UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"

Jimena Alejandra Lama Sarmiento

DISSERTAÇÃO

AVALIAÇÃO DA RESPOSTA INFLAMATÓRIA, BIOMINERALIZAÇÃO E CAPACIDADE DE REPARO TECIDUAL DO IODOFÓRMIO E HIDRÓXIDO DE CÁLCIO

Araçatuba – SP

2019

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Dissertação apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP como parte dos requisitos para obtenção do título de Mestre em Ciência Odontológica, área de concentração em Endodontia.

Orientador: Prof. Assoc. Eloi Dezan-Junior

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Não se esqueça:

NUNCA, sob nenhuma circunstância, deixe de lado sua capacidade de confiar em você. Têm feito acreditar que você é fraco, mas no teu interior se concentra a mais grande das fortalezas.

500 vezes teu nome – Nacarid Portal

Sarmiento JAL. **Avaliação da resposta inflamatória, biomineralização e capacidade de reparo tecidual do iodofórmio e hidróxido de cálcio**, 2019. 50p. Dissertação (Mestrado em Endodontia) – Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araçatuba.

Resumo

Além da atividade antimicrobiana e biocompatibilidade de um curativo de demora, é oportuno que ele tenha também a capacidade de estimular a biomineralização e reparo dos tecidos periapicais. O objetivo deste estudo foi avaliar a resposta inflamatória, a capacidade de induzir biomineralização e reparo tecidual em tecido subcutâneo de ratos, causada pelas pastas de iodofórmio e hidróxido de cálcio. Foram utilizados 18 ratos Wistar albinos (n=6), que receberam implantes subcutâneos de tubos de polietileno com os seguintes materiais: hidróxido de cálcio + propilenoglicol (1:1) [$Ca(OH)_2$+P], hidróxido de cálcio + propilenoglicol + iodofórmio (2:1:1) [$Ca(OH)_2$+P+Iodo], iodofórmio + Carbowax (5:1) [Iodo+Carbow] and Carbowax [Carbow]. Tubos vazios extras foram utilizados como grupo controle. Após 7, 15 e 30 dias, os implantes foram removidos conjuntamente com tecido circundante. Foram utilizadas as colorações de hematoxilina-eosina (HE), Von Kossa (VK), técnica de luz polarizada (LP) e Picrosirius red (PSR). Para HE foram utilizados os scores de 0, poucas células inflamatórias; 1, menos de 25 células inflamatórias - reação leve; 2, entre 25 e 125 células inflamatórias - reação moderada; e 3, 125 ou mais células inflamatórias – reação severa (400x). A cápsula fibrosa foi considerada fina quando menor que 150 μm e espessa quando maior que 150 μm. A capacidade de mineralização foi analisada como positivo ou negativo com VK e como presente ou ausente sob LP. Para PSR as fibras imaturas apareciam em amarelo-esverdeadas e finas, enquanto as fibras maturas em tons vermelho-amareladas e espessas. Foi calculada proporção de fibras maturas para área analisada dividindo-se a percentagem de fibras maturas pela a percentagem de fibras imaturas. Foi utilizado o teste de Kruskal-Wallis com um nível de significância de 5%. Todos os grupos, excluindo o carbowax, exibiram escore 2 aos 7 dias e capsula fibrosa espessa, semelhante ao grupo controle. Após 15 dias todos os grupos, menos o controle, tiveram uma diminuição da espessura da cápsula fibrosa. Aos 30 todos os grupos apresentaram score 1 e uma cápsula fibrosa fina. Apenas os grupos que continham hidróxido de cálcio apresentaram tecido mineralizado em todos os períodos analisados. Aos 7 dias todos os grupos mostraram uma maior proporção de fibras imaturas. Aos 15 dias; somente os grupos controle, [$Ca(OH)_2$ + P] e [Carbow] incrementaram a proporção de fibras maduras/imaturas. Aos 30 dias, o grupo [$Ca(OH)_2$ + P] foi o único que apresentou prevalência de fibras colágenas maturas, com diferencia significativa ($p < 0,05$). A hipótese nula foi parcialmente rejeitada. Todos os grupos mostraram biocompatibilidade. Apenas as pastas contendo Ca (OH) 2 induziam a biomineralização dos tecidos. A adição do iodofórmio retarda a capacidade de reparação tecidual.

Palavras-chaves: Iodoformium. Hidróxido de cálcio. Inflamação. Colágeno

Lista de Abreviações

HE - Hematoxylin – Eosin

VK - Von Kossa

PL - Polarized Light

PSR - Picrosirius Red

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Sumário

Abstract

The antimicrobial activity and biocompatibility of a root canal dressing are important requirement, but it is also interesting that it has the ability of stimulate biomineralization and repair of the periapical tissues. The objective of this study was to evaluate the inflammatory response, biomineralization and tissue repair induction in subcutaneous tissue of rats caused by iodoform and calcium hydroxide. Eighteen Wistar rats (n=6) received subcutaneous implants with iodoform and calcium hydroxide pastes. Extra empty tubes were used as control group. After 7, 15 and 30 days, the implants were removed with surrounding tissue. Kruskal-Wallis test was used with a significance level of 5%. At 7 days, all groups excluding carbowax, showed moderate inflammatory reaction and thick fibrous capsule. At 15 days, all groups excluding control decrease their fibrous capsule thickness. At 30 days all groups presented mild reaction and fine fibrous capsule. Only the groups containing calcium hydroxide presented mineralized tissue in all periods analyzed. At 7 days all groups showed a higher proportion of immature fibers. At 15 days, only [$Ca(OH)_2$ + P] and [Carbow] groups increased their proportion of mature / immature fibers. At 30 days, the [$Ca(OH)_2$ + P] group was the only that presented prevalence of mature collagen fibers, with significant difference ($p < 0.05$). The null hypothesis was partially rejected. All groups showed biocompatibility. Only the groups containing $Ca(OH)_2$ induced the biomineralization of the tissues. The addition of iodoform delay the ability of tissue repair.

