Early Response of Mechanically Exposed Dental Pulps of Swine to Antibacterial-Hemostatic Agents or Diode Laser Irradiation

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Objectives: *The purpose of this study was to compare the effectiveness of an antibacterial and hemostatic agent to diode laser irradiation in the healing of mechanically exposed porcine pulps.* ***Materials and Method:*** *The experiment required three adult swine (Sus scrofa domestica, Yorkshire) with 36 teeth prepared with occlusal penetrations into the pulpal tissues. The preparations were performed under general anesthesia and the pulps were exposed using high speed instrumentation with rubber dam isolation and a disinfected field. Following instrumentation the coronal pulpal tissue was amputated and immediately treated with ferric sulfate and chlorhexidine semi-gel (12), diluted Buckley' formocresol solution (12) for 5 minutes or laser irradiation with a diode laser (12). After treatment, hemostasis was obtained and a ZOE base applied to the treated pulps (36). The pulpal bases were all covered with a RMGI (Fuji II LC). The tissue samples were collected at 4 weeks (28 days). Following fixation, the samples were de-mineralized, sectioned, stained and histologically graded with a scale of 0-4.* ***Results:*** *The treatment groups were statistically different with the Laser Treated Group demonstrating the least inflammation.* ***Conclusion:*** *Pulpotomy treatment with the KaVo Gentle Ray Diode Laser demonstrated significantly less inflammation than the other two pulpal therapy modalities. The ferric sulfate and chlorhexidine mixture demonstrated the greatest inflammation as histologically graded. Also, the histological sections of pulpotomized swine teeth treated with the ferric sulfate and chlorhexidine mixture presented with black pigmented areas in the pulp and surrounding tissue. The formocresol group (clinical standard) and the diode laser group did not present with the black precipitate.*

Keywords: *pulp exposures, pulp response, bacteria, swine*
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INTRODUCTION

For many decades, pulpotomy treatment of primary teeth was based on the theory of devitalizing the remnant pulpal tissue, mainly utilizing formocresol for fixation of the pulpal stumps. However, the application of this chemical does not produce true fixation of the pulp, but only creates a state of chronic inflammation.[1] Because of this property, it cannot be considered a biological medication as it does not promote pulpal tissue healing. In addition, it is toxic, mutagenic, and considered carcinogenic, with great capability of diffusion, being able to reach the periapical tissues[2] and the adjacent permanent tooth follicle.[3] The ideal material for pulp treatment would be bactericidal, non injurious to the pulp or adjacent oral structure and would promote regeneration of the pulp tissue without interfering with the physiological process of root resorption. New substances, considered being more biocompatible, such as dehydrated bone, collagen, morpho-genetic proteins and allogenic dentin matrix have been proposed, as well as non-pharmacological techniques for the treatment of the exposed pulp, including laser and electro-surgery. However, the results confirming the long term success of these materials and techniques are not yet available, and thus an ideal agent for pulp therapy in primary teeth has not yet been identified.[4–6]

Ferric sulfate, a hemostatic agent, has also been proposed for the vital pulpotomy technique.[7] No difference was found between the ferric sulfate and diluted formocresol groups in regards to inflammation, abscess formation, or inflammatory root resorption. Questions remained as additional studies demonstrated the presence of inflammation and other

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adverse histological reactions in what were deemed clinically successful responses.[8] On the other hand, long term follow up studies have demonstrated that there are no significant differences between the clinical and radiographic success rate of ferric sulfate or formocresol treated primary molars.[9] Loh *et al* [10] evidence-based assessment concluded that, in carious primary molars with reversible coronal pulpitis, pulpotomies with either ferric sulfate or formocresol would have indistinguishable clinical and radiographic success rates. In addition, Deery concluded from the clinical data that ferric sulfate was significantly more successful than formocresol.[11] Radiographic data indicated no significant difference between the two medicaments. However, there are no studies claiming any anti-microbial action for ferric sulfate.

