

The biocompatibility of a new endodontic paste used in dental trauma

Biocompatibilidade de uma nova pasta utilizada em traumatismo dentário

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Resumo

Objetivo: O presente estudo investigou a reação em tecido subcutâneo de ratos, por um curto período, de uma nova pasta endodôntica, obtida a partir da associação entre o hidróxido de cálcio [Ca(OH)₂], clorexidina gel 2% (CHX) e óxido de zinco (ZnO), em comparação à pasta controle, e as substâncias isoladamente. **Material e método:** Foram utilizados vinte e quatro ratos wistar machos. Tubos de polietileno contendo os materiais foram implantados no tecido conjuntivo dorsal de ratos. Tubos vazios foram utilizados como controle. Os materiais utilizados foram: Pasta de Ca(OH)₂ + CHX + ZnO em duas proporções diferentes (2: 1: 2) e (2: 1: 3), pasta controle, Ca(OH)₂ + CHX (2:1), e as substâncias isoladas. Após intervalos de tempo de 2, 3, 7 e 15 dias, os animais foram sacrificados e as amostras preparadas para processamento histológico. Os dados foram avaliados qualitativamente de acordo com os níveis de reparo dos tecidos e do grau de inflamação. **Resultado:** No segundo dia observou-se que todos os materiais causaram uma resposta inflamatória grave. No terceiro dia, as pastas de Ca(OH)₂ + CHX + ZnO (2: 1: 2) e (2: 1: 3) mostraram uma redução no grau de inflamação. No sétimo dia, o tecido encontrava-se mais organizado, com a presença de fibras colágenas em todos os materiais. Após 15 dias, verificou-se que a pasta de Ca(OH)₂ + CHX + ZnO (2: 1: 2) mostrou áreas sem infiltrado inflamatório. **Conclusão:** As pastas experimentais, Ca(OH)₂ + CHX + ZnO, nas duas proporções avaliadas (2: 1: 2) e (2: 1: 3), mostraram resultados favoráveis em relação à biocompatibilidade, quando avaliada por curto período de tempo.

Descritores: Teste de materiais; clorexidina; hidróxido de cálcio; óxido de zinco; toxicidade.

Abstract

Objective: This study investigated the short-term subcutaneous tissue reaction of a new endodontic paste, based on the association among calcium hydroxide [Ca(OH)₂], 2% chlorhexidine gel (CHX) and zinc oxide (ZnO) in comparison with a control paste, and the individual substances alone. **Material and method:** Twenty-four male wistar rats were used. Polyethylene tubes containing the materials were implanted into the dorsal connective tissue of rats. Empty implanted tubes served as controls. The materials used were: Ca(OH)₂+CHX+ZnO paste in two different proportions (2:1:2) and (2:1:3), a control paste, Ca(OH)₂+CHX (2:1), and the substances alone. After time intervals of 2, 3, 7 and 15 days, the animals were euthanized and the specimens prepared for histological processing. Data were evaluated qualitatively according to the levels of tissue repair and the degree of inflammation. **Result:** On the second day all the materials were observed to cause a severe inflammatory response. On the third day, the pastes Ca(OH)₂+CHX+ZnO (2:1:2) and (2:1:3) showed reduction in degree of inflammation. On seventh day the tissue was already more organized with presence of collagen fibers in all materials. After 15 days it was found that compound Ca(OH)₂+CHX+ZnO (2:1:2) showed areas with no inflammatory infiltrate. **Conclusion:** The experimental pastes, Ca(OH)₂+CHX+ZnO in two different proportions (2:1:2) and (2:1:3), showed favorable results with respect to biocompatibility, when evaluated for a short-term.

Descriptors: Materials testing; chlorhexidine; calcium hydroxide; zinc oxide; toxicity.

INTRODUCTION

Worldwide, 20–30% of 12-year-old children experience dental trauma that may lead to damage of the developing dentition¹. When an injury damages Hertwig's root sheath or causes pulp necrosis, root development may be arrested^{2,3}. Incomplete root maturation results in thinner and weaker dentinal canal walls that may predispose these teeth to fracture⁴ and wide-open apices, making it difficult to achieve an optimal apical seal⁵⁻⁷.