Keywords: iodoformium, calcium hydroxide, inflammation, collagen

Introduction

Different studies have shown that after the biomechanical preparation there is still a big number of microorganisms present within the root canal system due to the complex anatomy that facilitates the permanence of them in critical areas such as isthmus, apical deltas and accessory root canals, where neither the instruments nor the auxiliary chemical substances can reach[1,2]. Literature shows that about 40-60% of teeth that received endodontic treatment are still contaminated[3]. Calcium hydroxide is one of the most used intracanal dressing during endodontic therapy, due to its antibacterial[4, 5] and reparative properties trough to the dissociation of the calcium and hydroxyl ions. Calcium ions, in contact with carbon dioxide of the tissues, form calcium carbonate molecules, which are the responsible for stimulating the differentiation in cells with mineralizing capacity[6]; furthermore, it helps to reduce the levels of carbon dioxide needed for anaerobic bacteria respiration, providing also an antibacterial effect [7, 8]. On the other hand, hydroxyl ions provide an alkaline pH, favoring the conditions of the environment for tissue repair by inhibiting the osteoclastic activity and inactivating the enzymes of bacteria plasmatic membrane, altering the transport of nutrients and organic components to the interior of the cell[5-7]. Some of the most used formulations of calcium hydroxide are prepared with serum, propylene glycol and polyethylene glycol. In 1994 Holland *et al.* suggested the use of a paste conformed by calcium hydroxide, propylene glycol and iodoform. The propylene glycol is one of the most appropriate vehicles[9], for not presenting toxicity and having a viscous consistency, allowing a slower release of calcium and hydroxyl ions and therefore the dressing stays longer in the root canal space, exercising the two properties already mentioned. The use of iodoform improves the radiopacity of calcium hydroxide, making able to verify the correct filling of the dressing inside the root canal[10], and appearing as a radiopaque image.

Iodoform, besides being evaluated as a radiopacifier, has been also studied as a root canal filling cement in deciduous teeth[11], and as root canal dressing. This material has showed antimicrobial activity against *Enterococus faecalis*[12, 13] and *Candida albicans*[14] which are common species found in persistent infections[1]. Iodoform mechanism action consists in the release of iodine, which can oxidize irreversibly and inactivate vital metabolic compounds, such as proteins, by its antimicrobial action [15, 16.] Formulations using calcium hydroxide with iodoform have been also demonstrated to be efficient antimicrobials against strains present in periapical lesions[17].

Some authors have proposed the use of other medications different from calcium hydroxide to be employ as intracanal dressing. This is the case of iodoform + Carbowax (polyethylene glycol with a higher molecular mass, used as a vehicle) association, which was compared with the calcium hydroxide + propylene glycol paste[18]. The study evaluated the inflammatory response of the tissue in wounds made on rat's back, using experimental periods between 3-11 days. The authors concluded that, the association of iodoform + Carbowax have a better tissue response than the calcium hydroxide paste. However, the methodology used in this experiment was not according to the biocompatibility evaluation standards for any material, stablished by ISO[19].

The biocompatibility of a material use in the endodontic treatment is an important requirement[5,20,21], but it is also interesting that it has the ability of induce biomineralization[15, 16, 22] and repair of the periapical tissues. Because of this, the objective of this study was to evaluate the inflammatory response, biomineralization ability and collagen fiber maturation in subcutaneous tissue of rats caused by iodoform and calcium hydroxide.

The null hypothesis was that, there was not significant difference between iodoform an calcium hydroxide pastes in terms of inflammation, biomineralization and collagen fibers maturation.

Methodology

A total of eighteen Wistar albino rats, (250-300 g) were used. They were housed in temperature-controlled rooms receiving solid diet (Guabi Nutrilabor, Mogiana Alimentos SA, Brazil) and water *ad libitum*. The care of the animals was according to the Ethical Committee, approved by relevant guidelines (CEUA protocol 00425-2018).

Seventy-two polyethylene tubes with 1.5 mm external diameter and 10 mm length were made and filled them with the following experimental materials: calcium hydroxide + propylene glycol (1:1) [$Ca(OH)_2$+P], calcium hydroxide + propyleneglycol + iodoform (2:1:1) [$Ca(OH)_2$+P+Iodo], iodoform + Carbowax (5:1) [Iodo+Carbow] and Carbowax [Carbow]. Extra empty tubes were used as the control group.

The surgical procedure was performed following previous studies[5, 21, 38]. After administration of xylazine (10mg/ kg Rhobifarma Indústria Farmacêutica Ltda,

Hortolândia, Brazil) and ketamine (25 mg/kg União Química Farmacêutica Nacional S/A, São Paulo, Brazil) intramuscular anesthesias, the backs of the animals were shaved, antisepsis was obtained with 5% iodine solution, and a 1-cm incision was formed in a head-tail orientation with no. 15 Bard-Parker blade (BD, Franklin Lakes, NJ). The skin was reflected to create two pockets on the right side (upper and lower) and another two pockets on the left side of the incision (upper and lower), totalizing four experimental sites. Four polyethylene tubes, containing the described materials, were implanted in the dorsal region of each animal in the created pockets in opposite directions (upper right, upper left, lower right, and lower left), and the skin was closed with a 4/0 silk suture (Johnson & Johnson Produtos Prossionais Ltda, São José dos Campos, Brazil).

After 7, 15 and 30 days, the animals were euthanized by an anesthetic overdose. Polyethylene tubes were removed with the surrounding tissues and fixed in10% formalin solution at a pH7.0. The fixed specimens were processed and embedded in paraffin and serially sectioned into 5-μm cuts for staining with hematoxylin-eosin and picrosirius red, and 10-μm cuts for staining with the Von Kossa technique, used to observe mineralization as it darkly stains mineralized structures. Some slices were kept unstained for examination under polarized light to observe the presence of birefringent structures. Histologic analysis was performed by a single calibrated operator in a blinded manner under ×400 light microscopy (DM 4000 B; Leica, Wetzlar, Germany). Tissue reactions at the open end of the tubes were scored according to previous studies[5, 21, 38] as follows: 0, few inflammatory cells or no reaction; 1, less than 25 cells and mild reaction; 2, between 25 and 125 inflammatory cells and moderate reaction; and 3, 125 or more inflammatory cells and severe reaction. Fibrous capsules were considered thin when <150 μm and thick when > 150 μm, as exemplified in Fig. 1. The Hematoxylin-Eosin staining allowed the evaluation of the contact area between the subcutaneous tissue and the tested material (at the lower center of the polyethylene tube) and one of the scores was attributed, classifying the tissue response as mild, moderate, or severe for each experimental time period of 7, 15 and 30 days. Calcification was recorded as positive or negative by Von Kossa staining and present or absent under PL

The maturation level of collagen fibers was analyzed by PSR under polarized light microscopy (DM 4000 B; Leica, Wetzlar, Germany). The program QWin was used (400x magnification; Leica QWin V3; Leica Microsystems), allowing the selection of

corresponding colors for each type of collagen fiber. After color selection, the program automatically calculated the marked area of each collagen type. Greenish-yellow fibers are considered immature and thin, whilst yellowish-red fibers are considered mature and thick [24]. The values of red and green fiber obtained by the QWin program were converted into percentage in relation to the total area. Then, the percentage of mature fibers was divided by the percentage of immature fibers, in order to obtain the mature/immature fibers proportion. Thus, values < 1 (50%:50%) indicated prevalence of immature fibers whereas values > 1 indicated the predominance of mature fibers.

