 different filling materials based on chemical compounds like calcium hydroxide, ZOE, Kri paste, Vitapex, and Vaseline on 13 infected deciduous teeth, employing the agar diffusion technique. As in the present study, they measured the diameters of the inhibition halos obtaining means, standard deviations, and ranks; they concluded that Vitapex and calcium hydroxide mixed with water had minimal antibacterial effects; their best results were exhibited by calcium hydroxide with CPC, ZE + CPC and ZOE + formocresol. However, the authors did not clearly specify which microorganisms were employed, unlike our study in which identified anaerobic bacteria were tested. Pabla *et al*[28] evaluated the antimicrobial efficacy of ZOE, iodoform, Kri paste, Maisto paste, and Vitapex over aerobic and anaerobic bacteria (*S aureus, S viridans, S faecalis, B melaninogenicus* and polymicrobial samples isolated from nonvital deciduous teeth, using the agar diffusion technique to show that Maisto paste was the best material, followed by iodoform, ZOE, and Kri paste. Vitapex exhibited the lowest efficacy, as occurred in our research.

Among the various filling materials available for primary teeth at present, and for the purposes of this research, we decided to use Ultrapex, constituted mainly by calcium hydroxide and iodoform, as the control group due to its reported therapeutic advantages.[6,7] In reference to the antibacterial activity of iodoform pastes (Vitapex, Ultrapex), Nurko and García Godoy[8] have reported that to compensate for the incomplete pulp canal debridement caused by the complex anatomy, it is necessary to destroy the remnant microorganisms in the pulp canal and provide an environment unsuitable for bacterial growth; however, according to the results of the present study, Ultrapex did not exhibit any antimicrobial potential against the anaerobic bacteria isolated from the necrotic primary root canals. These findings are similar to those of Tchaou *et al,*[24,30] who reported that these pastes exhibited a minimal antibacterial effect, exerting activity only against *P buccae, P intermedia, P melaninogenica* and, although weakly, against *S morbillorum*. Their results coincide with ours only with respect to *P melaninogenica*.

Similarly, Reddy and Ramakrishna[23] studied the antimicrobial efficacy of several filling materials, evaluating their bactericidal ability through the halos of inhibition, and concluded that the tested iodoform paste (Metapex) was effective only over *S aureus* and *P micros*. In our study, we employed the same category classification, although our results were different: only *A naeslundii* and *Bifidobacterium* showed low susceptibility, and for the remaining microorganisms were null; 12 out of 15 polymicrobial samples studied were classified under the null category and 3 under low. Our investigation employed these polymicrobial samples since pulpal infection has been attributed to multiple types of microorganisms.[5,10,12,24]

Bacterial samples used in this study were collected from necrotic primary teeth and handled under strict anaerobic conditions in a special chamber, with controlled atmosphere and temperature, also taking into account those precautions necessary for avoiding contamination.[20] Similar studies[23,28,31] used different handling and control methods, such as Gen Bag and Gas Pak Systems. Likewise, we worked with bacteria extracted directly from pulp canals, unlike other researchers who employed preserved or reference microorganisms, as did Cox *et al*[2] and Barkhordar *et al.*[31] On the other hand, Tchaou *et al*[24,30] first extracted the primary teeth, stored them in the chamber, then collected the bacterial content from their apices. It is important to collect microorganisms directly from the root canals so as to reproduce the actual conditions in which anaerobic bacteria grow, thus obtaining conditions closer to those existing in the ecological sites inside the necrotic teeth. Brook[19] concluded that some microbiological studies have been carried out on microbiota from necrotic primary teeth; however, the quality can vary because the anaerobic techniques employed are not always optimal.

