

Endodontic Irrigants: A Comprehensive Review

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Abstract

Irrigation has been performed in conjunction with endodontic therapy for many years. One of the primary reasons for irrigating the root canal system is to ensure cleanliness of the canals prior to obturation. This cleanliness involves both elimination of microorganisms and removal of organic matter. Sodium hypochlorite [NaOCl] is the most commonly used solution in root canal treatments, despite its risk for complications. Some other commonly used irrigants include chlorhexidine, MTAD, EDTA and citric acid. This review discusses the available literature on root canal irrigants and the possible complications during their usage.

Keywords: antimicrobial, disinfection, irrigants, smear layer, sodium hypochlorite.

INTRODUCTION

The main aim of a Root canal treatment is the complete removal of the connective tissue and the destruction of residual microorganisms found in infected root canals and an effective seal in order to prevent recolonization of the root canal system with bacteria. [1] Thus the primary endodontic treatment goal must be to ensure root canal disinfection and to prevent re-infection. Irrigating solutions play a very large role in disinfecting the root canals. The complexity of the root canal system, presence of numerous dentinal tubules in the roots, invasion of the tubules by microorganisms, formation of smear layer during instrumentation and presence of dentin as a tissue are the major obstacles in achieving the primary objectives of complete cleaning and shaping of root canal systems. [2]. However many mishaps can occur while cleaning and shaping the root canals with irrigating solutions. Some of the commonly used irrigants are Sodium Hypochlorite (NaOCl), chlorhexidine, ethylenediaminetetraacetic acid (EDTA), and a mixture of tetracycline, an acid and a detergent (MTAD).

HISTORY

The first listed literature about the need for frequent irrigation of the root canal was advocated by Taft . He recommended the use of a 'deodorising agent' like chloride of sodium. [3]. The early literature describes various methods for obtaining a clean canal using a variety of flushing agents and medicaments. Schreir (1893) introduced potassium and sodium metals into canals for removal of necrotic pulp. [4] 20-5-% aqueous solution of sulphuric acid applied on a cotton pledget and sealed into the root canal for 24-48 hours was introduced by Callahan (1894) . A saturated solution of bicarbonate soda was then introduced into the root canals thereby producing an effervescent action and forcing debris to the surface. [5] In the late 20th century, studies conducted by Grossman and Meiman in 1941 led to introduction of the combined

use of double strength sodium hypochlorite and hydrogen peroxide to wash out fragments of pulp tissue and dentinal shavings after mechanical instrumentation. This was published later in 1943 by Grossman. [6] At present sodium hypochlorite have been recommended for day to day clinical practice.

IDEAL PROPERTIES OF AN IRRIGANT [7]

- Tissue/ debris solvent
- Low toxicity
- Low surface tension
- Lubricant
- Sterilization/ disinfection
- Removal of smear layer
- Have a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms
- Inactivate endotoxin.
- Systemically nontoxic, noncaustic to periodontal tissues.
- Low cost, easy availability, shelf life.

SODIUM HYPOCHLORITE

Sodium hypochlorite is the most widely used endodontic irrigant as it is an effective antimicrobial and has tissue-dissolving capabilities. It has low viscosity allowing easy introduction into the canal, an acceptable shelf life, is easily available and inexpensive. The antibacterial and tissue dissolution action of hypochlorite increases with its concentration, but this is accompanied by an increase in toxicity. NaOCl was introduced by Dakin when he used 0.5%-0.6% NaOCl solution (Dakins solution) for the irrigation of wounds in soldiers during World War I. [8] Concentrations ranging from 0.5% - 5.25% are widely used. [9] However for clinical use concentrations between 0.5% and 1% is recommended. [9,10]. Mechanism of action of sodium hypochlorite is that the free chlorine in NaOCl dissolves vital and

necrotic tissue by breaking down proteins into amino acids. [11]

