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“Júlio de Mesquita Filho”
Faculdade de Odontologia de Araraquara

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Root canal irrigation: physicochemical and biological properties of calcium hypochlorite and effects of the GentleWave system in endodontic treatment

Araraquara
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Thesis presented to São Paulo State University (Unesp), School of Dentistry, Araraquara, to obtain the PhD degree in Dentistry, in the area of Endodontics.

Supervisor: Gisele Faria, PhD

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Eric Hernán Coaguila Llerena

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Judging Commission

Thesis for obtaining the PhD degree in Dentistry

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CURRICULAR DATA

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I dedicate this work:

To God, in whose omnipresence, infinite benevolence and unquestionable will, I know that there is no impossible. God gave me nothing I wanted, he gave me everything I needed.

To my family, because it is through them that I learned to remain humble in victory and elegant in defeat. I know that the tireless pursuit of the realization of my dreams and the consolidation of the principles that guide my life are a consequence of what I learned at home. And, without a doubt, it is through them that I learned that I should never give up because God provides enough strength.

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“O que dá o verdadeiro sentido ao encontro é a busca, e é preciso andar muito para se alcançar o que está perto”

José Saramago*

* Saramago J. Todos os nomes. Lisboa: Editorial Caminho; 1997.

Coaguila Llerena EH. Irrigação do canal radicular: propriedades físico-químicas e biológicas do hipoclorito de cálcio e efeitos do sistema GentleWave no tratamento endodôntico [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2022.

RESUMO

Parte 1: Na publicação 1, o objetivo foi avaliar as propriedades físico-químicas e a penetrabilidade nos túbulos dentinários do hipoclorito de cálcio [$Ca(OCl)_2$] a 2,5% associado aos surfactantes cloreto de benzalcônio (BAK), cetrimida (CTR), tween 80 (T80) e triton X-100 (TR100), na concentração micelar crítica (CMC), em comparação ao hipoclorito de sódio (NaOCl) a 2,5%. Foram avaliados tensão superficial, pH, conteúdo de cloro livre (FAC), íons cálcio livres (Ca^{2+}) e a penetrabilidade nos túbulos dentinários. A adição de surfactantes reduziu a tensão superficial das soluções ($p<0,05$), e não alterou o pH e o FAC ($p>0,05$). Os surfactantes, principalmente BAK, aumentaram a disponibilidade de Ca^{2+} em $Ca(OCl)_2$ ($p<0,05$). $Ca(OCl)_2$ apresentou menor penetrabilidade nos túbulos dentinários que NaOCl ($p<0,05$) e a adição de surfactantes àquele não aumentou a sua penetrabilidade nos túbulos dentinários. Na publicação 2, o objetivo foi avaliar o mecanismo de citotoxicidade da solução de $Ca(OCl)_2$ em fibroblastos L929 e o seu efeito na biologia de osteoblastos-*like* (Saos-2), em comparação com NaOCl. Fibroblastos L929, expostos a $Ca(OCl)_2$ e NaOCl, foram avaliados quanto ao metabolismo celular, integridade dos lisossomos, tipo de morte, alterações no citoesqueleto e na ultraestrutura. O efeito das soluções sobre a atividade da fosfatase alcalina (ALP) foi determinada em Saos-2. O $Ca(OCl)_2$ promoveu maior viabilidade celular e menor porcentagem de apoptose e necrose do que o NaOCl ($p<0,05$). $Ca(OCl)_2$ e NaOCl diminuíram o metabolismo celular e a integridade dos lisossomos, induziram ruptura de microtúbulos e filamentos de actina, promoveram alterações do retículo endoplasmático rugoso e nas cristas mitocondriais, e não induziram a atividade da ALP. Concluiu-se que, embora a adição de surfactantes ao $Ca(OCl)_2$ não aumentou a sua penetrabilidade nos túbulos dentinários, eles promoveram menor tensão superficial, sem alterações nos valores de pH e FAC, além de maior disponibilidade de Ca^{2+} na solução. Adicionalmente, NaOCl e $Ca(OCl)_2$ apresentaram o mesmo mecanismo de citotoxicidade e não induziram atividade de ALP, porém, $Ca(OCl)_2$ foi menos citotóxico que NaOCl. **Parte 2:** Na publicação 3, o objetivo foi descrever, por meio de revisão de literatura, os efeitos do sistema GentleWave (GW) no tratamento endodôntico. GW mostrou resultados *in vitro* semelhantes ou melhores que a irrigação utrassônica passiva (PUI) sob diferentes aspectos. Clinicamente, embora GW tenha mostrado resultados promissores, ainda são necessários mais estudos. Na publicação 4, o objetivo foi avaliar a eficácia de remoção de biofilme multiespécie do GW e PUI. Molares inferiores humanos com configuração tipo II de Vertucci na raiz mesial foram inoculados com placa dental e incubados. As raízes mesiais foram instrumentadas até 20.06 (V-Taper) para o grupo GW, e até 35.04 (Vortex Blue) para o grupo PUI. Raspas de dentina foram obtidas pré e pós-tratamento para a análise por reação em cadeia da polimerase quantitativa (qPCR) e por sequenciamento do gene rRNA 16S (Next Generation Sequencing - NGS). Não houve diferença na remoção de biofilme entre GW e PUI ($p>0,05$). Pode-se concluir que mais pesquisas, principalmente clínicas, são necessárias para estabelecer se GW apresenta vantagens sobre outros métodos de irrigação.