Apexification is the process that allows the formation of a calcified barrier across the open apex, after pulp necrosis, thus creating a suitable environment within the root canal and periapical tissues⁸. Various materials have been used to induce apical closure, among them calcium hydroxide and mineral trioxide aggregate (MTA).

Calcium hydroxide [$\text{Ca}(\text{OH})_2$] has been the material of choice for apexification since 1964 when Kaiser first reported its capacity to induce physiological closure of immature pulpless teeth^{9,10}. However the use of this material presents some disadvantages. Calcium hydroxide paste should usually be replaced every three months, which requires multiple visits with heavy demands on patients and operators, inevitable clinical costs. In addition, there is the increased risk of tooth fracture when using $\text{Ca}(\text{OH})_2$, since many dressing changes are necessary until a calcified barrier is formed¹¹. An alternative treatment to $\text{Ca}(\text{OH})_2$ apexification is to fabricate an artificial apical barrier with MTA, which allows immediate obturation of the canal. However, the high costs and difficulties in handling the material have restricted its widespread use^{8,12}.

The disadvantages of both calcium hydroxide and MTA have encouraged a search for alternatives. A promising alternative to achieve the goal of apexification is the mixture of this well-known medication, calcium hydroxide, with 2% chlorhexidine gel and zinc oxide. This may be a low cost, easy to use, providing a mineralizing material with high radiopacity and no need for periodic exchanges^{11,13}.

The aim of this study was to investigate the short-term subcutaneous tissue reactions of a new endodontic paste, based on the association among calcium hydroxide [$\text{Ca}(\text{OH})_2$], 2% chlorhexidine gel (CHX) and zinc oxide (ZnO) in comparison with a control paste, and the individual substances alone.

MATERIAL AND METHOD

Twenty-four male Wistar rats (*Norvegicus albinus*) weighing approximately 200-250 g were used. The rats were housed in cages under climate-controlled conditions (12 h light/12 h dark; thermostatically regulated room temperature) with free access to food and tap water during the course of the study. The animals were randomly divided in two groups:

Group 1: In these animals, the substances evaluated in isolation were: G1A: $\text{Ca}(\text{OH})_2$ + Saline solution (2:1); G1B: 2% chlorhexidine gel (CHX); G1C: Zinc oxide (ZnO) + Saline solution (2:1). Empty polyethylene tubes were used as negative control.

Group 2: In this group, the experimental and control pastes were evaluated. Control paste (G2A) was the association between $\text{Ca}(\text{OH})_2$ and CHX in a ratio/proportion of 2:1. The experimental

pastes consisted of the association between $\text{Ca}(\text{OH})_2$ +CHX+ZnO in two different proportions 2:1:2 (G2B) and 2:1:3 (G2C). Empty tubes were also used as control.

One of the ends of all the polyethylene tubes were closed with the use of sterile heated clinical tweezers, and then filled with the materials under aseptic conditions immediately before use and implantation.

Before tube implantation, the animals were anaesthetized with Xylazine (Anasedan® - AgribRANDS do BRASIL Ind. e Com. LTDA - 25 mg/Kg or 0.25 ml/Kg) and ketamine (Dopalen® - AgribRANDS do BRASIL LTDA - 100 mg/Kg or 1 ml/Kg), and their dorsal region was shaved and cleaned with iodinated alcohol. A 1 cm wide incision was made in the dorsal region, followed by tissue divulsion, and the tube was inserted into the connective tissue and the skin edges were sutured (Vicryl; Johnson & Johnson: Ethicon Inc., New Brunswick, NJ, USA). The surgery was performed under aseptic conditions to minimize the trauma to the implant area. The animals were allowed free access to standard chow and water during the experimental periods and were periodically observed for local, systemic and behavioral abnormalities.