Statistical Analysis

Data were collected and analyzed by a single, calibrated and blinded operator. The SigmaPlot 12.0 program was used for the statistical analyses. Non-parametric data did not pass the Shapiro Wilk test. Due to this, the Kruskal–Wallis test was used for all statistical analyses and were applied at a significance level of 5% ($p < 0.05$).

Results

Control group

A moderate inflammatory reaction (score 2) was observed in the 7- and 15-day period (Figure 1 A,B and Table 1). The inflammatory infiltrate present was composed generally by lymphocytes and macrophages in a thick fibrous capsule. After 30 days, the capsule surrounding the opening of the tube decreased in thickness and exhibited a lower inflammatory infiltrate (score 1) (Figure1 C). The control group was negative for the VK staining and no birefringent structures were observed under PL (Figure 2 a, b, c)

In terms of the amount of collagen fibers, there was a higher proportion of immature than mature fibers on 7 (0.619),15 (0.805) and 30 (0.724) days. (Table 2).

Calcium hydroxide + propylene glycol group [$Ca(OH)_2$ + P]

On days 7 and 15 a moderate inflammatory reaction (score 2) (Figure 1 D, E and Table 1) was presented, and it was reduced (score 1) until day 30 (Figure 1 Ff and Table

1). The fibrous capsule at the opening of the tube was thick in the first two experimental period, and thin by the end (Figure 1 d, e and Table 1). VK staining was positive and birefringent structures were observed under LP in all analyzed periods (Figure 2 Dd, Ee, Ff)

Picrosirius Red staining showed prevalence of immature fibers at 7 and 15 days and a notable increase of mature collagen fibers at 30 days (Figure 3 D-F), obtaining the highest proportion of mature fibers in comparison with the others experimental groups ($p<0.05$).

Calcium hydroxide + propylene glycol + iodoform group [$Ca(OH)_2$ + P + Iodo]

The two first analyzed periods presented a moderate inflammatory reaction (score 2), and a thick inflammatory fibrous capsule with the presence of macrophages, lymphocytes, giant cells, and some large blood vessels profiles (Figure 1 Gg and Hh). On day 30 the intensity of the inflammation was reduced (score 1) in 50% of the sample (Figure 1 I and Table 1) and fibrous capsule was thin, similar to the control group (Figure 1i). VK staining was positive and birefringent granulations were present in all analyzed periods under PL (Figure 2, Gg, Hh, Ii).

In all experimental periods there was a prevalence of immature fibers (Figure 4 G-I).

Iodoform + carbowax group [Iodo + Carbow]

A moderate inflammatory reaction (score 2) was observed in the 7- and 15-day period. The inflammatory infiltrate present was composed generally by macrophages and some giant cells contained in a fibrous capsule, which was thick (Table 1 and Figure 1 Jj,Kk) At the end of the experiment, the fibrous capsule reduced its thickness, presenting a mild inflammatory reaction in 3 of 6 specimens (score 1) (Table 1 and Figure 2 Ll). The other samples presented moderate to severe inflammatory reaction (score 2) (Table 1 and Figure 1 Ll). VK staining was negative and no birefringent structures were observed under LP in all analyzed periods (Figure 2 Jj, Kk, Ll).

There was a prevalence of immature collagen fibers in all periods, mainly on day 15 (Figure 3 J-L).

Carbowax group [Carbow]

This experimental group was the one that presented the highest inflammatory reaction on day 7 consisting in macrophages and lymphocytes in a thick fibrous capsule (score 3) (Table 1 and Figure1 Mm). On day 15 there was a reduction of the inflammatory reaction (score 2) and the thickness of the fibrous capsule in half of the specimens were classified as thin (Table 1 and Figure 1 Nn). At the end of the experiment, a mild inflammatory reaction was present and the fibrous capsules of all analyzed specimens were thin (Table 1 and Figure 1 Oo). VK staining was negative and no birefringent structures were observed under LP in all analyzed periods (Figure 2 Mm, Nn and Oo).

Immature collagen fibers appeared in higher proportion during all the analyzed periods, especially at 30 days (Figure 3 M-O).

Comparison among groups

All the groups, excluding the carbowax, exhibited a moderate inflammatory reaction (score 2) and a thick fibrous capsule on day 7, similar to the control group, which is normal due to the trauma produced at the surgical process ($p>0.05$). At 15 days, the fibrous capsule of some groups became thin, and on day 30 there was observed a mild reaction (score 1) and a thin fibrous capsule in all groups, the same as the control; which demonstrated biocompatibility of all the pastes ($p>0.05$). Only groups with calcium hydroxide in their composition presented mineralized tissue at the open of the tubes with the Von Kossa staining and under polarized light in all analyzed periods. The PSR analysis showed that all groups had a higher proportion of immatures fibers at 7 days, since all the values for this period were < 1 (Table 2). At 15 days; the control, [$Ca(OH)_2$ + P] and [Carbow] group had an increase in the mature/immature fiber proportion, unlike the others groups. At 30 days, [$Ca(OH)_2$ + P] was the only group that had prevalence of mature fibers, presenting significant difference with all others ($p<0.05$).

Table 1 – Inflammatory score of each group, fibrous capsule thickness, Von Kossa and Polarized light

	Score					Capsule			
(N = 6)	**0**	**1**	**2**	**3**	**Median***	**Thick**	**Thin**	**VK**	**PL**
Group									
7 days									
Control	**0**	**1**	**4**	**1**	**2[a]**	**6**	**0**	**-**	**absent**
$Ca(OH)_2$ +P	**0**	**1**	**4**	**1**	**2[a]**	**6**	**0**	**+**	**present**
$Ca(OH)_2$+P+Iodo	**0**	**0**	**5**	**1**	**2[a]**	**6**	**0**	**+**	**present**
Iodo+Carbow	**0**	**0**	**4**	**2**	**2[a]**	**6**	**0**	**-**	**absent**
Carbow	**0**	**0**	**2**	**4**	**3[b]**	**6**	**0**	**-**	**absent**
15 days									
Control	**0**	**2**	**3**	**1**	**2 [a]**	**6**	**0**	**-**	**absent**
$Ca(OH)_2$ +P	**0**	**1**	**5**	**0**	**2 [a]**	**4**	**2**	**+**	**present**
$Ca(OH)_2$+P+Iodo	**0**	**1**	**4**	**1**	**2 [a]**	**3**	**3**	**+**	**present**
Iodo+Carbow	**0**	**0**	**4**	**2**	**2 [a]**	**5**	**1**	**-**	**absent**
Carbow	**0**	**0**	**4**	**2**	**2 [a]**	**3**	**3**	**-**	**absent**
30 days									
Control	**1**	**5**	**0**	**0**	**1 [a]**	**0**	**6**	**-**	**absent**
$Ca(OH)_2$ +P	**0**	**4**	**2**	**0**	**1 [a]**	**0**	**6**	**+**	**present**
$Ca(OH)_2$+P+Iodo	**0**	**3**	**3**	**0**	**1.5 [a]**	**0**	**6**	**+**	**present**
Iodo+Carbow	**0**	**4**	**2**	**0**	**1 [a]**	**0**	**6**	**-**	**absent**
Carbow	**0**	**4**	**2**	**0**	**1 [a]**	**0**	**6**	**-**	**absent**