Palavras chave: Biofilmes. Hipoclorito de cálcio. Hipoclorito de sódio. Permeabilidade da dentina. Sequenciamento de nucleotídeos em larga escala. Tensoativos. Teste de materiais.

Coaguila Llerena EH. Root canal irrigation: physicochemical and biological properties of calcium hypochlorite and effects of the GentleWave system in endodontic treatment [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2022.

ABSTRACT

Part 1: In publication 1, the aim was to assess the physicochemical properties and the penetration into dentinal tubules of calcium hypochlorite [$Ca(OCl)_2$] at 2.5% associated with the surfactants benzalkonium chloride (BAK), cetrimide (CTR), tween 80 (T80) and triton X-100 (TR100), at critical micellar concentration (CMC), compared to sodium hypochlorite (NaOCl) at 2.5%. Surface tension, pH, free available chlorine (FAC), free calcium ions (Ca^{2+}) and penetration into dentinal tubules were evaluated. The addition of surfactants reduced the surface tension of the solutions ($p<0.05$), and did not change the pH and FAC ($p>0.05$). Surfactants, mainly BAK, increased the Ca^{2+} availability in $Ca(OCl)_2$ ($p<0.05$). $Ca(OCl)_2$ showed lower penetration into dentinal tubules than NaOCl ($p<0.05$) and the addition of surfactants to $Ca(OCl)_2$ did not increase its penetration into dentinal tubules. In publication 2, the aim was to assess the cytotoxicity mechanism of $Ca(OCl)_2$ solution on L929 fibroblasts and its effect on osteoblast-like (Saos-2) biology, compared to NaOCl. Cellular metabolism, lysosome integrity, type of cell death, changes in cytoskeleton and ultrastructure of L929 fibroblasts, exposed to $Ca(OCl)_2$ and NaOCl, were evaluated. The effect of the solutions on alkaline phosphatase (ALP) activity was determined in Saos-2. $Ca(OCl)_2$ promoted higher cell viability and lower percentage of apoptosis and necrosis than NaOCl ($p<0.05$). $Ca(OCl)_2$ and NaOCl decreased cellular metabolism and lysosome integrity, induced disruption of microtubules and actin filaments, promoted changes in the rough endoplasmic reticulum and mitochondrial cristae, and did not induce ALP activity. It was concluded that, although the addition of surfactants to $Ca(OCl)_2$ did not increase its penetration into dentinal tubules, they promoted lower surface tension, without changes in pH and FAC values, in addition to higher Ca^{2+} availability. Additionally, NaOCl and $Ca(OCl)_2$ showed the same mechanism of cytotoxicity, and they did not induce ALP activity; however, $Ca(OCl)_2$ was less cytotoxic than NaOCl. **Part 2:** In publication 3, the aim was to describe, through a literature review, the effects of the GentleWave system (GW) in endodontic treatment. GW showed similar or better *in vitro* results than passive ultrasonic irrigation (PUI) in different aspects. Clinically, although GW has shown promising results, further studies are still needed. In publication 4, the aim was to assess the effectiveness of multispecies biofilm removal of GW and PUI. Human mandibular molars with Vertucci type II configuration in the mesial roots were inoculated with dental plaque and incubated. Mesial roots were instrumented up to 20.06 (V-Taper) for GW group, and up to 35.04 (Vortex Blue) for PUI group. Dentin shavings were obtained pre- and post-treatment for analysis by quantitative real-time Polymerase Chain Reaction (qPCR) and 16S ribosomal RNA gene sequencing (Next Generation Sequencing - NGS). There was no difference in biofilm removal between GW and PUI ($p>0.05$). It can be concluded that more research, mainly clinical, is needed to establish whether GW has advantages over other irrigation methods.