Sodium hypochlorite has been demonstrated to be an effective agent against a broad spectrum of bacteria and to dissolve vital as well as necrotic tissue. [12] Beside their wide-spectrum, nonspecific killing efficacy on all microbes, hypochlorite preparations are sporicidal, virucidal [13], and show far greater tissue dissolving effects on necrotic than on vital tissues [14] However studies have proven its toxic effects on vital tissues as well. The pH level of NaOCl varies from 11-12. Decreasing the concentration of the solution reduces its toxicity, antibacterial effect and ability to dissolve tissues. [15] Increasing the temperature of a less concentrated solution helps in improving its effectiveness [10]. Several studies revealed that warmed NaOCl solutions dissolved organic tissues better and exhibited greater anti microbial efficacy compared to non heated solutions [16-20]

The major disadvantages of this irrigant are its cytotoxicity when injected into periradicular tissues, foul smell and taste, ability to bleach clothes and ability to cause corrosion of metal objects [21]. In addition, it does not kill all bacteria [22-25] nor does it remove all of the smear layer. [26] It also alters the properties of dentin. [27,28]. Sodium hypochlorite is generally not utilized in its most active form in a clinical setting. For proper antimicrobial activity, it must be prepared freshly just before its use. [29,30] In the majority of cases, however, it is purchased in large containers and stored at room temperature while being exposed to oxygen for extended periods of time. Exposure of the solution to oxygen, room temperature and light can inactivate it significantly. [30] Studies have shown the susceptibility of *Candida Albicans* to NaOCl. [31-34].

Studies conducted by M. Hülsmann & W. Hahn reported that following extrusion of irrigant the patient experienced a sharp, severe pain and a rapidly increasing swelling. [35] . Studies have also been reported where patients were admitted with a swelling that comprised the area between the periorbital region and the mandibular angle, with hematoma formation in the infraorbital region due to sodium hypochlorite extrusion in the upper left first premolar. [36]. Most of the hypochlorite accidents are due to incorrect determination of endodontic working length, iatrogenic widening of the apical foramen, lateral perforation, or wedging of the irrigating needle. Clearly, precautions must be undertaken to prevent such mishaps.

CHELATOR SOLUTIONS

Although sodium hypochlorite appears to be the most desirable single endodontic irrigant, it cannot dissolve inorganic dentin particles and thus prevent the formation of a smear layer during instrumentation [37]. In addition, calcifications hindering mechanical preparation are frequently encountered in the canal system. Chelating agent is defined as a chemical

which combines with a metal to form chelate. Chelating agents such as ethylenediamine tetraacetic acid (EDTA) [38] and citric acid [39] have therefore been recommended as adjuvants in root canal therapy. In addition to their cleaning ability, chelators may detach biofilms adhering to root canal walls [40]. This may explain why an EDTA irrigant proved to be highly superior to saline in reducing intracanal microbiota [41], despite the fact that its antiseptic capacity is relatively limited [42]. The effect of EDTA on dentin depends on the concentration of EDTA solution and length of time it is in contact with dentin. EDTA has self limiting action, forms a stable with calcium and dissolve dentin.

Citric acid is used in concentrations ranging from 1-40% in endodontic practice to remove smear layer after root canal preparation. 10% citric acid have been proven to be more effective in removing smear layer and dentine dissolution when compared with EDTA and also has antimicrobial effects. [43-45]. EDTA and citric acid should never be mixed with sodium hypochlorite because both EDTA and citric acid interact strongly with NaOCl.

Antiseptics such as quaternary ammonium compounds (EDTAC [38]) or tetracycline antibiotics (MTAD [46]) have been added to EDTA and citric acid irrigants, respectively, to increase their antimicrobial capacity. EDTAC shows similar smear-removing efficacy as EDTA, but it is more caustic [42]. As for MTAD, resistance to tetracycline is common. MTAD is able to safely remove the smear layer and is effective against *Enterococcus fecalis*. It also helps to open the dentinal tubules and allow the antimicrobial agents to penetrate the entire root canal system. [2]

Both citric acid and EDTA immediately reduce the available chlorine in solution, rendering the sodium hypochlorite irrigant ineffective on bacteria and necrotic tissue [47]. Irrigation with 17% EDTA for one minute followed by a final rinse with NaOCl is the most commonly recommended method to remove the smear layer. [11]

Hydroxyethylidene bisphosphonate (HEBP), also called etidronate, is a decalcifying agent, has recently been suggested as a possible alternative to citric acid or EDTA [47,48]. Its chelating properties are advantageous in such a way that it shows only short term interference with sodium hypochlorite. HEBP prevents bone resorption and is used systemically in patients suffering from osteoporosis or Paget's disease [49]. However, its efficacy endodontic irrigation is yet to be proved.

