

# Behavior of subcutaneous tissue of rats in response to infected dentine associated with different endodontic irrigants

## Comportamento do tecido subcutâneo de ratos frente à dentina infectada e associada a diferentes irrigantes endodônticos

### Abstract

**Purpose:** To compare rat subcutaneous connective tissue reaction to dentine contaminated with *Enterococcus faecalis* associated with 0.9% sterile saline, 5.25% sodium hypochlorite (NaOCl) and 2% chlorhexidine gel (CHX).

**Methods:** Dentine was crushed into powder and inoculated with *E. faecalis*. Tested substances were mixed with contaminated dentine and placed in polyethylene tubes. Ten male Wistar rats had their backs divided into four quadrants that received an implant containing one of the tested substances. An empty tube was used as a control. Five rats were randomly distributed for evaluation at time intervals of 24 hours and 72 hours. Tissue samples were histologically processed. Tissue reactions to experimental groups were evaluated under optical microscopes.

**Results:** Groups of 5.25% NaOCl induced greater inflammatory response after 24 hours and 72 hours. Compared to groups of 2% CHX, the groups of 0.9% sterile saline showed milder inflammatory reactions after 24 hours and more severe after 72 hours.

**Conclusion:** The results indicate that 5.25% NaOCl group showed a higher inflammatory reaction to rat subcutaneous connective tissue and the 2% chlorhexidine group showed the least reaction.

**Key words:** Chlorhexidine; root canal irrigants; sodium hypochlorite; subcutaneous tissue

### Resumo

**Objetivo:** Comparar a resposta do tecido subcutâneo de ratos frente a dentina contaminada com *Enterococcus faecalis* associado ao soro fisiológico 0.9%, hipoclorito de sódio 5.25% (NaOCl) ou clorexidina gel 2% (CHX).

**Metodologia:** Foi realizada a contaminação de dentina em pó com *E. faecalis*. As substâncias testadas foram misturadas com a dentina contaminada e inseridas em tubos de polietileno. Dez ratos Wistar tiveram os dorsos divididos em quatro quadrantes e cada quadrante recebeu um tubo com cada uma das misturas testadas. Um tubo vazio foi utilizado como controle. Os ratos foram distribuídos em dois grupos para avaliação no período de 24 e 72 horas. Os tecidos foram processados histologicamente e as reações teciduais foram avaliadas sobre microscopia de luz.

**Resultados:** Os grupos de NaOCl 5.25% promoveram maiores reações inflamatória após 24 e 72 horas. Quando comparado com os grupos de CHX 2%, os grupos de soro fisiológico 0.9% mostraram inflamação moderada após 24 horas e severa após 72 horas.

**Conclusão:** Os resultados indicaram que o grupo de NaOCl 5.25% apresentou maior reação inflamatória aos tecidos subcutâneos de rato e que o grupo de CHX 2% apresentou menor reação inflamatória.

**Palavras-chave:** Clorexidina; irrigantes endodônticos; hipoclorito de sódio; tecido conjuntivo

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Received: January 10, 2012

Accepted: August 13, 2012

**Conflict of Interests:** The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

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## Introduction

One of the goals of endodontic therapy is the removal of all vital or necrotic tissue, microorganisms, and microbial by-products from root canal system (1,2). The mechanical action of endodontic instruments may remove most bacteria found in the canal space; however, in several situations the complete debridement of the root canal system is complicated by the presence of a complex anatomy (1). This complex anatomy can provide ideal locations for organic residues and bacteria lodged deep inside isthmuses, accessory canals, deltas, and dentinal tubules that cannot be reached even after careful mechanical instrumentation (3). To aid in the removal of debris and the disinfection of these areas, the use of various intracanal irrigants has been advocated (4).

Sodium hypochlorite (NaOCl) is the most used irrigant in endodontic therapy, and its antimicrobial property has been widely reported, acting also as a lubricant for instrumentation and flushing loose debris from root canals (5,6). Although NaOCl has these great qualities, it is known to be extremely irritating to the periapical tissues, especially at higher concentrations (3). Spilling of NaOCl beyond the foramen may cause inflammatory reaction in the periapical tissues and severe pain (7).

