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Impact of infected and noninfected human dentine debris on bone healing in rats

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Abstract

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Aim To evaluate *in vivo* the bone tissue response of rats to varying amounts of infected and noninfected dentine debris.

Methodology Bone tissue reactions were evaluated histologically in 42 Wistar rats after 7, 30 and 60 days. For each animal, three surgical cavities were prepared on the femur and filled with varying amounts (5, 10 or 20 mg) of infected or noninfected dentine debris pellets. In the negative control group, the surgical cavities were not filled. At the end of each experimental period, the animals were euthanized. The samples were processed histologically and analysed using a light microscope. The presence and the severity of inflammatory reaction, as well as hard tissue deposition were evaluated. Data were subjected to statistical analysis and the effects of the dependent variables calculated using nonparametric tests Kruskal–Wallis and Mann–Whitney *U* with due Bonferroni corrections at $P = 0.05$.

Results At 7 days, the presence of infected debris significantly increased the histopathological scores for neutrophils ($P < 0.05$), and abscess formation ($P < 0.05$). Noninfected debris scored significantly higher for lymphocyte infiltrate compared with the control group and infected debris ($P < 0.05$). Both infected and noninfected debris equally triggered eosinophil cells compared with no-dentine ($P < 0.05$). As for giant cells and macrophages, no difference was detected amongst the dentine groups ($P > 0.05$). Hard tissue deposition was similar regardless of the presence or the bacteriological status of the dentine ($P = 1.00$). None of the above histopathological parameters was significantly influenced by the amount of debris ($P > 0.05$). For all parameters evaluated, at 7 days of analysis, the inflammatory response was significantly more intense compared with 30 and 60 days ($P < 0.05$). Inflammatory parameters were scored similarly for the evaluated groups after 30 and 60 days ($P > 0.05$). However, hard tissue deposition has significantly increased after 30 days ($P < 0.05$). No difference was seen between 30 and 60 days of analysis ($P = 1.00$) for all histological parameters evaluated.

Conclusion The assumption that the amount of extruded debris may negatively affect the inflammatory response of bone tissue was not validated in the present *in vivo* animal study. Infected dentine may trigger acute inflammatory parameters especially during the first 7 days of contact with the tissue; however, in the long term, these negative effects are mitigated.

Keywords: bone tissue response, debris extrusion, endodontics, infected dentine.

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Introduction

Root canal chemomechanical preparation is one of the most important steps for successful root canal treatment (Farzaneh *et al.* 2004, de Chevigny *et al.* 2008). However, during instrumentation, undesirable

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side effects include dentine chips, pulp tissue fragments, necrotic tissue, microorganisms and irrigants that may be transported apically and extruded into the periradicular tissues (Seltzer & Naidorf 1985, Siqueira *et al.* 2002). Apical extrusion following root canal preparation may trigger inflammatory reactions in the periapical region and subsequent postoperative pain, swelling, (Siqueira 2003, Parirokh *et al.* 2012) and flare-up, possibly delaying or impairing the healing process (Siqueira 2003).

In this sense, root canal instrumentation systems have been routinely scrutinized and ranked by several laboratory studies according to the amount of extruded debris created during root canal preparation (Tinaz *et al.* 2005, Costa *et al.* 2017, Labbaf *et al.* 2017, Western & Dicksit 2017). There is a relatively large body of evidence indicating that endodontic instrumentations systems, notably the mechanized ones, create variable quantities of apically extruded debris (Gutmann & Gao 2012). These results are usually associated with the design, size and taper of the instrument, as well as its kinematics, finally leading to the conclusion that the amount of apically extruded debris is directly related to the instrumentation system and technique chosen for root canal preparation (Koçak *et al.* 2013, Sowmya *et al.* 2014, Caviedes-Bucheli *et al.* 2016).