The agar diffusion method used in this study is one of the most often used methods for antimicrobial activity assessment. This method has been used in endodontics for evaluate antibacterial effect of sealers, pastes and root canal irrigating solutions. Also, facultative anaerobic and aerobic microorganism have been used to assess different materials with antibacterial activity using this method.[32-40]

An alternative to the before-mentioned materials for primary pulp canal treatment is the LSTR technique. This method is based on the principle of bacterial reduction or removal through the employment of a mixture of 3 antibacterial agents, ciprofloxacine, metronidazole, and minocycline, which are especially indicated for sterilizing infected primary root canals; therefore, repair of the damaged tissues can be expected if those lesions are disinfected. The mixture has been tested, obtaining high clinical success rates (>90%) on infected primary root canals having periradicular lesions, with or without physiological root resorption. The authors placed the drug mixture directly on the previously enlarged canal orifice, without mechanical canal preparation, and with a mean follow-up period of 680 days.[11] Sato *et al*, Hoshino *et al,* and Takushige *et al* consider the bactericidal properties of the mixture as the main factor in achieving disinfection of the dentin, canal, and alveolar bone lesions, even in the case of large periradicular infections.[11-13]

There are a few reports about the antibacterial effect of the 3-mix paste against anaerobic microorganisms specifically isolated from primary canals. Sato *et al*[11] measured the antibacterial effect of the paste only against *E coli*, which was deposited into small cavities prepared parallel to the root canal walls in 9 teeth; no bacteria were recovered 48 hours after application of the drug mixture. It is important to note that, in our work, such microorganisms were not identified in the samples obtained from the selected necrotic teeth. Another study by Hoshino *et al*[12] showed the antimicrobial effect of the 3-mix paste (with and without the addition of rifampicin); they isolated pathogenic microorganisms from the infected root canal's dentin walls, carious dentin, and infected pulps, and evaluated in vitro the bactericidal efficacy by measuring bacterial recovery, a different method than the one employed in the present work. Takushige *et al*[13] assessed the clinical efficacy of the LSTR technique as an endodontic treatment in primary teeth without making a microbiological analysis, obtaining highly successful results. They report the 3-mix paste in a ratio (potency) of 1:3:3 prepared on the two ways: using a mixure of macrogol and propylene glycol and using a canal sealer. In the present study we modified the mixure using a 3-mix paste in a ratio of 1:1:1 with propylene glycol and zinc oxide as vehicles. We carried out tests on both polymicrobial and monomicrobial cultures, observing that, according to the Reddy and Ramakrishna classification,[23] 12 out of 15 polymicrobial samples showed an inhibition halo in the presence of the modified 3-mix paste in the medium and high categories; the other 3 showed no inhibition halo (null category). When tested on single microorganisms, it was found that *P acnes*, *G morbillorum, P melaninogenica,* and *B ovatus theralotaomicron* exhibited the largest inhibition zones (high category); on the other hand, *C albicans* and *C ramosum* showed no bactericidal activity. Some bacteria, such as *Bifidobacterium spp*, *Bifidobacterium spp 1, S intermedius, A viscosus,* and *Clostridium spp,* exhibited heteroresistance.

An interesting finding in our research was the detection and isolation of *C albicans* from the necrotic primary root canals, which was previously reported only by Tchaou *et al,*[30] who also mentioned that Vitapex did not show any antibacterial effect against this fungus. In our study, *C albicans* did not exhibit, as expected, an inhibition halo when it was tested with either paste, coinciding with the findings previously reported on the minocycline effects over the fungus.[41] However, more studies are required to confirm the presence of *C albicans* in necrotic primary root canals and justify the addition of an antifungal agent to the modified 3-mix paste.

In this study, the microorganism most commonly isolated was *Bifidobacterium spp2*; furthermore, this bacterium exhibited resistance to bactericidal activity when both studied materials were tested. Nevertheless, *Bifidobacterium spp2* has not been previously identified and evaluated in other antimicrobial efficacy studies of canal filling materials on primary teeth.[1,6,7,19,31,41,42]

Although more clinical controlled studies are necessary to confirm the efficacy of the modified 3-mix paste, the results exhibited both *in vitro* and *in vivo* by this antibiotic mixture are highly promising as a feasible alternative in the pulp treatment of irreversibly infected or necrotic primary teeth.