At the end of each experimental time interval (2, 3, 7 and 15 days), the animals were anaesthetized again for implant removal together with the surrounding tissues (skin and subcutaneous connective tissue), and were sacrificed by anesthetic overdose. The biopsied tissues were fixed in 10% buffered formalin solution and then subjected to routine processing for histological analysis.

With a microtome (Leica RM 2025, Nussloch, Germany) five semi-serial histological sections, 5 μm thick, were made in the longitudinal direction in each block. The histological slides were stained with hematoxylin and eosin and analyzed using a light microscope (Carl Zeiss Microscopy, Göttingen, Germany) at 100, 200 and 400x magnification.

The histological sections were evaluated qualitatively, by two pathologists, according to the degree of inflammation and the levels of tissue repair.

With regard to Inflammatory infiltrate and related reactions, the magnitude of the infiltrate, predominant cell types present (macrophages, neutrophils, lymphocytes, plasma cells, eosinophils and multinucleated giant cells), and their distribution were observed subjectively. In addition, destructive changes, such as abscess formation, and vascular changes such as hyperemia were evaluated. With respect to the reparative phenomena, fibroblast proliferation and fibrosis were analyzed.

RESULT

Representative images of all groups in 2 days may be observed in Figure 1. Acute inflammatory infiltrate was observed in all substances and their associations. Among the isolated substances, zinc oxide showed the highest degree of inflammation followed by 2% chlorhexidine gel and calcium hydroxide. Among the associations, 2:1 has the lowest degree of inflammation followed by the similarity of 2:1:2 and 2:1:3. The associations have milder inflammation than substances isolated.