*different letters indicated $p < 0.05\%$

Table 2 – Proportion of the percentage of mature / immature collagen fiber of each analyzed experimental

	7D	15D	30D
Control	0.619 ± 0.237^{ab}	0.805 ± 0.189^{a}	0.724 ± 0.556^{ab}
$Ca(OH)_2$ +P	0.357 ± 0.365^{a}	0.400 ± 0.254^{ab}	$*1.539 \pm 0.707^{a}$
$Ca(OH)_2$+P+Iodo	0.486 ± 0.604^{ab}	0.394 ± 0.473^{ab}	0.559 ± 0.396^{b}
Iodo+Carbow	0.874 ± 1.450^{b}	0.321 ± 0.2075^{b}	0.605 ± 0.393^{ab}
Carbow	0.408 ± 0.506^{a}	0.601 ± 0.315^{ab}	0.088 ± 0.405^{b}

*values >1.00 indicate presence of more than 50% of mature fiber
Different letters indicate $p < 0.05\%$

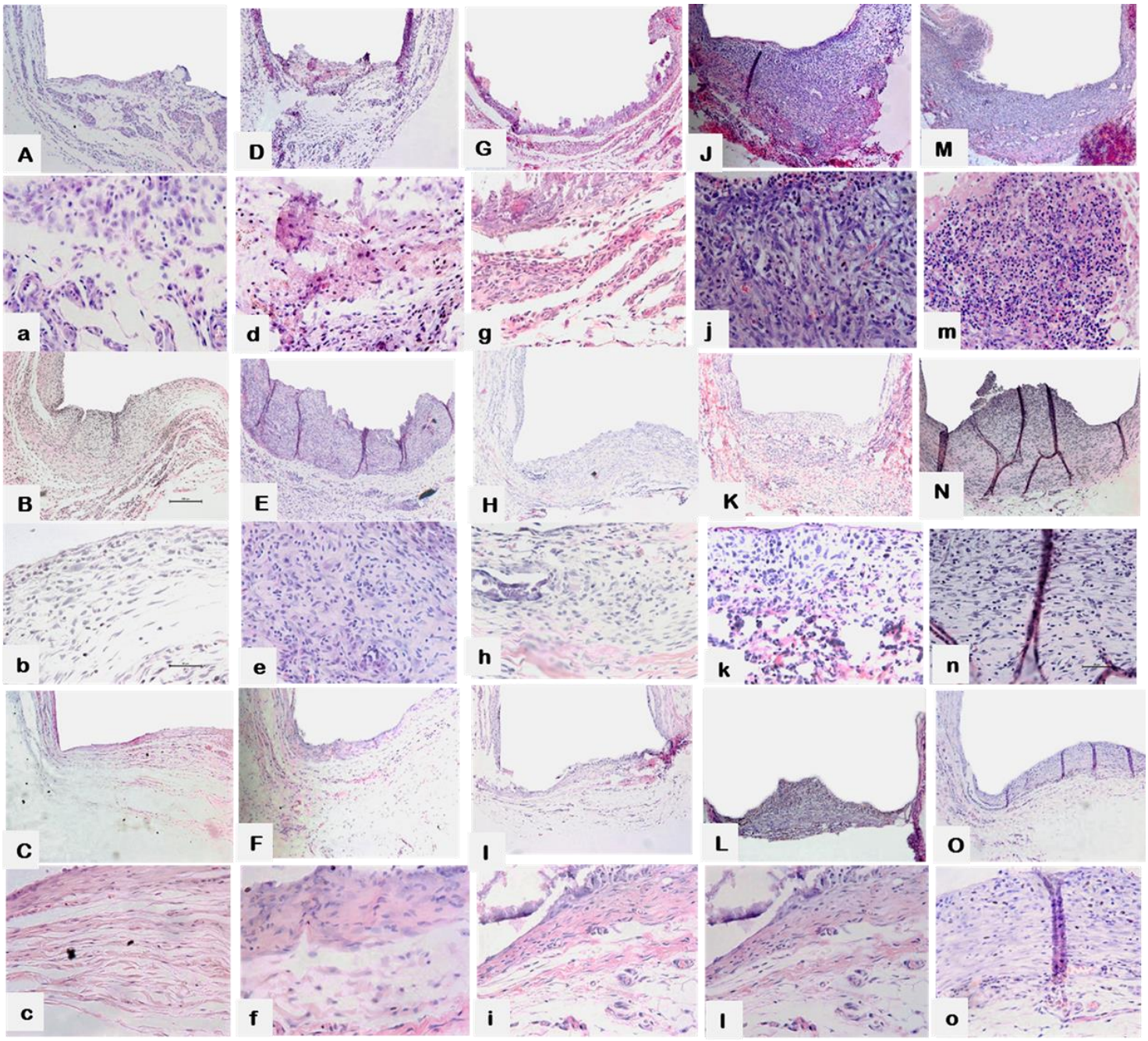


Figure 1 – Tissue reaction on experimental groups: Control Group: A- C (days 7, 15 and 30 HE, 100×) and a-c (days 7, 15 and 30, HE, 400×); $Ca(OH)_2$ +P: D-F (days 7, 15 and 30, HE, 100×) and d-f (days 7, 15 and 30, HE, 400×); $Ca(OH)_2$ + P + Iodo: G-I (days 7, 15 and 30, HE, 100×) and g-i (days 7, 15 and 30, HE, 400×); Iodo + Carbw: J-L (days 7, 15 and 30, HE, 100×) and j-l (days 7, 15 and 30, HE, 400×); CARBOW: M-O (days 7, 15 and 30, HE, 100×) and m-o (days 7, 15 and 30, HE, 400×).