Chlorhexidine has been widely used as a cavity and pulp canal disinfectant. Chlorhexidine has been demonstrated to be effective as a pulpal medicament or canal irrigant.[12–14] Both 2% chlorhexidine gluconate and 5.25% sodium hypochlorite have been shown to be effective in *in vivo* studies of infected teeth.[15–17] The addition of chlorhexidine to ferric sulfate would therefore appear to be an appropriate procedure. The mixture of the two pulpal medicaments was tested for antibacterial effectiveness utilizing the Kirby Bauer Disk diffusion method. The anti-bacterial effectiveness was similar to diluted Buckley's formocresol.[18]

Laser irradiation has been utilized for pulpotomy therapy for the last decade. Wilkerson *et al* [19] studied the use of the HGM PC argon laser for pulpotomy treatment of swine teeth. Three young pigs had 42 pulpotomies performed on primary teeth and the data collected at either 7 or 60 days. The data collection consisted of clinical impression, radiographic interpretations and the examination for reparative dentin formation. Huth *et al*[20] compared four different pulpotomy techniques in 107 children that were randomly assigned into treatment groups. Only the calcium hydroxide treatment group was not as significantly successful as the other groups. Odabas *et al* [21] utilized a Nd: YAG laser and the formocresol technique to perform pulpotomies and there were no statistically significant clinical and radiographic differences in the success rate between the treatment groups. Pulpotomy treatment of primary molars with lasers has been successful and recommended as an alternative to formocresol application.[22,23] Laser irradiation has been demonstrated in multiple studies to decrease the bacterial population both on implant surfaces and within root canal systems.[24–27] Noetzel *et al* demonstrated the efficacy of laser irradiation against a bacterial population however; it was less effective compared to both calcium hydroxide and gaseous ozone.[28] Additionally, Kuvvetli *et al* reported on the comparison of the Er: YAG laser to sodium hypochlorite with the NaOCl irrigation being significantly better, although the laser did achieve a significant reduction of bacteria compared to the control group.[29] Meire *et al* also demonstrated less effectiveness of Photo Activated Disinfection with either the Nd: YAG or KTP systems when compared to sodium hypochlorite.[30] On the other hand, a study by Gordon *et al* found a 99.7% reduction in bacteria with the use of a radial emitting tip and a two minute exposure.[27]

It is clear that further research is required to establish which of the existing pulp capping agents are most biocompatible and most effective at maintaining viability, with minimal inflammation, and the greatest likelihood of promoting pulpal repair and reparative dentin formation. This will a require a methodical approach wherein individual agents and procedures are compared in well-controlled studies designed to provide histological confirmation of the pulpal response patterns at early and late time periods after placement of the materials to be tested. Such studies will also serve as a base line against which to evaluate future agents and procedures. As a further step toward this long term goal, the current study was undertaken to compare the early pulpal response of swine molars within four weeks of pulp capping with a hemostatic and antibacterial agent compared to a formocresol control and also to laser diode treatment.

MATERIALS AND METHOD

The experiment required three swine selected from the Center for Advanced Surgical Education population at Northwestern University Medical School Research facility. The research project was presented to and approved by the Institutional Animal Research Committee of Northwestern University. Throughout the research project the animals were cared for according to International Standards for Animal Care. All pulpotomy procedures were performed in the animal Operating Rooms at the Research Facility. This was designed to be a "no-loss" study with the animals scheduled for additional surgical procedures at the Northwestern University Center for Advanced Surgical Education. The N-CASE procedures did not overlap with this study nor influenced this study results.

The swine pre-molars and molars were chosen for the study due to their anatomical similarity to human molars. In addition, the swine have four premolars in each quadrant, allowing for a larger sample size of similar teeth. On the other hand, the swine primary molars were not considered as research candidates as they are often exfoliated early or provide less than reproducible results. The preparations were performed under general anesthesia administered by an experienced animal anesthetist and with as sterile conditions as practical. The teeth were isolated with rubber dam, pumice prophylaxis performed and the operative area disinfected with providone-iodine. High speed handpieces (Dentex MS350) were utilized to create the preparations. The handpieces were autoclaved prior to each use. A new sterile #330 bur was used to make the preparation with sterile saline as the coolant. Cavity preparations bordered by enamel but extending onto the full occlusal surface of the teeth were created and a pulpotomy of the coronal tissue was accomplished from the center of the cavity with sterile, new #2 burs and spoon excavators. After the preparations were performed, the pulp chamber was rinsed with sterile saline to remove operative debris. The pulpal stumps were treated with either ferric sulfate with chlorhexidine semi-gel (experimental), irradiated with a diode laser (experimental) or diluted formocresol (Buckley's 1:5 dilution) applied to the pulp for five minutes (control group).