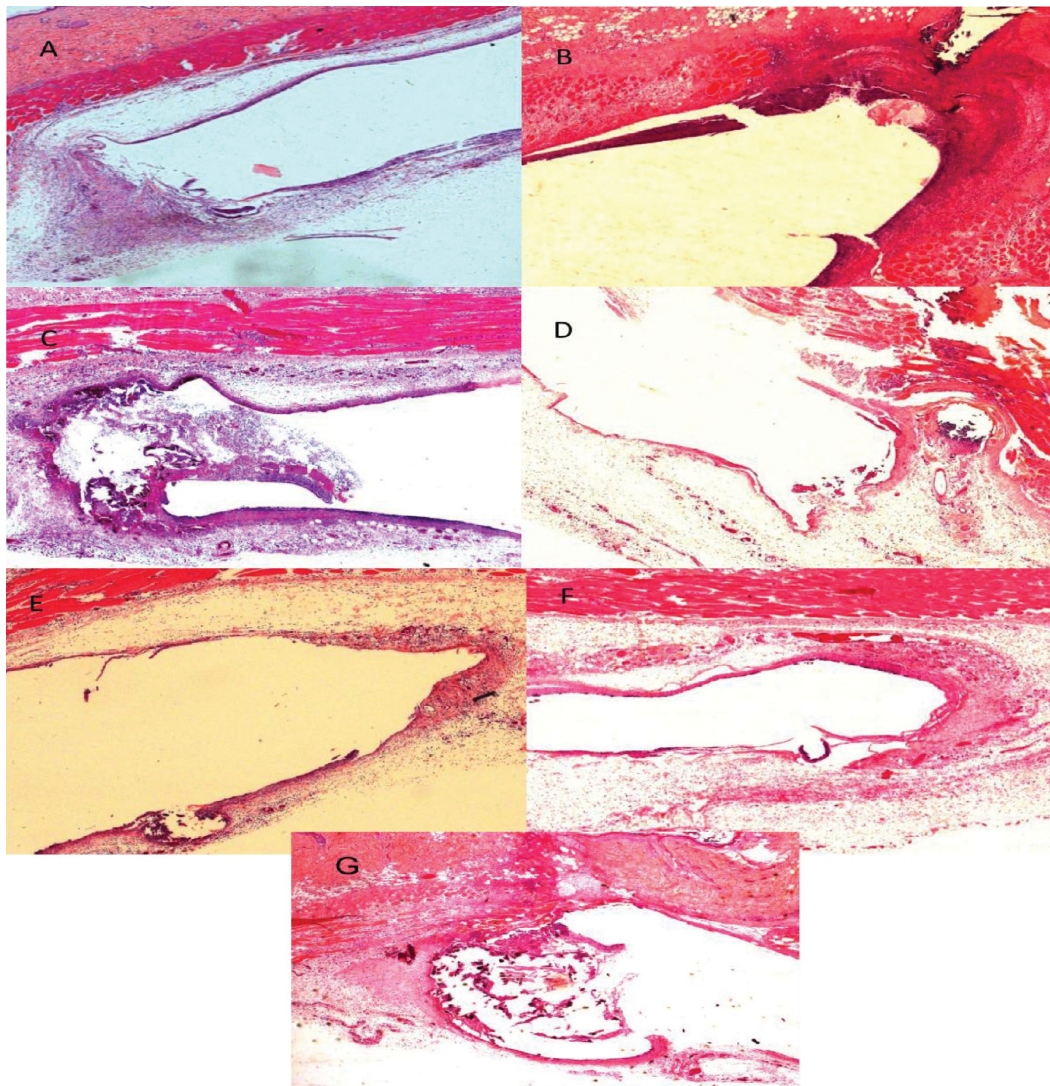


Figure 1. Representative images of all groups in 2 days. A) Negative control, B) calcium hydroxide, C) 2% chlorhexidine gel, D) zinc oxide, E) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:2) F) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:3) and G) Control paste, calcium hydroxide + 2% chlorhexidine gel (2:1). 88×90mm (600 × 600 DPI).

Figure 2 shows representative images of all groups in 3 days. Mixed inflammatory infiltrate was observed in all materials and their associations. Among the isolated compounds, zinc oxide has the highest degree of inflammation followed by calcium hydroxide and 2% chlorhexidine gel. Among the associations, 2:1:2 and 2:1:3 behave similarly being greater degree of inflammation than 2:1.

Representative images of all the experimental groups in 7 and 15 days may be observed in Figure 3 and Figure 4, respectively. In 7 days, a mononuclear inflammatory infiltrate was observed in all substances used in isolation, and pastes. Among the isolated compounds, zinc oxide had the highest degree of inflammation, followed by 2% chlorhexidine gel and calcium hydroxide. When the experimental and control pastes were compared, they were shown to be similar. In the time interval of 15 days, mononuclear cell infiltration and tissue repair were observed in all groups. Between the isolated compounds: zinc oxide presented inflammatory cells with the collection of PMN cells. The pastes had similar behavior.

DISCUSSION

The biocompatibility of endodontic materials has been evaluated by different methods, among which the most frequently used are in vitro cytotoxicity assays on cell or tissue cultures and biocompatibility tests by implantation of materials in the bone or subcutaneous connective tissue of experimental animals. Although cell culture methods provide some valuable information about the response of specific cells to a test material, they do not provide the full picture of how a tissue reacts to the material under in vivo conditions. In vivo implantation experiments provide more complete and clinically relevant information on the tissue response¹⁴.

In the present study, the biocompatibility of a new endodontic paste, composed of calcium hydroxide, 2% chlorhexidine and zinc oxide, was assessed. This paste is recommended as protocol in the Dental Trauma Service of Piracicaba Dental School (obturation paste without exchanges and coronal sealing) and it has shown clinical and radiographic success in traumatized immature teeth