Keywords: Biofilms. Calcium hypochlorite. Sodium hypochlorite. Dentin permeability. High-throughput nucleotide sequencing. Surface-active agents. Materials testing.

SUMMARY

1 INTRODUCTION

The control of infection is critical for the success of endodontic treatment[1], and the irrigation solution and its métodos de agitação used in chemo-mechanical preparation has an important role to achieve this goal[2].

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution due to its antimicrobial activity and organic dissolution capacity[3–5], being considered as "*gold standard*"[6]. However, NaOCl negatively alters the mechanical properties of dentine, such as microhardness, elastic modulus, resistance to flexion and fatigue[7], and can reduce the bond strength of root canal sealers[8] and some dentine adhesive materials[7,9]. Additionally, NaOCl does not provide adequate removal of the smear layer from the dentine surface[10], and when interacts with ethylenediaminetetraacetic acid (EDTA) causes deleterious effects to root canal dentine[11]. High concentrations of NaOCl are irritating when in contact with periapical tissues[12,13] and have a pronounced negative effect on the survival and differentiation of apical papilla stem cells, which can hinder pulp regeneration/revascularization[14]. The research for alternative irrigating solutions is focused on substances that have an antimicrobial effect, of organic dissolution, as well as biocompatibility.

Calcium hypochlorite [$Ca(OCl)_2$], a chlorine disinfectant[15], has been proposed as endodontic irrigant. It is available as granules powder and when prepared in aqueous solution, there is a release of hypochlorous acid and calcium hydroxide[4]: $Ca(OCl)_2 + 2\ H_2O \rightarrow 2HOCl + Ca(OH)_2$. Compared with sodium hypochlorite ($NaOCl + H_2O \rightarrow HOCl + NaOH$), there is a higher generation of hypochlorous acid[16]. The generated calcium hydroxide [$Ca(OH)_2$] could favour the antimicrobial activity of $Ca(OCl)_2$ [16]. In addition, calcium present in its composition, instead of sodium[17], could be associated to a possible osteogenic induction and subsequent mineralization in periapical tissues. The $Ca(OCl)_2$ solutions are highly alkaline (pH around 11-12), and higher surface tension than NaOCl[18]. $Ca(OCl)_2$ solution had a stable FAC up to 30 days, which is comparable to NaOCl stabilized with sodium hydroxide[18,19]. Studies have been performed assessing the $Ca(OCl)_2$ potential for tissue dissolution[4,20], efficacy against *Enterococcus faecalis*[20–22], effects on mechanical properties of root canal dentine[23,24], on composite resin microleakage[17], and cytotoxicity[25–28].