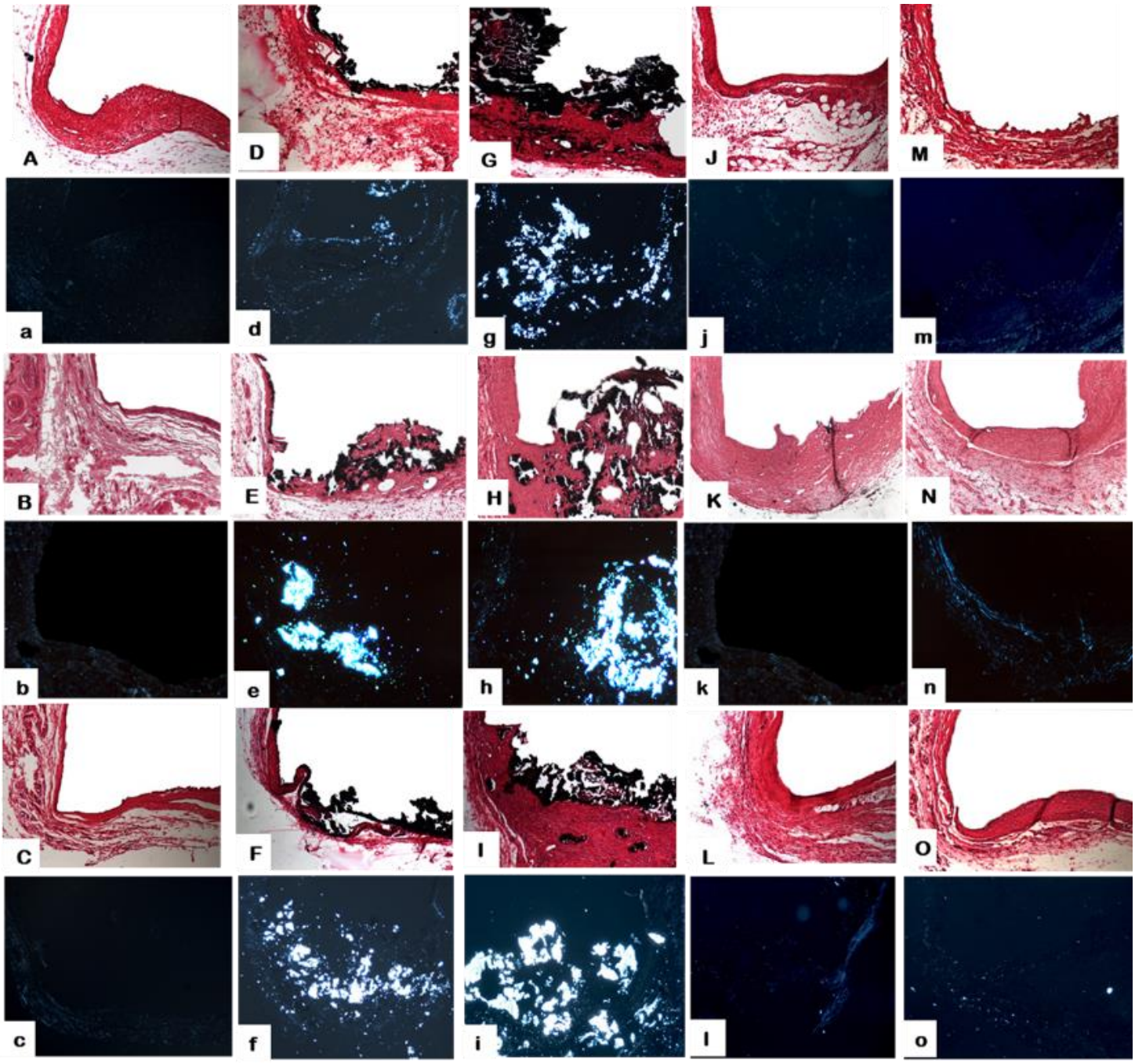
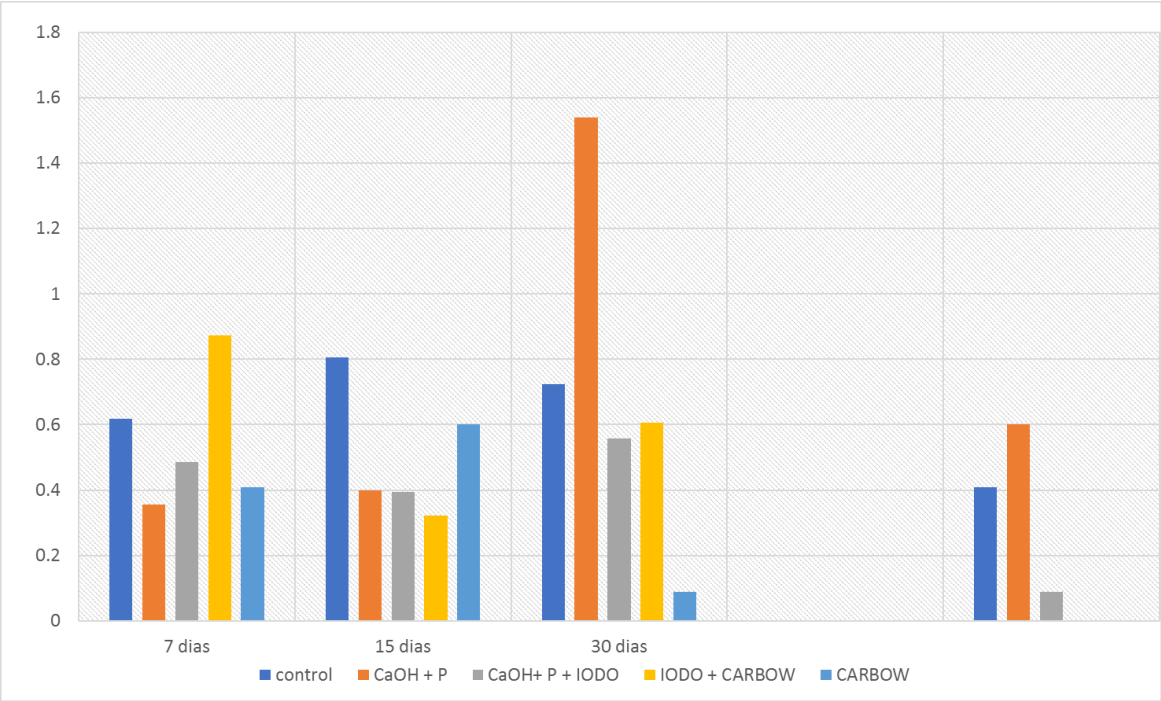


Figure 2 - Mineralization on experimental groups: Control group: A- C (days 7, P and 30; Von Kossa, 100×) and a-c (days 7, 15 and 30, polarized light, 100×); $Ca(OH)_2$ + P : D-F (days 7, 15 and 30; Von Kossa, 100×) and d-f (days 7, 15 and 30; polarized light, 100×); $Ca(OH)_2$+ P + Iodo: H-I (days 7, 15 and 30; Von Kossa, 100×) and h-i (days 7, 15 and 30; polarized light, 100×); Iodo + Carbow : J-L (days 7, 15 and 30; Von Kossa, 100×) and j-l (days 7, 15 and 30, polarized light, 100×); Carbow : M-O (days 7, 15 and 30; Von Kossa, 100×) and m-o (days 7, 15 and 30, polarized light, 100×).