CHLORHEXIDINE

Chlorhexidine is a cationic bisbiguanide. It is most stable in the form of its salt like Chlorhexidine gluconate. Aqueous solutions of 0.1 to 0.2% are recommended for chemical plaque control, while 2% is used for mechanical irrigation of root [50]. It is highly antimicrobial especially at pH 5.5-7.0, and is known for its long-lasting effectiveness even after the removal

of the solution and it does not provide any tissue dissolving properties. Commonly, Chlorhexidine is used in conjunction with NaOCl as an irrigant as it raises effectiveness of the irrigation protocol. However, being a highly reactive molecule, it creates problems when used in a multiple-irrigant regimen. When sodium hypochlorite and chlorhexidine are mixed, an orange-brown precipitate known as para-chloroaniline is formed, which might be carcinogenic, although that has not been substantiated. Clinically, its seen as a difficult-to-remove, orange-brown film on tooth structure where the reaction occurs. [51] The major advantages of chlorhexidine over NaOCl are its lower cytotoxicity and lack of foul smell and bad taste.

Despite the characteristics of chlorhexidine as an irrigating solution, cannot be used as a gold standard endodontic irrigant because its of its inability to dissolve necrotic tissue remnants [52], and is less effective on Gram-negative than on Gram-positive bacteria [53-55].

HYDROGEN PEROXIDE

Hydrogen peroxide is a clear odourless liquid. Hydrogen peroxide has various applications in dentistry. It is a highly unstable compound which decomposes by heat and light. It acts by releasing nascent oxygen upon which coming in contact with tissue enzymes produces bactericidal effect by interfering with bacterial metabolism. Also the rapid release of nascent oxygen creates effervescence or bubbling action which is said to aid in mechanical debridement by dislodging necrotic tissue and dentinal debris. [51] However higher concentrations of hydrogen peroxide is toxic to the tissues. Concentrations ranging from 1-30% are being used in dentistry [9] while 3-5% is preferred for endodontic treatments. Its active against bacteria, yeast and viruses [9], however its antimicrobial and tissue dissolving capacity is poor when compared to NaOCl. Combination of H₂O₂ and NaOCl have been proven to be less effective as irrigating solutions than while used individually, due to a chemical reaction that results in the release of oxygen. [56-58]. The advantage of rapid nascent oxygen production can also be a complication when the nascent oxygen reacts with blood and pulp debris and causes a pressure build up which may result in severe pain.

IODINE COMPOUNDS

Iodine compounds are bactericidal, fungicidal, virucidal, tuberculocidal and sporicidal. 2% iodine in 4% potassium iodide (IPI) has been used in endodontics. [9] It also shows low toxicity and has a decreased tendency to stain dentine. Main advantage of Iodine is that it is less irritating and toxic than Formocresol, Camphorated Monochlorophenol (FMCP), and Cresatin. However, its not the first choice an irrigant. Despite its antimicrobial effect, iodine is a very potent allergen thereby causing a risk for allergic reactions. Additionally, the substances found in the root canal

such as dentin powder, organic dentin matrix etc show an inhibitory effect on the action of IPI as an irrigant. [59,60]

PHENOLIC COMPOUNDS

Phenolic compounds are widely used in clinical dentistry as sedatives for the dental pulp, as disinfectants for caries, and as root canal medications. Para chloro phenol is a substitution product of phenol in which chlorine replaces one of the hydrogen atoms. Camphorated paramonochlorophenol have been found to be ineffective, toxic and irritating to the tissues and hence is not compatible as a root canal irrigant anymore. However its been shown that aqueous solution of para-chloro phenol penetrates deeper into the dentinal tubules than camphorated phenol. [61-64]

IRRIGATION TECHNIQUES AND DEVICES

Various irrigation techniques are available but its traditionally delivered to the canal system using an irrigating syringe and tip. Other methods include agitation with brushes, and manual dynamic agitation with files or gutta-percha points. The above mentioned methods are mechanical irrigation techniques. Nowadays rotary irrigation systems are also widely used such as rotary brushes, continuous irrigation during instrumentation, sonic and ultrasonic vibrations, and application of negative pressure during irrigation of the root canal system. [65].