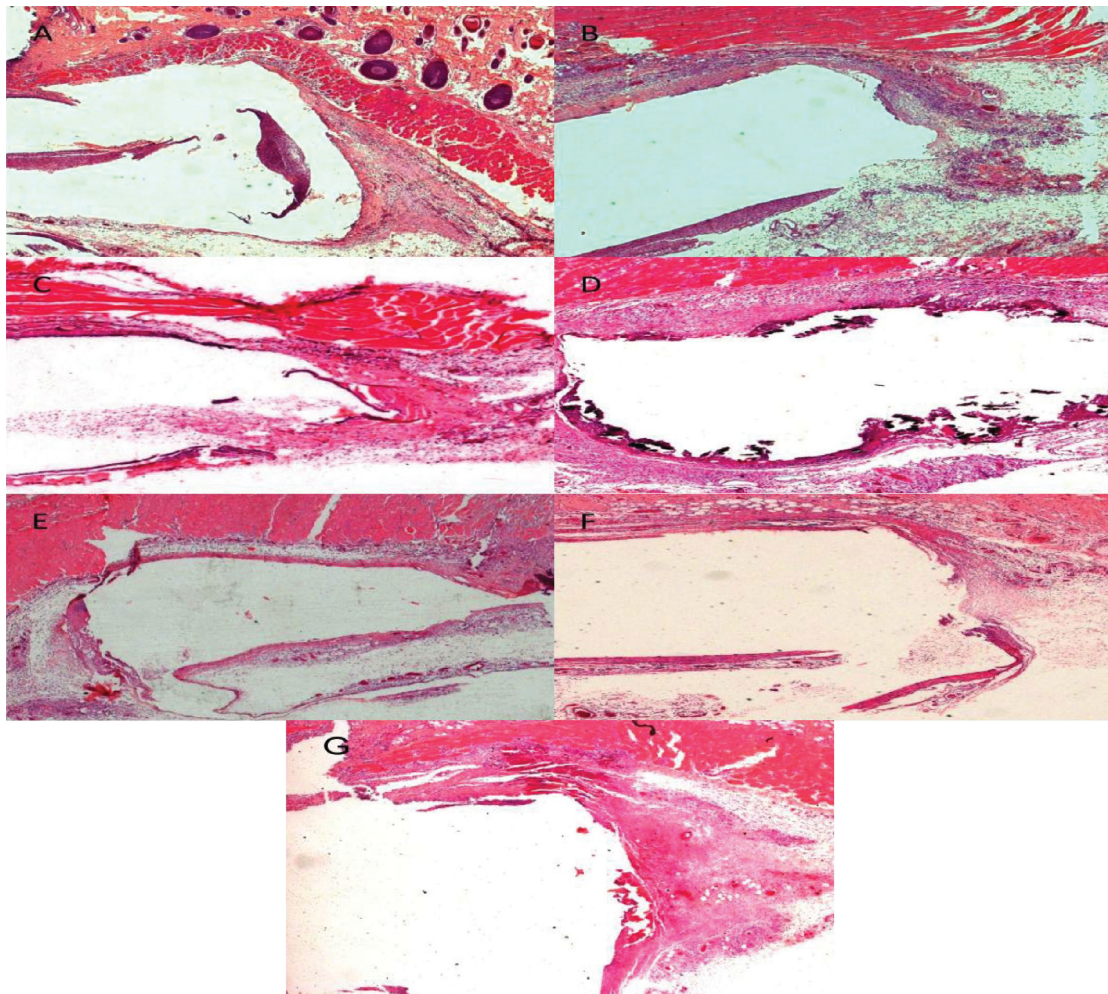


Figure 2. Representative images of all groups in 3 days. A) Negative control, B) calcium hydroxide, C) 2% chlorhexidine gel, D) zinc oxide, E) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:2) F) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:3) and G) Control paste, calcium hydroxide + 2% chlorhexidine gel (2:1). 76×67mm (600 × 600 DPI).

with apical barrier formation after nine months and reduction of all the signs and symptoms after treatment has been performed¹⁵. It has been suggested that the hydroxyl ions of this obturation paste have good diffusion into dentinal tubules¹⁶, and in thirty days, no pH alterations have been observed with this medication¹³. Another advantage is that it does not need to be changed during the period while apexification takes place, as no dissolution has been radiographically observed during the control visits. The fact that medicaments do not have to be removed is advantageous; for example to prevent infection during replacement of the medication and to reduce the time necessary for apical barrier formation. Moreover, radiographs have shown that the material did not undergo dissolution. It is likely that after nine months, only zinc oxide was present, since $\text{Ca}(\text{OH})_2$ would have undergone complete dissolution. The presence of zinc oxide would have worked as an inert sealing material, preventing contamination and allowing apical repair and barrier formation¹². Moreover, this paste is easily removed, when necessary¹⁵.