The ferric sulfate (Viscostat, Ultradent, Salt Lake City, Utah, USA) with chlorhexidine solution was applied for approximately two minutes and hemostasis was obtained. The experimental ferric sulfate and chlorhexidine mixture had been previously tested for its antimicrobial activity by use of a modified Kirby Bauer test. The concentration of the mixture was 1.2% chlorhexidine and 20% ferric sulfate mixed at equal volumes of each suspension, that is, 10 ml of the 20% ferric sulfate semi-gel had 10 ml of the 1.2% chlorhexidine solution added (50% mixture by volume). The KaVo GentleRay 980 diode laser was utilized at the factory pre-set values (pulsed) at 3.0 watt with a 300 micron fiber at 100 ms intermittent pulses. The exposure of the pulpal stumps was approximately two minutes as that was all the time necessary to achieve hemostasis. This exposure time would also reportedly (by manufacturer) be sufficient for sterilization of the pulpal chamber. The control group utilized Buckley's formocresol with a 1:5 dilution applied for 5 minutes on cotton pellets as a medicament due to its wide spread usage. A mixture of zinc oxide eugenol was applied to all the treated pulps after obtaining hemostasis. The cavity preparations were then conditioned and sealed with resin modified glass ionomer restorative cement (Fuji II LC).

Exposure of the pulpal cavity to the oral environment to encourage contamination of the pulpal tissues as was previously performed would not be allowed by the International Standards of Animal Care (Appendix D: International Guiding Principles for Biomedical Research Involving Animals 1985). This is due to the pain the experimental animal would suffer if a pulp is left exposed. The animals were fed a dental soft diet and the Veterinarian staff of the Center for Comparative Medicine regularly examined the swine for any post surgical complications.

The animals were cared for according to International Standards for Animal Care. The swine were observed for any changes in eating habits or signs of inflammation or suppuration in the oral tissues. The swine were to be medicated with analgesics if determined necessary by the care givers.

Table 1. Three Treatment Groups–each consisting of 12 teeth, swine premolars and molars

Swine 1
- UR-Ferric Sulfate Chlorhexidine ZOE
- UL- Laser diode ZOE
- LR- Laser Diode
- LL-Formocresol ZOE

Swine 2
- UR- Laser diode ZOE
- UL- Ferric Sulfate Chlorhexidine ZOE
- LR-Formocresol ZOE
- LL –Formocresol ZOE

Swine 3
- UR-Ferric Sulfate Chlorhexidine
- UL-Formocresol ZOE
- LR- Laser diode ZOE
- LL- Ferric Sulfate Chlorhexidine ZOE

The swine's' behavior was closely monitored and recorded. There were no behavioral changes noted in any of the animals. The swine were scheduled for procedures at Northwestern University Center for Advance Surgical Education after an experimental period of 28 days. Previous studies have reported that 28 days is a sufficient healing period to histologically discern if a technique for pulpal therapy is acceptable.[31-32]

The tissue samples were collected following surgical procedures performed at the Northwestern University Center for Advanced Surgical Education as this was a "no-loss" Center for Comparative Medicine cooperative study. After the N-CASE procedures were performed the extraction of the treated molars was accomplished and the swine were given an overdose of general anesthesia. The extracted molars were placed in 10% formalin for 48 hours. Decalcification of the extracted molars was done by placing the samples into a Hydrochloric Acid/EDTA solution (Prolocal, Sigma Scientific, Kalamazoo, MI). After dehydration, the samples were embedded in paraffin for sectioning. The tissues were cut into serial sections of 6 µm with a Leica BM 2025 microtome. The sections were then stained using Hematoxylin and Eosin.