It is always advised to flush the root canals with NaOCl throughout the cleaning and shaping process as it helps to increase the working time available for the irrigant and improves cutting efficiency of the instrument [66].

Some other approaches to improve root canal debridement include the use of laser light to induce lethal photosensitization on canal microbiota [67], irrigation using electrochemically activated water [68], and ozone gas infiltration into the endodontic system [69].

CONCLUSION

Endodontic success is greatly dependent on the elimination of micro organisms and removal of smear layer during cleaning and shaping. Care should be administered to the fact that the irrigant must be employed such that it can act to its full potential in the root canals. The choice of irrigants varies from practitioner to practitioner. No irrigant till date provides 100% elimination of bacteria and cleansing the root canal. However, despite the complications, NaOCl is the gold standard irrigant used in day to day clinical practice. Proper administration of the desired irrigant helps to achieve sufficient antimicrobial effect and thereby boosting the endodontic success.

REFERENCES

- [1] Garberoglio R, Becce C. Smear layer removal by root canal irrigants. A comparative scanning electron microscopic study. *Oral Surg Oral Med Oral Pathol*. 1994;78:359-67
- [2] Torabinejad M, Handysides R, Khademi A, Bakland LK. Clinical implications of the smear layer in endodontics: A review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;94:658-66.
- [3] Taft J. A practical treatise on operative dentistry. London: Trubner and Co, 1859:244.
- [4] Schreier EO. The treatment of infected root canals with kalium and natrium. *Dent cosmos* 1893; 38: 863.
- [5] Callahan JR. Sulphuric acid for opening root canals. *Dent Cosmos* 1894;36:329.
- [6] Grossman LI. Irrigation of the root canals. *J Am Dent Assoc* 1943;30:1915-7.
- [7] Edgar Schaffer. Irrigation of the root canal. *ENDO* 2007 ; 1 [1]: 11-27.
- [8] Dakin HD. On the use of certain antiseptic substances in the treatment of infected wounds. *Br Med J* 1915 ; 2:318-21.
- [9] Haapasalo M, Endal U, Zandi H, Coil J. Eradication of endodontic infection by instrumentation and irrigation solutions. *Endodontic topics* 2005; 10 :77-102.
- [10] Zehnder M. Root canal irrigants. *J Endod* 2006; 32: 489-398.
- [11] Johnson WT, Noblett WC. *Cleaning and Shaping in: Endodontics: Principles and Practice*. 4th ed. Saunders, Philadelphia, PA, 2009.
- [12] Senia ES, Marraro RV, Mitchell JL [1975] Rapid sterilization of gutta-percha cones with 5.25 per cent sodium hypochlorite and hydrogen peroxide versus normal saline solution. *Journal of Endodontics* 1, 136-40.
- [13] McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999;12:147-79.
- [14] Austin JH, Taylor HD. Behavior of hypochlorite and of chloramine-T solutions in contact with necrotic and normal tissue in vivo. *J Exp Med* 1918;27:627-33.
- [15] Johnson WT, Noblett WC. *Cleaning and Shaping in: Endodontics: Principles and Practice*. 4th ed. Saunders, Philadelphia, PA, 2009.
- [16] Sirtes G, Valtimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short term stability, pulp dissolution capacity and antimicrobial efficacy. *J Endod* 2005; 31: 669-671.
- [17] Cunningham WT, Balekjian AY. Effect of temperature on collagen dissolving ability of Sodium hypochlorite endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1980;49: 175-177.
- [18] Kamburis JJ, Barker TH, Barfield RD, Eleazer PD. Removal of organic debris from bovine dentine shavings. *J Endod* 2003; 29: 559-561.
- [19] Cunningham WT, Joseph SW. Effect of temperature on bactericidal action of sodium hypochlorite endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1980; 50: 569-571.
- [20] Abou-Rass M, Oglesby SW. The effect of temperature, concentration and tissue type on the solvent ability of sodium hypochlorite. *J Endod* 1981; 7: 376-377.
- [21] Gomes BP, Ferraz CCR, Vianna ME, Berber VB, Teixeira FB, de Souza-Filho FJ. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J* 2001;34:424-8.