Among the alternatives, chlorhexidine gluconate has been recommended as an auxiliary chemical substance (3,8); in addition to being relatively non-toxic when compared to NaOCl (9) it has excellent antimicrobial power and prolonged time of action within the canal, called substantivity (3). These properties may offer a clinical advantage of chlorhexidine over NaOCl in infected teeth postoperative against resistant microorganisms (3,8).

During canal instrumentation and irrigation, dentine chips, pulp tissue fragments, necrotic tissue, microorganisms, and intracanal irrigants may be extruded from the apical foramen (10). Nevertheless, little data is available about tissue reaction to endodontic irrigants associated with dentine chips and microorganisms. Thus, the aim of the present study was to compare the reaction of rat subcutaneous connective tissue in response to the implant of dentine infected with *Enterococcus faecalis* associated with 0.9% sterile saline, 5.25% sodium hypochlorite and 2% chlorhexidine gluconate gel.

## Methodology

Thirty teeth from the Teeth Bank of the Rio de Janeiro State University (UERJ) had the crown portion removed and the roots sanded to cementum removal. Dentine was broken into smaller particles, which were then ground through a Ball Mill (SPEX CertiPrep, Metuchen, NJ, USA). Particles were obtained with size between 0.2 and 20  $\mu\text{m}$ . Bacterial strains of *Enterococcus faecalis* (ATCC 29212, Rockville, MD, USA) were cultured in a TSB medium (Difco, MI, USA) for 24 hours to obtain the microbial pellet. After this growth, the strains were centrifuged and washed three times with buffered saline solution (PBS, Phosphate buffered saline)

and the pH was adjusted to 7.2. The strains were then diluted in saline solution to obtain 0.5 turbidity on the McFarland scale, corresponding to  $1.5 \times 10^8$  colony-forming units per milliliter.

The samples were divided into four groups according to the material to be tested:

- Group 1: empty tube (control group);
- Group 2: 0.9% sterile saline;
- Group 3: 5.25% sodium hypochlorite (B'Herzog – Rio de Janeiro, RJ, Brazil);
- Group 4: 2% chlorhexidine gluconate gel (Endogel® Essencial Pharma – Itapetininga, SP, Brazil).

For each group, 150  $\mu\text{L}$  aliquots of bacterial suspension were mixed with 2g of dentin powder until obtaining a homogenous pellet. Then, the contaminated dentin was mixed with 0.5 mL of the corresponding substance to the experimental group and placed inside polyethylene tubes.

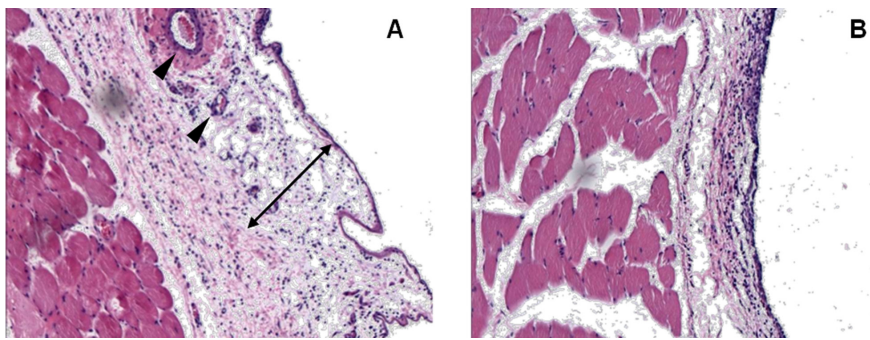
Ten Wistar rats weighing 250-270g were used for experiments. The animals were housed in a temperature-controlled environment with water and food *ad libitum*. All experiments were conducted in accordance with the National guidelines on the welfare of experimental animals and after approval by the Ethics in Research Committee of the Dental School of Rio de Janeiro State University. Under general anesthesia with xylazine (10 mg/kg body weight) and 5% ketamine hydrochloride (25 mg/kg body weight), the dorsal of each animal was divided into four quadrants and a tube containing a different sample was inoculated in each quadrant. After the implantation of the cylinders, the incisions were closed by simple suture.