Although of concern, the results of laboratory studies on debris extrusion during root canal treatment are conflicting (Ahn *et al.* 2016, Pedrinha *et al.* 2018). For example, reciprocating instruments in some studies may cause more debris extrusion than files used under continuous rotation (Bürklein *et al.* 2014, Borges *et al.* 2016, Toyoğlu & Altunbaş 2017, Uslu *et al.* 2018), whilst opposite results are also reported (Tinoco *et al.* 2013, De-Deus *et al.* 2015, Arslan *et al.* 2016). However, the phenomenon of apical extrusion in laboratory studies should not be taken in isolation, since clinically, the presence of periapical tissues may provide a physical natural barrier, limiting the apical extrusion of debris and irrigants (Bonaccorso *et al.* 2009), possibly contributing to reduce its overall impact *in vivo*. Furthermore, the claim that greater volumes of extruded debris would result in increased severity of the inflammatory response is not logical, as not only the amount of debris, but also the virulence and bacteriological status of the microorganisms and the host tissue resistance will be of importance (Elmsallati *et al.* 2009, De-Deus *et al.* 2015).

Even though there are a large number of laboratory studies demonstrating and quantifying the occurrence of apical extrusion with various endodontic preparation systems, there is no biological evidence to support the rational that the amount and the bacteriological status of dentinal debris influences bone tissue response. Therefore, the objective of this study was to evaluate longitudinally, *in vivo*, the bone tissue response of rats to varying amounts of infected and noninfected dentine debris. The core hypotheses tested are that the presence of infected dentine and a greater mass can trigger more severe inflammatory reactions and impair the healing rate of bone tissue in rats.

Materials and methods

The Research Ethics Committee for Animal Use of the Federal University of Pelotas, Brazil approved the study (Protocol n^0 23110.004329/2016-73). All procedures were carried out in accordance with institutional guidelines for animal care and use. Wistar rats (*Rattus norvegicus*; age, 4 months; weight, ~300 g) were used. Bone tissue reactions to infected and noninfected dentine debris were evaluated in 42 rats after three experimental periods (7, 30, and 60 days; $n = 14$ rats per period). Three cavities were prepared in each animal, to give a total of 126 cavities.

Surgical cavities were randomly allocated to the experimental and control groups (six cavities per group, per experimental time). The animals' tails were marked for individual identification. The rats were housed in plastic cages (two per cage) placed in ventilated racks (Alesco, Monte Mor, SP, Brazil) at 22 °C with a 12 h light/dark cycle (lights on between 7am to 7 pm). The animals were provided with a standard diet of rat chow (Nuvilab, Colombo, PR, Brazil) and filtered water *ad libitum* during the experiment.

Sample calculation

The minimum number of samples needed to identify differences between groups was determined using the G * Power 3.1 programme for Mac (one-way ANOVA test from the F family of tests). Due to the absence of previous studies that correlated the volume of extruded dentine with inflammatory tissue reaction, an average effect size of 0.7 was chosen. Other parameters included were: alpha-error = 0.05, beta-power = 0.8 and correlation between the repeated values of 0.5. The result indicated a minimum of 5 samples per group and per experimental time.

Production of infected and noninfected dentinal debris

Four freshly extracted molar teeth were collected, for reasons not related to this research. The teeth were kept in 0.5% sodium hypochlorite to remove soft tissue, rinsed in distilled water and autoclaved. The crowns of the teeth were removed with a diamond saw, and dentine debris was obtained by cutting the inner portion of the pulp chamber with a no. five spherical bur, driven in a slow-speed handpiece under irrigation. Dentine debris were then, immediately suspended in brain-heart infusion broth (BHI; Becton Dickinson, Sparks, MD, USA).

With the aim of keeping the dentine debris moist, in the noninfected debris group, they were suspended in sterile BHI (Becton Dickinson, Sparks, MD, USA), for 7 days before being used. For the infected debris group, subgingival plaque was collected from an adult, healthy donor and suspended in BHI. The dentine debris was incubated in this suspension using 24 well plates under anaerobic conditions (AnaeroGen; OXOID, Basingstoke, UK) at 37 °C for 1 week.

Before animal procedures, the excess BHI medium was removed using filter papers, the dentine debris was weighed in a precision scale and pellets of dentine debris were formed with the aid of a small plugger (Odous-De-Deus, Belo Horizonte, MG, Brazil).