- [22] Siqueira Jr JF, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, *in vitro*. *Int Endod J* 1997;30:279-82.
- [23] Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* 1997;30:297-306.
- [24] Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. *J Endod* 2000;26:751-5.
- [25] Shabahang S, Torabinejad M. Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth. *J Endod* 2003;29:576-9.
- [26] McCome D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. *J Endod* 1975;1:238-42.
- [27] Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J* 2001;34:120-32.
- [28] Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* 2001;34:113-9.
- [29] Clarkson RM, Moule AJ, Podlich HM. The shelf-life of sodium hypochlorite irrigating solutions. *Aust Dent J* 2001;46:269-76.
- [30] Piskin B, Turkun M. Stability of various sodium hypochlorite solutions. *J Endod* 1995;21:253-5.
- [31] Peciuliene V, Reynaud A, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of *Enterococcus faecalis* in previously root filled canals in a Lithuanian population. *J Endod* 2000; 26: 593-595.
- [32] Waltimo TM, Ørstavik D, Siren EK, Haapasalo M. *In vitro* susceptibility of *Candida Albicans* to four disinfectants and their combinations. *Int Endod J* 1999; 32: 421-429.
- [33] Radcliffe CE, Potouridou L, Quereshi R, Habahdeh N, Qualthrough A, Worthington H, Drucker DB. Antimicrobial activity of varying concentrations of sodium hypochlorite of the endodontic microorganisms *Actinomyces israelii*, *A. Maeslundii*, *Candida albicans* and *Enterococcus faecalis*. *Int Endod J* 2004; 37: 438-446.
- [34] Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FL. *In vitro* evaluation of the anti microbial activity of chlorhexidine and sodium hypochlorite. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;97:79-84.
- [35] M. Hülsmann & W. Hahn. Complications during root canal irrigation – literature review and case reports. *International Endodontic Journal* 2000; 33:186-193.
- [36] Maria Luisa Bosch-Aranda , Carlos Canalda-Sahli , Rui Figueiredo , Cosme Gay-Escoda. In an accidental sodium hypochlorite extrusion: A report of two cases. *J Clin Exp Dent*. 2012;4 [3]:e194-8.
- [37] Lester KS, Boyde A. Scanning electron microscopy of instrumented, irrigated and filled root canals. *Br Dent J* 1977;143:359-67.
- [38] Nygaard Østby B. Chelation in root canal therapy. *Odontol Tidskr* 1957;65:3-11.
- [39] Loel DA. Use of acid cleanser in endodontic therapy. *J Am Dent Assoc* 1975;90:148 -51.
- [40] Root Canal Irrigants *Matthias Zehnder, Dr. med. dent., PhD. JOE—Volume 32, Number 5, May 2006.*
- [41] Yoshida T, Shibata T, Shinohara T, Gomyo S, Sekine I. Clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant. *J Endod* 1995;21:592-3.
- [42] Patterson SS. In vivo and in vitro studies of the effect of the disodium salt of ethylenediamine tetra-acetate on human dentine and its endodontic implications. *Oral Surg Oral Med Oral Pathol* 1963;16:83-103.
- [43] Guttman JL, Saunders WP, Nguyen L, Gou IY, Saunders EM. Ultrasonic root-end preparation. Part 1. SEM analysis. *Int Endod J* 1994 ;27:318-324.
- [44] Machado- Silveiro LF, Gonzales Lopes S, Gonzales Rodriguez MP. Decalcification of the root canal dentine by citric acid, EDTA and Sodium Citrate. *Int Endod J* 2004;37:365-369.
- [45] Yamaguchi M, Yoshida K, Suzuki R, Nakamura H. Root canal irrigation with citric acid solution. *J Endod* 1996 ; 22: 27-29.
- [46] Torabinejad M, Khademi AA, Babagoli J, et al. A new solution for the removal of the smear layer. *J Endod* 2003;29:170 -5.
- [47] Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. *J Endod* 2005;31:817-20.
- [48] Zehnder M, Schicht O, Sener B, Schmidlin P. Reducing surface tension in endodontic chelator solutions has no effect on their ability to remove calcium from instrumented root canals. *J Endod* 2005;31:590 -2.
- [49] Russell RG, Rogers MJ. Bisphosphonates: from the laboratory to the clinic and back again. *Bone* 1999;25:97-106.