With regard to the different experimental periods, these periods are in accordance to American Dental Association¹⁷ to evaluate short-term subcutaneous tissue reaction. Additionally, according

to Andreollo et al.¹⁸ several authors performed experimental studies in rats and estimated 30 days of human life for every day life of the animal. Using this comparison, the present study evaluated approximately 15 months of human.

In the present study, in 2 days all groups showed a severe inflammatory response and absence of tissue repair. These results can be explained by the surgical trauma¹⁹. In 3 days, the inflammatory response was reduced and there was absence of tissue repair in all groups. No tissue repair was found because this experimental period is too short to evaluate tissue repair²⁰. In 7 days, the reduction of inflammatory infiltrate and the beginning of tissue repair was observed. This finding is in agreement with Sousa et al.²¹, Semenov et al.²² and Martínez Lalis et al.²³ who affirmed that 7 days is the initial period in which to evaluate inflammatory response and tissue repair. The 15-day period is associated with limited influence of the surgical procedures on tissue response to the materials^{21,24}. In the present study, the short experimental period could be a key reason why some of the biological effects of the test materials, such as complete reparative and inflammatory phenomena, were not observed. These effects may be observable in longer experimental periods, e.g. 60 or 90 days⁹.

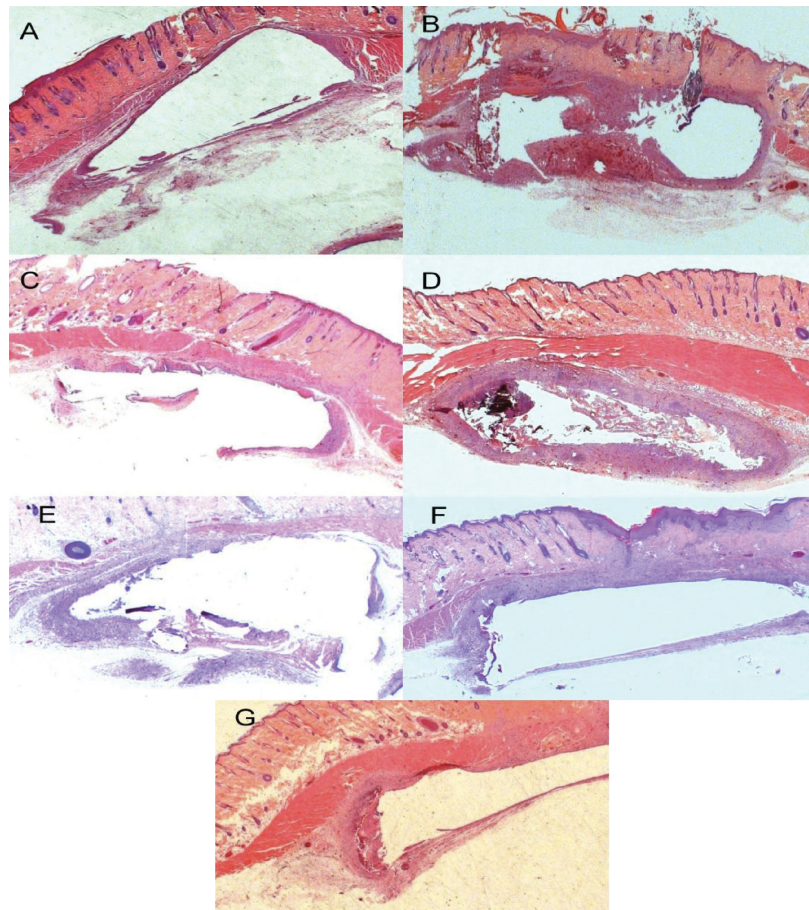


Figure 3. Representative images of all experimental groups in 7 days. A) Negative control, B) calcium hydroxide, C) 2% chlorhexidine gel, D) zinc oxide, E) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:2) F) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:3) and G) Control paste, calcium hydroxide + 2% chlorhexidine gel (2:1). 92×104mm (600 × 600 DPI).