Figure 4 – Evolution of the increase of mature collagen fibers trough the analyzed experimental periods



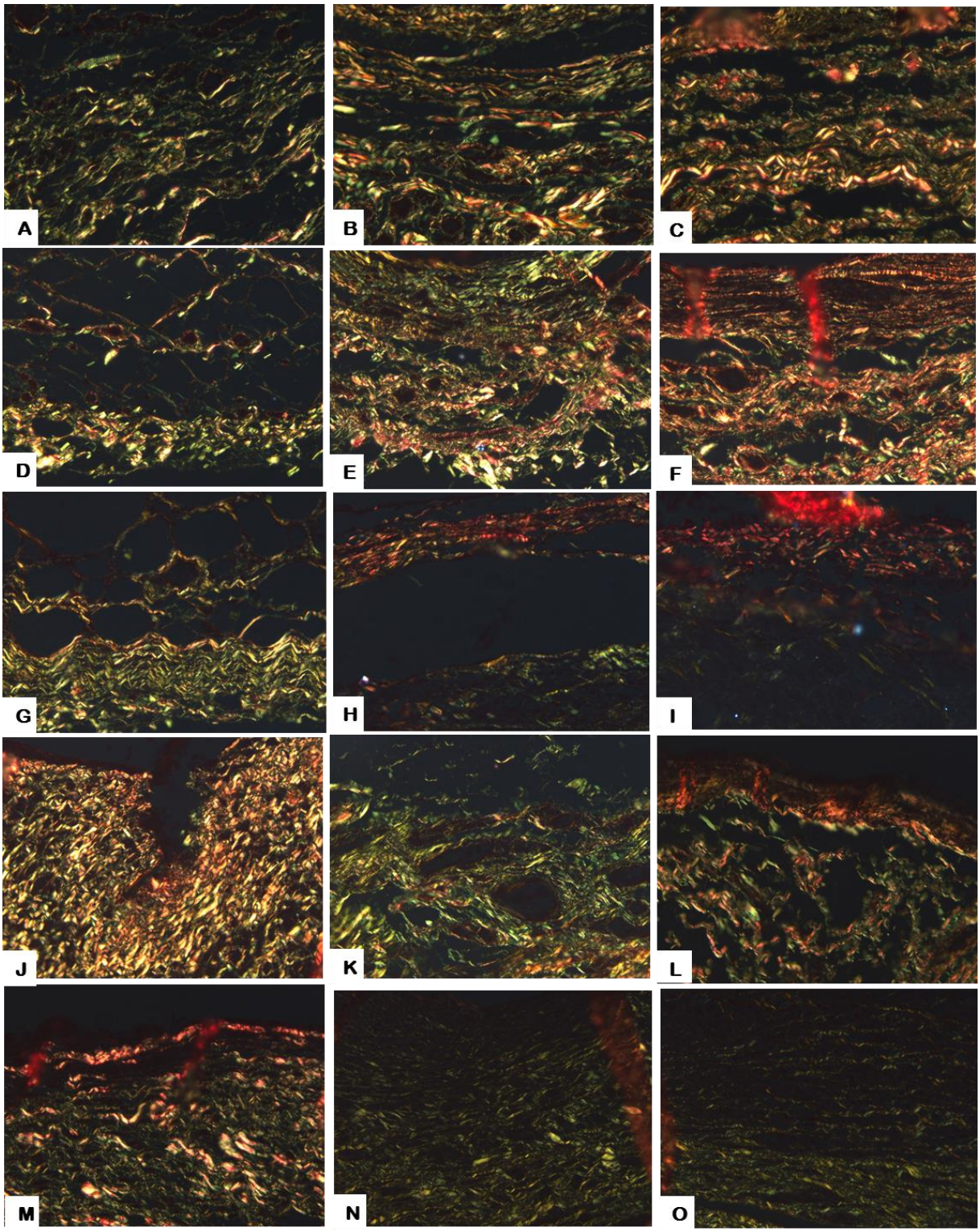


Figure 3 – Collagen fibres are shown as birefringent structures at the open end of the tubes. Greenish-yellow fibres indicate immature collagen, and yellowish-red fibres indicate mature collagen: Control Group: A- C (days 7, 15 and 30 HE, 400×); $Ca(OH)_2$ +P: D-F (days 7, 15 and 30, HE, 400×); $Ca(OH)_2$ + P + Iodo: G-I (days 7, 15 and 30, HE, 400×); Iodo + Carbow: J-L (days 7, 15 and 30, HE, 400×); Carbow: M-O (days 7, 15 and 30, HE, 400×).

Discussion

This study evaluated the biocompatibility, mineralization ability and tissue repair capacity of iodoform and calcium hydroxide pastes as intracanal dressings. Based on these results, the nulled hypothesis was partially rejected, since all the groups presented biocompatibility and only the pastes containing calcium hydroxide allowed biomineralization and a better tissue repair. The use of an intracanal dressing consists in the application of a drug inside the root canal for a longer period than a single visit, and with the purpose of having a therapeutic effect[25, 26]. Beside the fulfillment of the microbiological objective like, the eradication of microorganism that survive to chemical mechanical preparation and the inactivation of bacterial byproducts[27]; the medication to be used must stimulate the tissue repair by biomineralization[28-30].

The tissue repair process after root canal treatment consists in a series of biological events, that begins with the inflammation and finishes with the proliferation and remodeling phase[31], this was observed in our study. All the groups exhibited a moderate inflammatory reaction on day 7 similar to the control group, which is normal, due to the trauma produced at the surgical process[21]. Cintra *et al* (2017)[32] evaluated the tissue response of different endodontic sealers and observed that the principal histological criteria in regard of biocompatibility, is the absence of inflammation, what agrees with the present results at the end of the experiment (30 days), where was observed a mild reaction and a thin fibrous capsule in all the groups.

However, in relation to the objective that a root canal dressing besides to have antibacterial properties, should also stimulate the tissue repair by biomineralization [16, 22], early studies of Holland *et al.* (1971)[23] evaluated the answer of pulp stump and periapical tissues of several materials, concluding that solely the calcium hydroxide favored the deposition of cement in the periapical region. Based on these results, Bueno *et al.* (2016)[5] and Cosme-Silva *et al* (2019)[33] evaluated endodontic sealers added or forming calcium hydroxide respectively. They observed that these cements besides being biocompatible, also induced biomineralization.

In this study the addition of iodoform to the [$Ca(OH)_2$ + P] paste did not interfere in the mineralization process, agreeing with previous studies of Ordinola-Zapata *et al*. (2015)[34] and Kuwa *et al*. (2014)[35] who evaluated the effect of radiopacifying agents (within them the iodoform) on the pH level and calcium ion release. On the other hand, the [Iodo + Carbow] paste did not induced deposition of mineralized tissue at the open of

the tube in all the analyzed periods (Figure 2). According previous studies it is necessary to have deposition of mineralized tissue at the periapical tissues after the endodontic treatment in order to have a biological seal[21, 23, 28, 32, 36, 37]. Thus, the only compound that has been proven by several studies, which is able to stimulate the formation of hard tissue, is the calcium hydroxide.