CONCLUSION

The bactericidal effect of modified 3-mix paste was superior to Ultrapex, with a significant statistical difference, against anaerobic microorganisms isolated from infected root canals of primary teeth.

ACKNOWLEDGMENTS

This work was supported partially by PIFI 2010-11, C12-FAI-03-45.45, CONACYT 103311, 162651 grants. We would like to thank Norman Wahl, for his assistance in editing this manuscript.

REFERENCES

1. Mandel ID. Oral infections: Impact on human health, well-being, and health-care costs. Compend Contin Educ Dent, 25: 881–90, 2004.
2. Cox ST, Hembre JH, McKnight JP. The bactericidal potential of various endodontic materials for primary teeth. Oral Surg Oral Med Oral Pathol, 45: 947–54, 1978.
3. Rodd HD, Waterhouse PJ, Fuks AB, Fayle SA, Moffat MA. UK National Clinical Guidelines in Paediatric Dentistry. Int J Paediatr Dent, 16 (Suppl 1): 15–23, 2006.
4. Fuks AB. Pulp therapy for the primary and young permanent dentition. Dent Clin North Am, 44: 571–96, 2000.
5. Tronstad L. Recent development in endodontic research. Scan J Dent Res, 100: 52–9, 1992.
6. Kubota K, Golden BE, Penugonda B. Root canal filling materials for primary teeth: A review of the literature. J Dent Child, 69: 93–7, 1992.