- [50] Zamany A, Safavi K, Spångberg LS. The effect of chlorhexidine as an endodontic disinfectant. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;96:578–81.
- [51] Endodontic Irrigants by David Carter, DMD.
- [52] Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. *J Endod* 2004;30:785–7.
- [53] Davies GE, Francis J, Martin AR, Rose FL, Swain G. 1:6-Di-4--chlorophenyldiguanidohexane [hibitane]; laboratory investigation of a new antibacterial agent of high potency. *Br J Pharmacol Chemother* 1954;9:192– 6.
- [54] Hennessey TS. Some antibacterial properties of chlorhexidine. *J Periodontal Res Suppl* 1973;12:61–7.
- [55] Emilson CG. Susceptibility of various microorganisms to chlorhexidine. *Scand J Dent Res* 1977;85:255– 65.
- [56] Heling I, Chandler MP. Antimicrobial effects of irrigant combinations within dentinal tubules. *Int Endod J* 1998; 31: 8-14.
- [57] Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus Faecalis* from the root canal, *in vitro*. *Int Endod J* 1997; 30: 279- 282.
- [58] Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. *J Endod* 1987; 13: 47-51.
- [59] Portenier I, Haapasalo H, Orstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of iodine potassium iodide and chlorhexidine digluconate against *Enterococcus Faecalis* by dentin, dentin matrix, type-I collagen, heat- killed microbial whole cells. *J Endod* 2002 ;28: 634-637.
- [60] Haapasalo HK, Siren EK, Waltimo TM, Orstavik D, Haapasalo MP. Inactivation of local root canal medicaments by dentine- an *in vitro* study. *Int Endod J* 2000; 33: 126-131.
- [61] Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms *in vitro*. *J Endod* 2006 ; 32: 434-437.
- [62] Torneck CD. Reaction of hamster tissue to drugs used in sterilization of the root canal. *Oral surg Oral Med Oral Pathol* 1961;14:730-747.
- [63] Powell DL, Marshall FJ, Melfi RC. A histopathologic evaluation of tissue reaction to the minimum effective doses of some endodontic drugs. *Oral Surg Oral Med Oral Pathol* 1973;36:261-272.
- [64] Fager FK, Messer HH. Systemic distribution of champhorated monochlorophenol from cotton pellets sealed in pulp chambers. *J Endod* 1986;12:225-230.
- [65] Gu L, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR et al. Review of Contemporary Irrigant Agitation Techniques and Devices. *J Endod* 2009;35:791-804.
- [66] Tepel J, Schafer E, Hoppe W. Kunststoffe als Modellmaterial In der Endodontie. *Dtsch Zahnärztl Z* 1993; 48;736- 738.
- [67] Kimura Y, Wilder-Smith P, Matsumoto K. Lasers in endodontics: a review. *Int Endod J* 2000;33:173– 85.
- [68] Solovyeva AM, Dummer PM. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. *Int Endod J* 2000;33:494 – 504.
- [69] Deltour MM, Vincent J, Lartigau G. Effet lethal de l’ozone sur certaines souches de bactéries aerobes dans un modèle de chambre pulpaire. *Rev Odontostomatol Midi Fr* 1970;15:278–84.