When used as irrigating solution in extracted teeth contaminated with *E. faecalis*, 2.5% $Ca(OCl)_2$ showed antimicrobial efficacy similar to 2.5% NaOCl[22], regardless of the use of passive ultrasonic irrigation (PUI)[21] or sonic activation using Vibringe system[29]. Another study showed that 2.5% $Ca(OCl)_2$ was more effective against *E. faecalis* than 2.5% NaOCl when used during chemo-mechanical preparation with Reciproc R40 file[30]. The 5% $Ca(OCl)_2$ has the same organic dissolution capacity as 5.25% NaOCl after 35 and 60 minutes of contact with the tissue[4]. This organic dissolution capacity gradually increases with time and with an increase in its concentration[31]. On the other hand, $Ca(OCl)_2$, does not have the ability to dissolve inorganic tissue and consequently does not remove the smear layer[16].

Regarding the effects on root canal dentine surface, $Ca(OCl)_2$ changes dentine roughness in a similar manner to NaOCl[23]. When used before an acetone-based adhesive system, $Ca(OCl)_2$ does not affect microleakage compared to NaOCl. However, the contact of $Ca(OCl)_2$ on dentine increases the amount of calcium and phosphorus ions, which can be beneficial for the mineralization process and for the formation of an amorphous calcium phosphate phase within the hybrid layer during bonding procedures[17]. Study showed that 6% NaOCl negatively affects the flexural strength, ultimate tensile strength and fracture resistance of dentine, while 6% $Ca(OCl)_2$ does not alter those properties[24].

Regarding $Ca(OCl)_2$ cytotoxicity, studies that used methyl-thiazole-tetrazolium - MTT[26–28] or trypan blue and scratch assay[25] do not show consensus. A study found no difference between $Ca(OCl)_2$ at a high concentration (5%) and NaOCl at a low concentration (0.5%) in L929 fibroblasts, that is, $Ca(OCl)_2$ had more cytocompatibility than NaOCl[27], which was corroborated by a study that used both solutions at the same concentration, 2.5%[26]. Another study that used 3T3 cells revealed that both $Ca(OCl)_2$ and NaOCl promoted similar cytotoxicity at 3h and 6h; however, at 24h, $Ca(OCl)_2$ promoted higher cell cytotoxicity[28]. On the other hand, no differences were founded between $Ca(OCl)_2$ and NaOCl in 3T3 fibroblasts[25].

In teeth with pulp necrosis, microorganisms can penetrate areas that are difficult to clean mechanically such as isthmus, ramifications, lateral or accessory canals, apical deltas and dentinal tubules[32]. It has been reported that the biofilm of *E. faecalis* and *Porphyromonas gingivalis* can invade dentinal tubules up to 500 μm deep[33]. Thus, the depth that irrigants penetrate into dentinal tubules is an important factor in endodontic treatment[34]. The penetration depth of NaOCl into dentinal tubules can be affected by concentration[33,34], contact time, temperature[34,35], agitation, use of gel form [36] and surface tension of the irrigant[35,37].

Inside root canal, a high surface tension can hinder the penetration of the irrigating solution into dentinal tubules, regions of isthmus and anatomical irregularities, reducing its antibacterial effectiveness[38]. It has been reported that a decreased surface tension can increase the penetration of the irrigating solution into inaccessible areas of the root canal system including dentinal tubules, improving the disinfection[35,37,38]. Substances that reduce the surface tension of irrigants, which are called surfactants, including cetrimide (CTR), benzalkonium chloride (BAK), triton X-100 (TR100) and tween 80 - T80 has been associated to irrigating solutions[36,39–42].

CTR is a cationic surfactant that significantly reduces the surface tension of NaOCl[38] and has antimicrobial activity[43]. Studies have shown that 0.2% CTR eradicated *E. faecalis* biofilm[43], improved the antimicrobial effect of 2% chlorhexidine against polymicrobial biofilm[42] and did not affect the 2.5% NaOCl activity against *E. faecalis* biofilm[44].