7. Silva LA, Leonardo MR, Oliveira DS, Silva RA, Queiroz AM, Hernández PG, Nelson-Filho P. Histopathological evaluation of root canal filling materials for primary teeth. Braz Dent J, 21: 38–45, 2010.
8. Nurko C, Garcia-Godoy N. Evaluation of a calcium hydroxide/iodoform paste (Vitapex®) in root canal therapy for primary teeth. J Clin Pediatr Dent, 3: 289–93, 1999.
9. Mortazavi M, Mesbahi M. Comparison of zinc oxide and eugenol and Vitapex for root canal treatment of necrotic primary teeth. Int J Paediatr Dent, 14: 417–24, 2004.
10. Trairatvorakul C, Chunlasikaiwan S. Success of pulpectomy with zinc oxide-eugenol vs calcium hydroxide/iodoform paste in primary molars: A clinical study. Pediatr Dent, 30: 303–8, 2008.
11. Sato I, Kurihara-Ando 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, 29: 118–24, 1996.
12. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, Iwaku M. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J, 29: 125–30, 1996.
13. Takushige T, Cruz EV, Asgor A, Hoshino E. Endodontic treatment of primary teeth using a combination of antibacterial drugs. Int Endod J, 37: 132–8, 2004.
14. Dahlen G. Microbiology and treatment of dental abscesses and periodontal-endodontic lesions. Periodontol 2000, 28: 206–39, 2002.
15. Aas JA, Paster BJ, Stokes LN. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol, 43: 5721–32, 2005.
16. Tomie-Karovie K, Jelinek E. Comparative study of the bacterial flora in the surroundings, the root canals and sockets of deciduous molars. Int Dent J, 21: 375–88, 1971.
17. Edwards S, Nord CE. Identification and characterization of microorganisms isolated from infected primary teeth. J Int Assoc Dent Child, 3: 15–8, 1972.
18. Manzur A, Gonzalez AM, Pozos AJ, Siva-Herzog D. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: A randomized clinical trial. J Endod, 33: 114–8, 2007.
19. Brook I. Microbiology and management of endodontic infections in children. J Clin Pediatr Dent, 28: 18–25, 2003.
20. Ledezma-Rasillo G, Flores-Reyes H, Gonzalez-Amaro AM, Garrocho-Rangel A, Ruiz-Rodríguez MS, Pozos-Guillen AJ. Identification of cultivable microorganisms from primary teeth with necrotic pulps. J Clin Pediatr Dent, 34: 329–34, 2010.
21. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol, 20: 340–9, 1965.
22. Siqueira JF Jr. Taxonomic changes of bacteria associated with endodontic infections. J Endod, 29: 619–23, 2003.
23. Reddy S, Ramakrishna Y. Evaluation of antimicrobial efficacy of various root canal filling materials used in primary teeth: A microbiological study. J Clin Pediatr Dent, 31: 193–9, 2007.
24. Tchaou WS, Turng BF, Minah G, Coll JA. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials. Pediatr Dent, 17: 351–5, 1995.
25. Onçağ O, Hoşgör M, Hilmioğlu S, Zekioğlu O, Eronat C, Burhanoğlu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J 36: 423–32, 2003.
26. Zehnder M. Root canal irrigants. J Endod 32: 389–98, 2006.
27. Ruiz-Esparza CL, Garrocho-Rangel A, Gonzalez-Amaro AM, Flores-Reyes H, Pozos-Guillen AJ. Reduction in bacterial loading using 2% chlorhexidine gluconate as an irrigant in pulpectomized deciduous teeth: A preliminary report. J Clin Pediatr Dent 35: 265–70, 2011.
28. Pabla T, Gulati MS, Mohan U. Evaluation of antimicrobial efficacy of various root canal filling materials for primary teeth. J Indian Soc Pedod Prev Dent, 15: 134–40, 1997.
29. Önçaġ Ö, Gogulu D, Uzel A. Efficacy of various intracanal medicaments against *Enterococcus faecalis* in primary teeth: An *in vivo* study. J Clin Pediatr Dent, 30: 233–6, 2006.
30. Tchaou WS, Turng BF, Minah G, Coll JA. Inhibition of pure cultures of oral bacteria by root canal filling materials. Pediatr Dent, 18: 444–9, 1996.
31. Barkhordar R. Evaluation of antimicrobial activity in vitro of ten root canal sealers on *Streptococcus sanguis* and *Streptococcus mutans*. Oral Surg Oral Med Oral Pathol, 68: 770–2, 1989.
32. Badr AE, Omar N, Badria FA. A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. Int Endod J, 44: 51–58, 2011.
33. Ribeiro CS, Scelza MF, Hirata Júnior R, Buarque de Oliveira LM. The antimicrobial activity of gray-colored mineral trioxide aggregate (GMTA) and white-colored MTA (WMTA) under aerobic and anaerobic conditions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 109: e109–12, 2010.
34. Sainulabdeen S, Neelakantan P, Ramesh S, Subbarao CV. Antibacterial activity of triclosan incorporated glass ionomer cements—an in vitro pilot study. J Clin Pediatr Dent, 35: 157–61, 2010.
35. Hollanda AC, Estrela CR, Decurcio Dde A, Silva JA, Estrela C. Sealing ability of three commercial resin-based endodontic sealers. Gen Dent, 57: 368–73, 2009.
36. Lin YH, Mickel AK, Chogle S. Effectiveness of selected materials against *Enterococcus faecalis*: part 3. The antibacterial effect of calcium hydroxide and chlorhexidine on *Enterococcus faecalis*. J Endod, 29: 565–66, 2003.
37. Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifácio KC, Ito IY. In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. J Endod, 26: 391–4, 2000.
38. Siqueira JF Jr, de Uzeda M. Intracanal medicaments: evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. J Endod, 23: 167–9, 1997.
39. Barbosa CA, Gonçalves RB, Siqueira JF Jr, De Uzeda M. Evaluation of the antibacterial activities of calcium hydroxide, chlorhexidine, and camphorated paramonochlorophenol as intracanal medicament. A clinical and laboratory study. J Endod, 23: 297–300, 1997.
40. Tobias RS. Antibacterial properties of dental restorative materials: a review. Int Endod J, 21: 155–60, 1988.
41. Siqueira JF Jr, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 97: 632–41, 2004.
42. Grossman LI. Antimicrobial effect of root canal cements. J Endod, 6: 594–7, 1980.