A representative section of each sample, demonstrating a maximal cross-sectional area of the pulp, was selected for histological analysis. The slides were coded and evaluated at both 63X magnification and at 160X magnification by two examiners who were unaware of the treatment history of the samples. The samples were identifiable by number only. The microscopic evaluation focused on the extent of inflammation (hyperemia, and the density and distribution of inflammatory cellular infiltrate, as well as the specific cellular constituents) and necrosis (frank necrosis and presence or absence of odontoblasts). The extent of reparative dentin formation and calcified bridge formation in each sample was also noted. The degree of inflammation appreciated in each sample, was graded and documented using a 4-point scale as shown in Table 2.

RESULTS

Table 2. Ranking for Inflammation and Bridge Formation

Inflammation-
- 0- none or a few inflammatory cells present
- 1- slight amount of inflammation
- 2- moderate inflammation
- 3- severe inflammation, micro-abscesses
- 4- necrosis or abscess formation

Representative sections were reviewed by two examiners who ranked the inflammation as outlined previously in the Materials and Methods. The rankings were tabulated with the following mean ranks; Laser Group- 0.9, Formo Group- 1.6 and Ferric Group- 2.0. The Laser Group as a whole demonstrated slight inflammation, the formocresol group slight to moderate inflammation and the ferric sulfate group moderate inflammation. The data was submitted to the Kruskall-Wallis Test (non-parametric, independent) for statistical analysis.

The treatment groups were statistically different with the

Table 3. Rank Sums and Mean Ranks for Treatment Groups: Non-parametric Independent – Kruskall-Wallis Test p= 0.0072

Group	N	Rank Sum	Mean Rank
Laser	**12**	**136.5**	**11.38**
Formo	**12**	**235.0**	**19.58**
Ferric	**12**	**294.5**	**24.54**

Laser Treated Group demonstrating the least inflammation (p=0.0072). An example of the Laser Treated Group would be Figure 1. The Formocresol Group demonstrated less inflammation than the Ferric Sulfate Group but more inflammation than the Laser Treated Group. An example of the Formocresol Group is Figure 2. The Ferric Sulfate Group not only demonstrated more inflammation and a greater association with necrosis than the other Groups it also showed areas of black deposits in the radicular pulpal tissues as demonstrated in Figure 3.

In addition, a one way ANOVA of the data demonstrated a statistically significant difference between the groups, p=.0059 with DF=2. The data is summarized in Table 4.

DISCUSSION

Table 4. 1-way ANOVA of the Groups Laser, Form and Ferric. Statistical Analysis by Analyze-it software.

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Groups	n	Mean	SE	Pooled SE	SD
Laser	12	1.75	0.265	0.284	0.92
Formo	12	2.63	0.338	0.284	1.17
Ferric	12	3.13	0.239	0.284	0.83

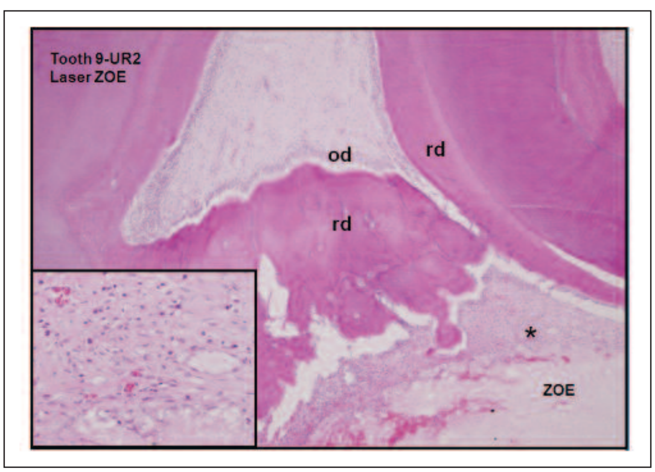

Figure 1. This section is of a sample treated with the diode laser and zinc oxide eugenol. The pulpal tissue demonstrates a mild diffuse chronic inflammatory cellular infiltrate in the region of the asterisk (higher power view shown in insert at lower left), and a dentinal bridge at this level of sectioning.

Figure 2. Section of porcine pulp tissue treated with diluted Buckley's formocresol and zinc oxide eugenol. The pulpal tissue is labeled as "p," dentin as "d," odontoblasts as "od" and reparative dentin as "rd." This sample demonstrated very mild inflammation.