BAK is a cationic surfactant widely used in the medical field as a preservative for eye solutions[45] that has been mixed with NaOCl. The addition of 0.008% BAK reduced the contact angle and surface free energy, and had no effect on FAC, cytotoxicity and antimicrobial effectiveness of 2.4% NaOCl[46]. The mixture of 0.008% BAK with 6% NaOCl was more effective in eliminating *E. faecalis* from the root canal compared to 6% NaOCl alone[47].

T80 is a nonionic surfactant present in the composition of Biopure MTAD (Dentsply Sirona Endodontics, York, PA, USA)[48] and TR100, another nonionic surfactant, is present in Chlor-Xtra (Vista Dental Products, Racine, WI, USA)[49,50]; in both cases manufacturers do not disclose their concentrations. A previous study shows that ChlorXtra has lower surface tension than NaOCl without surfactant[38]. Another study revelead that the addition of 0.1% BAK, TR100 and T80 in 5% NaOCl reduced surface tension and did not change the pH and FAC in comparison to 5% NaOCl[39].

There is no consensus in the literature about the impact of surface tension on penetration of irrigating solutions into dentinal tubules[35–37]. Two studies showed that NaOCl solutions with surfactant had higher penetration into dentinal tubules[35,37], while another study showed that this addition had no effect on the penetration of NaOCl[36]. According to available literature, $Ca(OCl)_2$ had a higher surface tension than NaOCl at the same concentrations - 0.5%, 1%, 2.5% and 5.25%[18]. However, it is not known if $Ca(OCl)_2$ penetrates into dentinal tubules less than NaOCl. In addition, it is not known if the addition of surfactants would decrease the surface tension and increase the penetration of $Ca(OCl)_2$ into dentinal tubules, without changing the pH and FAC.

For the selection of irrigating solution for root canal treatment it must be considered not only its antimicrobial effectiveness and organic dissolution capacity, but also its possible cytotoxic effects since it may come in contact with periapical tissues[51], which may influence the prognosis of root canal treatment. This becomes even more critical in regenerative endodontics procedures, which are performed in immature teeth. This is because the irrigating solution also contacts the periapical tissues, which are essential for endodontic regeneration[52]. It is also necessary to consider the physicochemical properties of irrigating solutions such as pH, surface tension, FAC, as well as the ability to penetrate areas not touched by the instruments, which are fundamental factors for the disinfection of the root canal system.

As previously mentioned, the control of infection is necessary to promote the healing of periapical tissues affected by apical periodontitis[53]. However, disinfection may be challenging when bacteria are organized in multispecies matrix-enclosed communities, called biofilms, especially in teeth with complex anatomies. These bacterial structures can colonize the canal walls[54], ramifications and isthmuses[32].

To improve irrigation effectiveness, technologies such as ultrasonic activation have been used. Specifically, passive ultrasonic irrigation (PUI) improves the removal of debris, smear layer[55,56], and bacterial biofilm[57] from root canals. However, even with the use of ultrasonic activation associated with NaOCl, microorganisms, debris and even pulp tissue may remain in the isthmus, apical third[58], oval/flattened canals[59] and root canal curvatures[60]. Another aspect to be considered is that if a small apical enlargement is performed during conventional chemical-mechanical preparation, a large amount of bacterial biofilm and necrotic tissue may remain in the root canal[61].

The GentleWave (GW), a new system that combines multisonic and negative apical pressure (Sonendo Inc, Laguna Hills, CA, USA), was introduced on the US market in 2014, and represents a type of endodontic device developed for cleaning and disinfection of the root canal[62]. According to its manufacturer, GW can be used in situations that need only minimal instrumentation, instead of using conventional instrumentation[63].