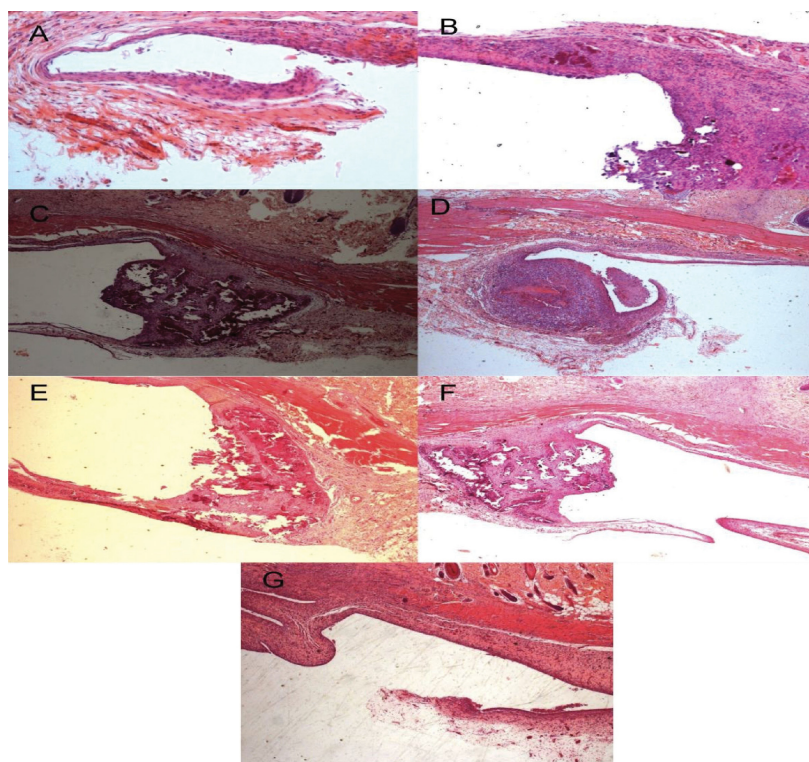


Figure 4. Representative images of all experimental groups in 15 days. A) Negative control, B) calcium hydroxide, C) 2% chlorhexidine gel, D) zinc oxide, E) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:2) F) Experimental paste, calcium hydroxide + 2% chlorhexidine gel + zinc oxide (2:1:3) and G) Control paste, calcium hydroxide + 2% chlorhexidine gel (2:1). 82×77mm (600 × 600 DPI).

As regards the groups evaluated, zinc oxide presented the highest degrees of inflammatory infiltration, as has previously been observed, due its cytotoxicity²⁵. Although this substance presented good results with respect to tissue repair, probably due to its anti-oxidant power, anti-inflammatory potential and tissue repair activity^{26,27}. Calcium hydroxide and 2% chlorhexidine gel showed similar behavior as regards inflammatory infiltrate. However in relation to the reparative phenomenon, chlorhexidine showed better results than Calcium hydroxide. These results may be associated with the biocompatibility of chlorhexidine^{22,28}.

When comparing the control paste, Ca(OH)₂+CHX with the experimental pastes, Ca(OH)₂+CHX+ZnO (2:1:2) and (2:1:3), with respect to inflammatory infiltrate, in 15 days, all pastes showed good results, with the experimental paste, Ca(OH)₂+CHX+ZnO

(2:1:2), showing areas without inflammatory infiltrate. As regards tissue repair, the experimental pastes showed better results than the control paste. This result may be associated with the zinc oxide, which presented low tissue irritation and induced good tissue repair⁹. When comparing the two experimental pastes, the amount of zinc oxide did not significantly influence the biocompatibility of the pastes.

CONCLUSION

In the short-term subcutaneous tissue reaction, the present study showed that the experimental pastes appear to be biocompatible. Further researches are needed to evaluate the biocompatibility of these pastes for longer periods of time.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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