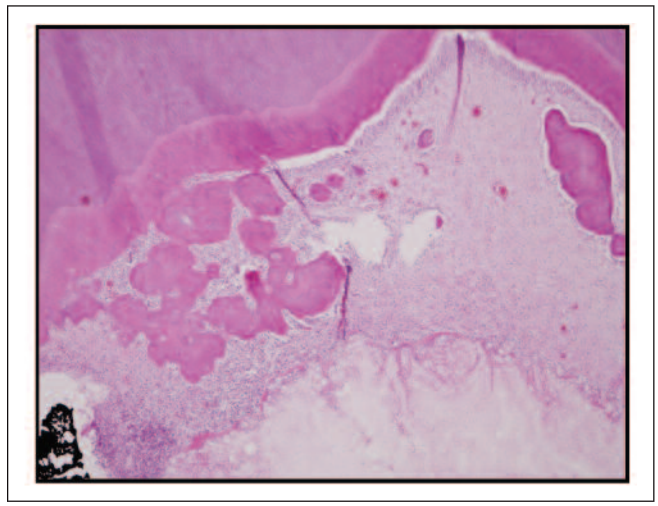

Figure 3. This sample treated with ferric sulfate and chlorhexidine solution demonstrates moderate to severe inflammation. A micro-abscess is present in close proximity to black granules which may represent a ferric related precipitate (lower left).

Pulpotomy therapy is one of the most important treatment techniques necessary to maintain and preserve the dentition. For example, pulpotomy therapy may be used in young permanent teeth either in case of traumatic exposure of the pulpal tissue or when an immature tooth with an un-developed root structure has a vital pulp but with a carious exposure.[33] Therefore, research continues to discern new and better medicaments suitable for pulpal therapy. The mixture of 20% ferric sulfate and 1.2% chlorhexidine did not perform as well in pulpal application as was hypothesized although the experimental mixture did provide acceptable hemostasis within a clinically acceptable time interval. It is very possible that the 1.2% chlorhexidine was not concentrated enough, especially after dilution with the ferric sulfate, as endodontic medicaments typically have 2.0% concentration.[13–15] Perhaps there is an un-expected chemical reaction between the ferric sulfate and the chlorhexidine. It is possible that another anti-microbial medicament, such as, benzyl ammonium chloride would be an acceptable additive to ferric sulfate to provide anti-bacterial effectiveness and maintain the hemostatic feature.

Perhaps the combination of the two components created the poor result. It may be that if the 20% ferric sulfate had been applied for hemostasis, and then the 1.2% chlorhexidine utilized to remove clot debris plus disinfection, a more acceptable histological result would have been produced. Gentle irrigation to remove coagulation debris may be a necessary step for the ferric sulfate pulpotomy technique that has yet to be emphasized to the clinician. Although the mixture had been determined to be anti-microbial, this obviously does not necessarily imply any direct improvement in healing or reduction in inflammation resulting from the surgical amputation, the pulpotomy procedure itself.

Laser treatment of pulpal tissue is rapidly becoming commonplace as the diode laser units are now extremely economical and found in many dental offices. The diode laser has been advocated for treatment of anything from apthous ulcers to periodontal disease. It is now utilized by dental hygienists rather routinely and for soft tissue surgery by dentists, both specialists and general dentists. All of the diode laser units have a pulpotomy setting, but the rationale for these settings is unclear. The setting advocated for the KaVo Gentle Ray was apparently sufficiently correct to allow for successful pulpal treatment. Ideally, animal and clinical studies should be performed to determine the exact settings for achieving the least amount of pulpal damage as determined by histological evaluation. The authors were surprised that the laser group histological results were apparently superior to the other techniques studied. Large clinical studies should be undertaken prior to wide spread adoption of the laser diode for pulpotomy therapy. In addition, long term studies will need to demonstrate that the laser diode pulpotomized teeth maintain viability and present without any unforeseen future complications.

The observance of microscopic black “ferric” deposits raises the question of their potential biological significance. These deposits may or may not contribute to pulpotomy failure or success but the end result of less than acceptable healing would more or less indicate that the deposits do not contribute to healing but may be the result of increased inflammation or result in increased inflammation. Further studies are definitely indicated to determine the nature of the deposits, and their contribution, if any, to inflammation of the treated pulps.