GW uses high-speed fluid dynamics to deliver the irrigants into the root canal system without requiring the tip of the instrument to enter the root canals. The irrigant is delivered through a handpiece to the end of a nozzle placed in the sealed pulp[64], and the excess of irrigant is simultaneously removed from the chamber by the built-in vented suction through the handpiece into a waste canister inside the console[65]. GW creates a powerful shear force, which causes hydrodynamic cavitation in the form of a cavitation cloud. The implosion of thousands of microbubbles creates an acoustic field of broadband frequencies that travels through the procedure fluid into the entire root canal system[66].

Regarding microbial reduction, a study using real-time PCR and bacterial cultures in molars, revealed that GW promoted higher reduction of total microbial DNA than CUI[67]. In anterior teeth, GW promoted less bacterial reduction than PUI, as previously shown using next generation sequencing (NGS)[68].

Traditionally, culture methods have been used to assess the bacterial composition and decontamination of the root canal system [69]. This method allows a semi- or absolute quantification of culturable bacteria. However, a significant amount of microorganisms in the root canal space cannot be cultured under laboratory conditions. The development of The Human Genome Project [70] allowed the subsequent development of databases (i.e., SILVA) for use in conjunction with NGS technologies [71,72]. NGS is a fifth-generation laboratory tool of microbiological analysis for the study of endodontic infections. This method provides vast information about bacterial communities and their profiles[53,73]. To date, the decontamination efficacy of infected root canals irrigated with GW in molars has not been provided using a relevant infection model.

Studies have been conducted to assess other types of GW effects in endodontic treatment. The GW promoted a significantly fast rate of bovine muscle dissolution than conventional syringe irrigation (CSI), continuous ultrasonic irrigation (CUI) and negative-pressure irrigation[62]. The root canal treatment using GW was not associated with extrusion[65] since GW creates a negative pressure at the apical foramen, irrespective of canal instrumentation size[64]. GW removed more debris than CSI in minimally and conventionally instrumented canals[74]; as well as more debris than CUI, but no more than PUI in conventionally instrumented canals[75]. Additionally, GW showed a greater removal of calcium hydroxide paste in comparison to CSI and PUI, in the root canals submitted to conventional instrumentation[76]. A prospective multicenter clinical study showed that the treatment of teeth with large periapical lesions with GW resulted in a 97.7% success rate after 12-month re-evaluation[66]. The association of GW with 3% NaOCl without root canal instrumentation cleaned the organic matter (tissue remnants) and dentin debris even in irregular areas, especially in middle and apical thirds of premolars[77]. Regarding the penetration of NaOCl into dentinal tubules, the use of GW promoted greater penetration of 3% NaOCl than PUI and CUI[78].

Currently, GW costs approximately $80,000.00 per console, and $50.00 to $100.00 for a one-time use handpiece. However, several doubts have been raised in regard to GW: Is it worth investing in such high-cost equipment? Does it produce better results than conventional root canal treatment? What are the effects of GW on endodontic treatment? Has GW biofilm removal efficacy been evaluated using a relevant model?

4 CONCLUSIONS

Part 1:

-The addition of surfactants to $Ca(OCl)_2$ did not increase the penetration into dentinal tubules, but it did promote lower surface tension, without changing the pH or free available chlorine values, and higher availability of free calcium ions in $Ca(OCl)_2$ (Publication 1).

-Although $Ca(OCl)_2$ and NaOCl promoted the same cytotoxicity mechanism and did not stimulate ALP activity, $Ca(OCl)_2$ was less cytotoxic than NaOCl (Publication 2).

Part 2:

-Further research, mainly clinical, is needed to establish whether GentleWave has any advantages over other available irrigation methods (Publication 3).

-There was no difference between GentleWave and Passive Ultrasonic Irrigation (PUI) in the reduction of multispecies biofilm; bacterial reduction in mesial roots of mandibular molars prepared to 35.04 with PUI was similar to those prepared to 20.06 with a GentleWave (Publication 4).

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