In this study the presence of collagen fibers is a positive signal that indicates the repare of a traumatized area by the surgical process, in the same way as happens after a canal instrumentation[31]. The analysis of collagen fiber found in each period was made by Picrosirius red (PSR) staining. This is a specific method for collagen fiber detection, which is capable of distinguish different collagen fiber types, especially when this protein is present in small amounts or is too thin[38]. PSR is an elongated dye molecule which reacts with collagen, increasing his normal birefringence[19] and allowing to observe the differences in fiber color, greenish-yellow colors suggest that the collagen is poorly packed (immature fibers), whilst yellowish-red colors are originated from tightly packed fibers (mature fibers) [24.]

At 7 days all the groups presented prevalence of immature collagen fiber, the same as the control group. On day 15 it was expected to have a greater proportion of mature/immature fiber, however, the [Iodo+Carbow] group showed an evident decrease of this proportion from day 7 to day 15. This can be explained because, when the tissue is in contact with an irritating material, the poorly packed fibers (immature fibers) are the first to be affected and the tightly packed fibers (mature fibers) are more resistant to the aggression, thus they appeared in greater proportion at 7 days. Then, the production of collagen fibers starts again, so there is a greater evidence of immature young collagen fibers on day 15.

At 30 days, [$Ca(OH)_2$ + P] was the only group that had prevalence of mature fibers, presenting significant difference with the others ($p<0.05$) (Table 2). The addition of iodoform to the [$Ca(OH)_2$ + P] group not only did not influence positively in the tissue repair progress, but also delayed the maturation of collagen fibers. Estrella *et al*. (2006)[16] consider that the biological ability of iodoform, may only be a hypothesis, due to lack of investigations and that the addition of iodoform to the calcium hydroxide paste, would be only to enhance the radiopacity, which agreed with our results. A study performed with implants in subcutaneous tissue of rats conducted by de Morais *et al* (2006)[39] evaluated the tissue response of Portland cement with iodoform, concluding that the paste was

harmless to connective tissue. However, in this study it was only use the HE analyses, not allowing to observed the collagen fibers maturity.

Sometimes, the different mechanisms and properties of two different medicaments can minimize the action of one of them, instead of potentializing. In this sense, it could be inferred that the only benefit of the addition of iodoform would be to enhanced the radiopacity of the calcium hydroxide when use as an intracanal dressing, in agreement with the studies of Lourenço Neto *et al.* 2015[40] e Marques *et al.* 2015 [41.] On the other hand, our findings concord with previous research in which calcium hydroxide has important effects on tissue, accelerating the process of tissue repair[6]. Carbowax presented the highest levels of immature fibers at 30 days, which would mean a very slow repair rate.

Conclusion

According to the animal model used, all groups showed biocompatibility. Only the pastes containing $Ca(OH)_2$ induced biomineralization of tissues. The addition of the iodoform delay the tissue repair capacity.

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Anexos

Anexo A

Brazilian Oral Research Journal

Presentation of the manuscript

The manuscript text should be written in English and provided in a digital file compatible with "Microsoft Word" (in DOC, DOCX, or RTF format).

All figures (including those in layouts/combinations) must be provided in individual and separate files, according to recommendations described under the specific topic.
Photographs, micrographs, and radiographs should be provided in TIFF format, according to the recommendations described under the specific topic.

Charts, drawings, layouts, and other vector illustrations must be provided in a PDF format individually in separate files, according to the recommendations described under the specific topic.

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- This must indicate the specialty* or research field focused on in the manuscript.

*Anatomy; Basic Implantodontology and Biomaterials; Behavioral Sciences; Biochemistry; Cariology; Community Dental Health; Craniofacial Biology; Dental Materials; Dentistry; Endodontic Therapy; Forensic Dentistry; Geriatric Dentistry; Imaginology; Immunology; Implantodontology – Prosthetics; Implantodontology – Surgical; Infection Control; Microbiology; Mouth and Jaw Surgery; Occlusion; Oral Pathology; Orthodontics; Orthopedics; Pediatric Dentistry; Periodontics; Pharmacology; Physiology; Prosthesis; Pulp Biology; Social/Community Dentistry; Stomatology; Temporomandibular Joint Dysfunction.

- Informative and concise title, limited to a maximum of 110 characters, including spaces.

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Abstract: This should be presented as a single structured paragraph (but with no subdivisions into sections) containing the objective of the work, methodology, results, and conclusions. In the System if applicable, use the Special characters tool for special characters.

Keywords: Ranging from 3 (three) to 5 (five) main descriptors should be provided, chosen from the keywords registered at http://decs.bvs.br/ or http://www.nlm.nih.gov/mesh/MBrowser.html (no synonyms will be accepted).

Main Text

Introduction: This should present the relevance of the study, and its connection with other published works in the same line of research or field, identifying its limitations and possible biases. The objective of the study should be concisely presented at the end of this section.

Methodology: All the features of the material pertinent to the research subject should be provided (*e.g.*, tissue samples or research subjects). The experimental, analytical, and statistical methods should be described in a concise manner, although in detail, sufficient to allow others to recreate the work. Data from manufacturers or suppliers of products, equipment, or software must be explicit when first mentioned in this section, as follows: manufacturer's name, city, and country. The computer programs and statistical methods must also be specified. Unless the objective of the work is to compare products or specific systems, the trade names of techniques, as well as products, or scientific and clinical equipment should only be cited in the "Methodology" and "Acknowledgments" sections, according to each case. Generic names should be used in the remainder of the manuscript, including the title. Manuscripts containing radiographs, microradiographs, or SEM images, the following information must be included: radiation source, filters, and kV levels used. Manuscripts reporting studies on humans should include proof that the research was ethically conducted according to the Helsinki Declaration (*World Medical Association,* http://www.wma.net/en/30publications/10policies/b3/). The approval protocol number issued by an Institutional Ethics Committee must be cited. Observational studies should follow the STROBE guidelines (http://strobe-statement.org/), and the check list must be submitted. Clinical Trials must be reported according to the CONSORT Statement standard protocol (http://www.consort-statement.org/); systematic reviews

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Manuscripts reporting studies performed on animals must also include proof that the research was conducted in an ethical manner, and the approval protocol number issued by an Institutional Ethics Committee should be cited. In case the research contains a gene registration, before submission, the new gene sequences must be included in a public database, and the access number should be provided to BOR. The authors may use the following databases:

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Results: These should be presented in the same order as the experiment was performed, as described under the "Methodology" section. The most significant results should be described. Text, tables, and figures should not be repetitive. Statistically relevant results should be presented with enclosed corresponding p values.

Tables: These must be numbered and cited consecutively in the main text, in Arabic numerals. Tables must be submitted separately from the text in DOC, DOCX, or RTF format.