The moderate inflammation seen with the formocresol group is consistent with many previous studies. Although this technique of de-vitalizing pulpal tissue with Buckley’s formocresol is the most commonplace method for pulpotomy therapy, many question its overall safety.[1–3] Studies have long reported that the pulpal tissue was moderately inflamed by the caustic nature of the medicament.[6] Perhaps now, dental diode laser application will supplant the long revered and time honored formocresol pulpotomy for treatment of cariously exposed primary molars.

As with all treatment modalities, the pulpotomy technique will progress through an un-ending state of evolution, with slow and inevitable improvement. Animal studies with more biologic medicaments, and pulp capping materials may provide significant advancements, provided that the results are replicated in large, well designed and controlled, clinical studies.

CONCLUSION

Pulpotomy treatment with the KaVo Gentle Ray Diode Laser demonstrated significantly less inflammation than the other two pulpal therapy modalities. The histological sections of pulpotomized swine molars presented with moderate inflammation and black pigmented areas in the radicular pulp possibly a precipitate from the ferric sulfate and chlorhexidine mixture. Further research is necessary, including large clinical trials, to determine if the diode dental laser application will replace the formocresol pulpotomy technique for the treatment of cariously exposed primary molars.

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REFERENCES

1. Russo MC, Holland R, Okamoto T, de Mell W. In vivo fixative effect of formocresol on pulpotomized deciduous teeth of dogs. Oral Surg, 58: 706–14, 1984.
2. Pashley EL, Myers DR, Pashley DH, Whitford GM, Systemic distribution of 14C-formaldehyde from formocresol-treated pulpotomy sites. J Dent Res, Mar, 59(3): 602–8, 1980.
3. Waterhouse, P.J. Formocresol and alternative primary molar pulpotomy medicaments: a review. Endod Dent Traumatol 11: 157–62, 1995.
4. Fuks, A. Pulp therapy for the primary and young permanent dentitions. Dent Clin North Am, 44: n.5, 2000.
5. Ranly, DM. Pulpotomy therapy in primary teeth: new modalities for old relationales. Pediatr Dent, 16: 403–9, 1994.
6. Waterhouse, P.J. Nunn JH, Whitworth JM, Soames JV. Primary molar pulp therapy-histological evaluation of failure. Int J Paediatr Dent, 10, 313–321, 2000.
7. Ibricevic H, al-Jame Q. Ferric sulfate as pulpotomy agent in primary molars: twenty month clinical follow-up. J Clin Pediatr Dent Summer; 24(4): 269–72, 2000.
8. Peng l, Ye l, Guo X, Zhou X, Wang C and Li R. Evaluation of formocresol versus ferric sulpahte primary molar pulpotomy: a systematic review and meta-analysis. Int Endod J, Oct, 40(10): 751–7, 2007.
9. Ibricevic H, al-Jame Q. Ferric sulphate and formocresol in pulpotomy of primary molars: long term follow-up study. Eur J Paediatr Dent, Mar, 4(1): 28–32, 2003.
10. Loh A, O’Hoy P, Tran X, Charles R, Hughes A, Kubo K, Messer LB. Evidence-based assessment: evaluation of the formocresol versus ferric sulfate primary molar pulpotomy. Pediatr Dent, Sep-Oct, 26(5): 401–9, 2004.
11. Deery C. Formocresol and ferric sulfate have similar success rates in primary molar pulpotomy. In carious primary molar does a pulpotomy performed with ferric sulphate, compared with formocresol, result in greater clinical/radiographic success? Evid Based Dent, 6(3), 70, 2005.
12. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine glu-