Animal procedures

This methodology was adapted from Assmann *et al.* (2015). Briefly, animals were anaesthetized by intraperitoneal injection with 0.008 mL 100 g^{-1} ketamine (Virbac do Brasil, São Paulo, SP, Brazil) and 0.004 mL 100 g^{-1} 2% xylazine hydrochloride (Virbac do Brasil), and 0.03 mL 100 g^{-1} tramadol hydrochloride by subcutaneous injection. The right femur was used for the intervention. Trichotomy was performed and the area was disinfected with alcohol iodine solution. A 5-cm long incision was made on the skin, tissues were separated by layers and the periosteum was incised with a no. 15 Surgical Scalpel Blade. Three circular cavities, 2 mm in diameter, were prepared on the cortical surface of the femur, 3 mm from each other, by means of a slow-speed handpiece and a no. 5 round steel bur, measuring 1.6 mm in diameter (KG Sorensen, São Paulo, SP, Brazil) under constant irrigation with saline solution and aspiration.

The bur was positioned perpendicular to the femur and used until the bone marrow was approached. The surgical cavities were randomly designated to experimental groups, which were filled with varying weights (5, 10 or 20 mg) of either infected or noninfected dentine debris pellets. In the control group, the surgical cavities were not filled (Fig. 1).

Finally, the wound was sutured in layers (Nylon 4-0 Procare, Labor import, Osasco, SP, Brazil). After the experimental procedures, the animals were placed in cages until their recovery. Two animals were kept per house, with a cycle of 12 h day night^{-1}, temperature between 19–23 °C, relative air humidity between 40%–70%. To aid recovery, paracetamol (0.06 mg g^{-1} day^{-1}) was added to their drinking water for 72 h.

Euthanasia

The animals were euthanized 7, 30 or 60 days after the intervention (n = 6 per group at each time-point), by means of inhalation of isoflurane in a closed chamber until loss of vital signs and consequent cardiorespiratory arrest. The operated leg was disarticulated and dissected to isolate the femur. Then, with a low-speed diamond disc (KG Sorensen), the bone was transversally sectioned to separate each region with the surgical cavities. Each fragment was stored individually in a fixative solution consisting of 10% neutral-buffered formalin for 24 h.

The specimens were decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution for 4 weeks and then dehydrated, clarified and embedded in paraffin. Sections with 5-μm thickness were cut transversely to the long axis of the femur, mounted on slides and stained with haematoxylin-eosin.

Histological evaluation

Slices were analysed with a light microscope (DM3000 LED; Leica, São Paulo, Brazil), using 40, 100, 200 and 400x magnification. The presence of inflammatory response and the hard tissue deposition was evaluated in the H&E-stained sections by a previously calibrated experienced pathologist who was blinded to sample group assignment.

The cellular inflammatory component was determined by the presence of neutrophils, lymphocytes, eosinophils, macrophages and giant cells. These cellular events were then classified according to the following scale (Tavares *et al.* 2013): (0) Absent: Inflammation was either absent or within blood vessels; (1) Mild: Cells were present although sparse or in