Evaluations were made 24 and 72 hours after the inoculation. In each examination period, five animals were euthanized with an overdose of anesthesia. The surgical parts containing tubes wrapped in conjunctive tissue were excised, stored in 10% formalin solution for 48 hours, washed in running water and thereafter embedded in paraffin blocks using standard procedures. Six-micrometer-thick sections were obtained from the paraffin-embedded specimens and stained with hematoxylin and eosin for histomorphological analysis under optical microscopy with a Zeiss – Axioplan 2 optical microscope (Carl Zeiss AG, Germany).

The microscopic analysis of the inflammatory process after the tube implantation consisted of a description or notification of the inflammatory infiltrate presence or absence. The type of infiltrate, acute or chronic, their intensity and extent were also evaluated. Exudate characteristics could be assessed by edema, abscess, or micro abscess presence. The presence or absence of tissue necrosis was also described.

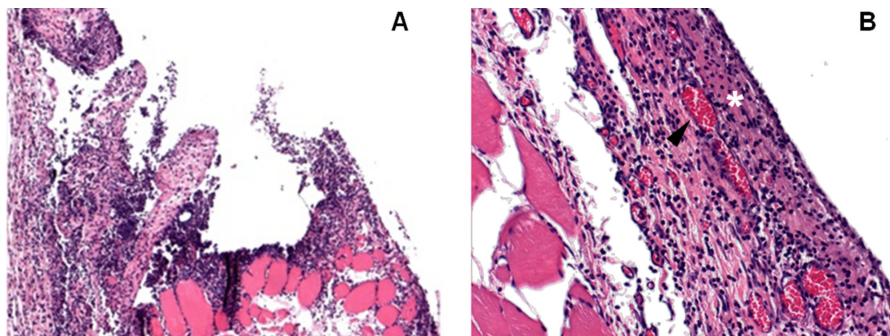
## Results

A qualitative analysis of histological results was performed, to determine if there was an inflammatory infiltrate in the periods studied, and if so, what type of inflammatory infiltrate was observed, to determine the biocompatibility of the materials studied.



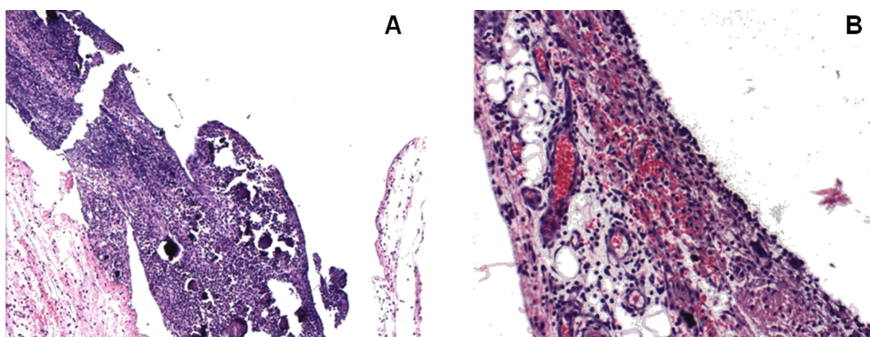
presence of blood vessels (arrowhead) in small number. **(B)** After 72 hours is possible to observe an edema reduction and chronic inflammatory infiltrate (H&E, original magnification X100).