Discussion: This must discuss the study results in relation to the work hypothesis and relevant literature. It should describe the similarities and differences of the study in relation to similar studies found in literature, and provide explanations for the possible differences found. It must also identify the study's limitations and make suggestions for future research.

Conclusions: These must be presented in a concise manner and be strictly based on the results obtained in the research. Detailing of results, including numerical values, etc., must not be repeated.

Acknowledgments: Contributions by colleagues (technical assistance, critical comments, etc.) must be given, and any bond between authors and companies must be revealed. This section must describe the research funding source(s), including the corresponding process numbers.

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References: Only publications from peer-reviewed journals will be accepted as references. Unfinished manuscripts, dissertations, theses, or abstracts presented in congresses will not be accepted as references. References to books should be avoided.

Reference citations must be identified in the text with superscript Arabic numerals. The complete reference list must be presented after the "Acknowledgments" section, and the references must be numbered and presented in Vancouver Style in compliance with the guidelines provided by the International Committee of Medical Journal Editors, as presented in Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.ncbi.nlm.nih.gov/books/NBK7256/). The journal titles should be abbreviated according to the List of Journals Indexed in Index Medicus (http://www.ncbi.nlm.nih.gov/nlmcatalog/journals). The authors shall bear full responsibility for the accuracy of their references.

Spelling of scientific terms: When first mentioned in the main text, scientific names (binomials of microbiological, zoological, and botanical nomenclature) must be written out in full, as well as the names of chemical compounds and elements.

Units of measurement: These must be presented according to the International System of Units (http://www.bipm.org or http://www.inmetro.gov.br/consumidor/unidLegaisMed.asp).

Footnotes on the main text: These must be indicated by asterisks and restricted to the bare minimum.

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Characteristics and layouts of types of manuscripts

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Limited to 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 8 (eight) figures and 40

(forty) references will be accepted. The abstract can contain a maximum of 250 words.

Layout - Text Files

- Title Page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Keywords: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: maximum of 40 references
- Figure legends

Layout - Graphic Files

- Figures: a maximum of 8 (eight) figures, as described above.

Short Communication

Limited to 10,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). A maximum of 2 (two) figures and 12 (twelve) references will be allowed. The abstract can contain a maximum of 100 words.

Layout - Text Files

- Title page
- Main text (10,000 characters including spaces)
- Abstract: a maximum of 100 words
- Descriptors: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: a maximum of 12 references
- Figure legends

Layout- Graphic Files

- Figures: a maximum of 2 (two) figures, as described above.

Critical Review of Literature

The submission of this type of manuscript will be

performed only by invitation of the BOR Publishing Commission. All manuscripts will be submitted to peer-review. This type of manuscript must have a descriptive and discursive content, focusing on a comprehensive presentation and discussion of important and innovative scientific issues, with a limit of 30,000 characters including spaces (considering the introduction, methodology, results, discussion, conclusion, acknowledgments, tables, references, and figure legends). It must include a clear presentation of the scientific object, logical argumentation, a methodological and theoretical critical analysis of the studies, and a summarized conclusion. A maximum of 6 (six) figures and 50 (fifty) references is permitted. The abstract must contain a maximum of 250 words.

Layout- Text Files

- Title page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Keywords: 3 (three)-5 (five) main descriptors
- Introduction
- Methodology
- Results
- Discussion
- Conclusion
- Acknowledgments
- Tables
- References: maximum of 50 references
- Figure legends

Layout - Graphic Files

- Figures: a maximum of 6 (six) figures, as described above.

Systematic Review and Meta-Analysis
While summarizing the results of original studies, quantitative or qualitative, this type of manuscript should answer a specific question, with a limit of 30,000 characters, including spaces, and follow the Cochrane format and style (www.cochrane.org). The manuscript must report, in detail, the process of the search and retrieval of the original works, the selection criteria of the studies included in the review, and provide an abstract of the results obtained in the reviewed studies (with or without a meta-analysis approach). There is no limit to the number of references or figures. Tables and figures, if included, must present the features of the reviewed studies, the compared interventions, and the corresponding results, as well as those studies excluded from the review. Other tables and figures relevant to the review must be presented as previously described. The abstract can contain a maximum of 250 words.

Layout - Text Files

- Title page
- Main text (30,000 characters including spaces)
- Abstract: a maximum of 250 words
- Question formulation
- Location of the studies
- Critical Evaluation and Data Collection
- Data analysis and presentation
- Improvement
- Review update
- References: no limit on the number of references
- Tables

Layout - Graphic Files

- Figures: no limit on the number of figures

Letter to the Editor

Letters must include evidence to support an opinion of the author(s) about the scientific or editorial content of the BOR, and must be limited to 500 words. No figures or tables are permitted.

Copyright transfer agreement and responsibility statements

The manuscript submitted for publication must include the Copyright Transfer Agreement and the Responsibility Statements, available in the online system and mandatory.

CHECKLIST FOR INITIAL SUBMISSION

- Title Page file (in DOC, DOCX, or RTF format).
- Main text file (Main Document, manuscript), in DOC, DOCX, or RTF format.
- Tables, in DOC, DOCX, or RTF format.
- Declaration of interests and funding, submitted in a separate document and in a PDF format. (if applicable)
- Justification for participation of each author, provided in a separate document and in a PDF format.
- Photographs, microradiographs, and radiographs (10 cm minimum width, 500 dpi minimum resolution) in TIFF format. (http://www.ncbi.nlm.nih.gov/pmc/pub/filespec-images/)
- Charts, drawings, layouts, and other vector illustrations in a PDF format.

- Each figure should be submitted individually in separate files (not inserted in the text file).

Publication fees

Authors are not required to pay for the submission or review of articles.

EXAMPLES OF REFERENCES

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Goracci C, Tavares AU, Fabianelli A, Monticelli F, Raffaelli O, Cardoso PC, et al. The adhesion between fiber posts and root canal walls: comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci. 2004 Aug;112(4):353-61.

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Anexo B

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Avaliação da resposta tecidual inflamatória de diferentes associações de iodofórmio"**, Processo FOA nº 00425-2018, sob responsabilidade de Eloi Dezan Júnior apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 14 de Agosto de 2018.

VALIDADE DESTE CERTIFICADO: 14 de Agosto de 2019.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 14 Setembro de 2019.

CERTIFICATE

We certify that the study entitled **"Evaluation of inflammatory tissue response of different association of iodoform"**, Protocol FOA nº 00425-2018, under the supervision of Eloi Dezan Júnior presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on August 14, 2018.

VALIDITY OF THIS CERTIFICATE: August 14, 2019.
DATE OF SUBMISSION OF THE FINAL REPORT: September 14, 2019.

Prof. Ass. Dr. Leonardo Perez Faverani
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
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