conate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod, Jun, 20(6): 286–8, 1994.
13. White RR, Hays GL, Janer L. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod, Apr, 23(4): 229–31, 1997.
14. Leonard M, Tanomaru F, Silva LA, Nelson F, Bonifacio K. Ito I. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod, 199 Mar, 25(3): 167–1.
15. Ercan E, Ozekinci T, Atakul F, Gul K. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: in vivo study. J Endod, Feb, 30(2): 84–7, 2004.
16. Estrela C, Ribeiro RG, Estrela CR, Pecora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Braz Dent J, 14(1): 58–62, 2003.
17. Dumani A, Yoldas O, Isci AS, Koksal F, Kayar B, Polat E. Disinfection of artificially contaminated Resilon cones with chlorhexidine and sodium hypochlorite at different time. Oral Surg Oral Med Oral Pathol Oral Radiol, Mar, 103(3): 82–5, 2007.
18. Cannon M, Wagner CJ, Thobaben Z, Zheng X, Johnson RV. Pilot Study to measure inhibition zones of pulpotomy medicaments. J Dent Res, sp is 88 (A), 87th General Session, 2009.
19. Wilkerson MK. et al., Effects of the argon laser on primary tooth pulpotomies in swine. J. Clin. Laser Med Surg, Feb, 14(1): 37–42, 1996.
20. Huth KC et al., Effectiveness of 4 pulpotomy techniques-randomized controlled trial. J Dent Res, Dec, 84(12): 1144–8, 2005.
21. Odabas ME et al., Clinical radiographic, and histopathological evaluation of Nd:YAG laser pulpotomy on human primary teeth. J Endod, Apr, 33(4): 415–21, 2007.
22. Saltzman B et al., Assessment of a novel alternative to conventional formocresol-zinc oxide eugenol pulpotomy for the treatment of pulpally involved human primary teeth: diode laser-mineral trioxide aggregate pulpotomy. Int J Paediatric Dent, Nov, 15(6): 437–47, 2005.
23. Liu JF. Effects of Nd:YAG laser pulpotomy on human primary molars. J Endod, May, 32(5): 404–7, 2006.
24. Hauser-Gerspach I, Stubinger S, Meyer J. Bacetericidal effects of different laser systems on bacteria adhered to dental implants sufaces: an in vivtro study comparing zirconia with titanium. Clin Oral Implants Res, Jan 13, 2010.
25. Eldeniz A, Ozer F, Hadimli H, Eganis O. Bactericidal efficacy of Er,Cr:YSGG laser irradiation against Enterococcus faecalis compared with NaOCI irrigation: an ex vivo pilot study. Int Endod J, Feb, 40(2): 112–9, 2007.
26. Kustarci A, Sumer Z, Altunbag D, Kosum S. Bactericidal effect of KTP laser irradiation against Enterococcus faecalis compared with gaseous ozone: an ex vivo study. Oral Surg Oral med Oral Pathol Oral Radiol Endod, May, 107(5): e73–9, 2009.
27. Gordon W, Atabakhsh V, Meza F, Doms A, Nissan R, Risolu I, Stevens R. The antimicrobial efficacy of the erbium, chromium:yttrium-scandium-gallium-garnet laser with radial emitting tips on root canal dentin walls infected with Enterococcus faecalis. J Am Dent Assoc, Jul, 38(7): 992–1002, 2007.
28. Noetzel J, Nonhoff L, Bitter K, Wagner J, Neumann K, Kielbassa AM. Efficiay of calcium hydroxide, Er:YAG laser of gaseous against Enterococcus faecalis in root canals. Am J Dent, Feb, 22(1): 14–6, 2009.
29. Kuvvetli S, Sandalli, Topcuoglu N, Kulekci G. Antibacterial efficacy of diode and Er:YAG laser irradiation in experimentally contaminated primary molar root canals. J Clin Pediatr Dent, Fall, 34(1): 43–6, 2009.
30. Meire M, De Prijck K, Coenye T, Nelis HJ, De Moor RJ. Effectiveness of different laser systems to kill Enterococcus faecalis in aqueous suspension and in an infected tooth model. Int Endod J, Apr, 42(4): 351–9, 2009.
31. Cannon M, Cernigliaro J, Vieira AE, Pericinoto C, Jurado R, Effects of Antibacterial Agents on Dental Pulps Of Monkeys Mechanically Exposed and Contaminated. J Clin Ped Dent, 33 (1): 21–28, 2008.
32. Cannon M, Gerodias I, Vieira AE, Percinoto C, Jurado R, Application of a Light Cured Pulp Capping Material: An Adjunct to the Practice of Minimally Invasive Dentistry. Dental Research and Applications, (1) 1: 60–64, 2007.
33. Trope M et al, Capping the inflamed pulp under different clinical conditions. J Esthet Restor Dent, 2002: 14(6): 349–57.