**Fig. 1.** Reaction of rat subcutaneous connective tissue to the empty tube (control group). **(A)** After 24 hours the subcutaneous tissue showed mild inflammatory infiltrate, moderate edema areas (double-headed arrow) and



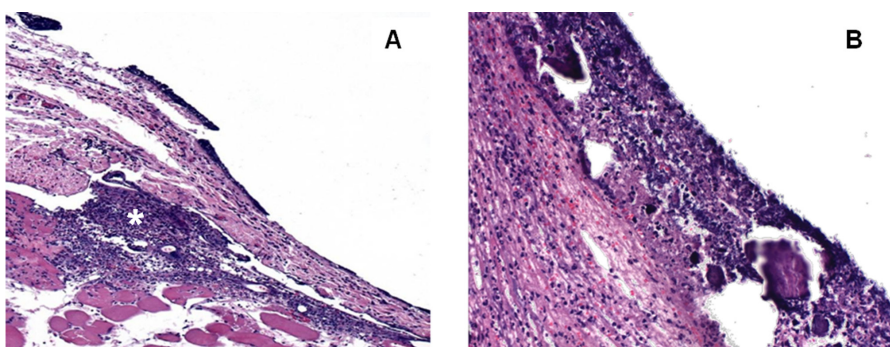
the assessed area presented an acute inflammation rich in neutrophils, mononuclear infiltrate severe edema, hyperemic blood vessels (arrowhead), small areas of tissue necrosis and micro abscesses (white star) (H&E, original magnification X200).

**Fig. 2.** Reaction of rat subcutaneous connective tissue to 0.9% sterile saline associated with infected dentine. **(A)** After 24 hours severe mononuclear and polymorph nuclear inflammatory infiltrate rich in neutrophils and intense edema areas (H&E, original magnification X100). **(B)** After 72 hours



area presented intense inflammatory infiltrate rich in neutrophils, large areas of edema, larger areas of tissue necrosis than in the 24 hours period, presence of abscesses and a large number of dilated and hyperemic blood vessels (H&E, original magnification X200).

**Fig. 3.** Reaction of rat subcutaneous connective tissue to 5.25% NaOCl associated with infected dentine. **(A)** After 24 hours subcutaneous tissue presented large areas of intense inflammatory infiltrate rich in neutrophils, severe edema, some areas of tissue destruction and many dilated and hyperemic blood vessels (H&E, original magnification X100). **(B)** After 72 hours the assessed



the assessed area showed intense inflammatory infiltrate rich in neutrophils and mononuclear cells, intense edema, larger areas of necrosis, low number of hyperemic blood vessels and no abscess (H&E, original magnification X200).

**Fig. 4.** Reaction of rat subcutaneous connective tissue to 2.0% Chlorhexidine gel associated with infected dentine. **(A)** After 24 hours it is possible to observe moderate to intense inflammatory infiltrate (white star), with presence of polymorphonuclear and mononuclear cells, severe edema, some areas of tissue necrosis and small number of hyperemic blood vessels (H&E, original magnification X100). **(B)** After 72 hours



### Empty tube (control)

As shown in Fig. 1, the histological findings in this group were similar in 24 hours and 72 hours. In the 24-hour period, the subcutaneous tissue showed mild inflammatory infiltrate, moderate edema areas, and presence of blood vessels in small number. In the 72-hour period an edema reduction and chronic inflammatory infiltrate presence were observed.

### Saline solution

In the 24-hour period, the histological section showed severe mononuclear and polymorph nuclear inflammatory infiltrate rich in neutrophils, and intense edema areas. In the 72-hour period, the assessed area presented an acute inflammation rich in neutrophils, mononuclear infiltrate, severe edema, hyperemic blood vessels, small areas of tissue necrosis, and micro abscesses (Fig. 2).

### Sodium hypochlorite

In the 24-hour period, the subcutaneous tissue presented large areas of intense inflammatory infiltrate rich in neutrophils, severe edema, some areas of tissue destruction, and many dilated and hyperemic blood vessels. In the 72-hour period, the assessed area presented intense inflammatory infiltrate rich in neutrophils, large areas of edema, larger areas of tissue necrosis than in the 24h period, a large number of dilated and hyperemic blood vessels, and presence of abscess (Fig. 3).

### Chlorhexidine

In the 24-hour period, moderate to intense inflammatory infiltrate was observed, with the presence of polymorphonuclear and mononuclear cells, severe edema, some areas of tissue necrosis, and a small number of hyperemic blood vessels. In the 72-hour period, the area assessed showed intense inflammatory infiltrate rich in neutrophils and mononuclear cells, intense edema, larger areas of necrosis, low number of hyperemic blood vessels, and no abscess (Fig. 4).

## Discussion

During endodontic treatment, debris, auxiliary chemical substances, and bacteria are often pushed out of the apex, coming into direct contact with the periapical vital tissues (9). Infection is considered the most significant factor in the flare-up pathogenesis (11,12). The organic tissue associated with microorganisms that spill into the apical region during the canal preparation may cause acute inflammation reaction and severe pain. *Enterococcus faecalis* was the microorganism selected for this study for several reasons: it is the key species in several persistent endodontic infections; it is more resistant to some auxiliary chemical substances than other microorganisms; it is able to be active in these substances; and it is easy to grow and to identify (13-15).

Good tolerance of subcutaneous connective tissue to polyethylene tubes was shown in the present study. Little

inflammatory response was observed in the control group. This inflammation could be caused by the aggression during tubes surgical implantation (9,16). In the saline solution group intense inflammatory response was observed within the first 24 hours. This response was more intense after 72 hours. The results suggest that despite the low inflammatory reaction of the saline solution, the association with pathogenic microorganisms can lead to intense inflammatory response, as this solution does not have antimicrobial action (17).

The substances 5.25% NaOCl and 2% chlorhexidine gel presented similar results at 24 hours. However, when compared to the 72-hour period, a more acute response was characterized in the hypochlorite group, with features not found in the chlorhexidine group, such as abscess formation, larger areas of necrosis, and more severe vascular changes. These results suggest the possibility of better antibacterial activity against *E. faecalis* of chlorhexidine compared to NaOCl. Previous studies reported that 2% chlorhexidine has a more effective antimicrobial action after several days of the chemomechanical preparation (10,18,19). This residual antimicrobial effect of chlorhexidine, called substantivity may be related to less tissue reaction caused after a 72-hour period. Another factor that may be attributed to this difference in inflammatory response between the two groups is the inability of chlorhexidine to dissolve vital and necrotic tissue (11,18,20), which makes it less aggressive than NaOCl. Tissue dissolution may cause severe effects, such as hemolysis, ulceration, inhibition of neutrophil migration, damage to endothelial, damage to fibroblast cells, facial nerve weakness, and necrosis if the solution is extruded during endodontic treatment (21-24).

When compared to the saline solution group, the chlorhexidine group results in the 72-hour period had differences similar to those found in the hypochlorite group. Again, the antimicrobial power and the chlorhexidine substantivity may be related to these results. However, in the 24-hour period, the chlorhexidine group showed greater irritation to tissues, with necrosis areas and inflammatory infiltrate more intense than the saline solution group. These results may be attributed to an initial cytotoxicity of chlorhexidine (21). Gomes-Filho et al. (9) suggest that the chlorhexidine gel may promote a greater inflammatory response in the conjunctive tissue because the tissues would take longer to absorb it than when it's in its liquid form.

The authors report that if the antimicrobial effect were the only major requirement for an irrigating solution, chlorhexidine would be the choice irrigant (25). However, it is known that the ability of tissue dissolution of the hypochlorite is of great importance in root canal instrumentation. Some clinicians consider the lower chlorhexidine toxicity as an advantage insufficient to compensate for its inability to dissolve tissues (25). Therefore, the antimicrobial properties of an irrigant should not be the only concern in the choice of a chemical auxiliary substance. Concerns about biocompatibility and the tissue dissolution ability also exist, especially when there is a greater possibility of clinical spilling of these substances.

## Conclusions

In conclusion, the results of the present study indicate that 5.25% NaOCl group showed the highest inflammatory reaction in rat subcutaneous connective tissue and the 2% chlorhexidine group showed the lowest.

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