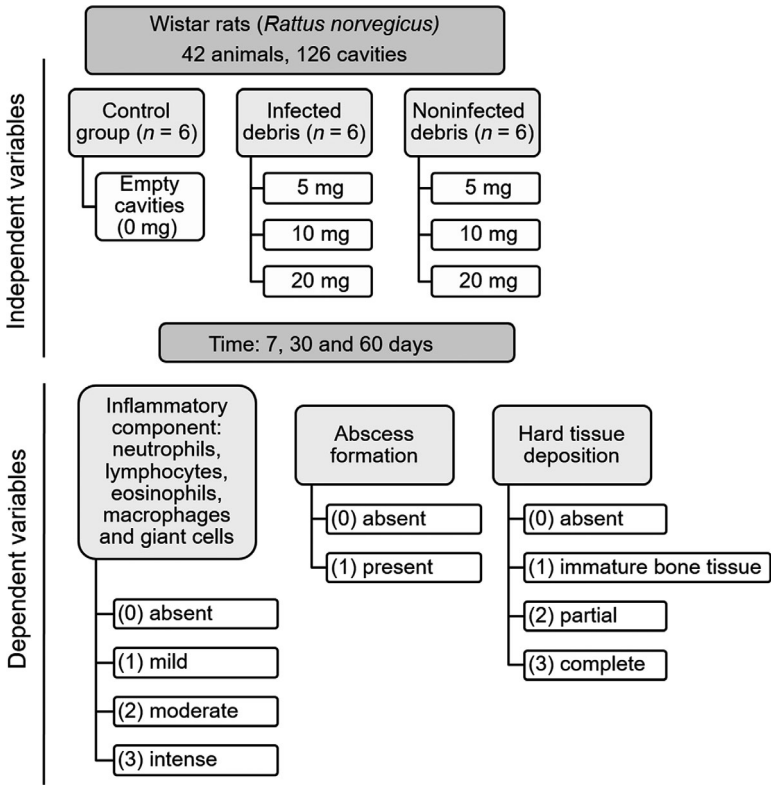

Figure 1 Flow chart illustrating the experiment design, with independent and dependent variables included in the study.

reduced clusters; (2) Moderate: Cells were present, but not dominating the microscopic field; (3) Intense: Cells were present in the form of an infiltrate within the surgical cavity. The abscess formation was also assessed and recorded as (0) absent or (1) present.

Hard tissue deposition was modified from the criteria used by Assmann *et al.* (2015), as follows: (0) Absent: no hard tissue deposition on the cavity region; (1) Formation of immature bone tissue, beginning the process of closure of the experimental defect; (2) Partial: partial close of cavity by hard tissue deposition; (3) Complete: total close of cavity by hard tissue deposition.

In order to test the intra-examiner variability, Kappa coefficient was obtained using a sample of 15 sections. The intra-examiner-weighted Kappa was calculated separately for the presence of inflammatory cells ($k = 0.89$, $P < 0.001$), abscess formation ($k = 1.00$, $P < 0.001$) and hard tissue deposition ($k = 1.00$, $P < 0.001$).

Statistical analysis

Categorical data were analysed statistically using SPSS statistical software (version 24.0, IBM, Chicago, IL, USA). Histopathological events were considered as the dependent variables, whilst debris (infected, noninfected and no-dentine), amount of debris (0, 5, 10 and 20 mg) and time of evaluation (7, 30 and 60 days) were considered as the independent variables. The effect of each independent variable over the dependent variables was studied individually by using a nonparametric test, Kruskal–Wallis with the due Bonferroni corrections. Pair-wise comparisons were also studied by means of a Mann–Whitney *U* test also with Bonferroni correction. All significances were accepted at $P = 0.05$. Box-plot graphs were constructed following interaction of the independent variables displaying significance at individual evaluations.

Results

The median scores obtained for the evaluated histopathological events, in each time interval, are presented in Table 1. Examples of histopathological parameters assessed within the different groups and experimental periods are shown in Figs 2, 3 and 4.

Regarding the presence and the bacteriological status of the debris inoculated in the surgical cavities,

there was a a significant difference amongst the groups for neutrophils ($P = 0.003$), lymphocytes ($P = 0.006$), eosinophils ($P = 0.048$) and abscess formation ($P = 0.003$). Further investigation revealed that, compared with no debris and noninfected debris, the presence of infected debris scored significantly higher for neutrophil infiltrate ($P = 0.005$, 0.015, respectively), and abscess formation ($P = 0.006$, 0.01, respectively). Noninfected debris significantly raised the severity of lymphocyte infiltrate compared with no-debris and infected debris ($P = 0.014$, 0.012, respectively). Both infected and noninfected debris triggered eosinophil cells equally compared with no-dentine ($P = 0.021$ for both). As for giant cells and macrophages, no difference was detected amongst the dentine debris groups ($P = 0.087$ and $P = 0.174$, respectively). Hard tissue deposition was similar regardless of the presence or the bacteriological status of debris ($P = 1.00$).

As for the effect of the weight of dentine debris over the histopathological parameters, Kruskal–Wallis revealed that none of them was significantly influenced by this variable ($P = 0.249$ for neutrophil infiltrate, $P = 0.204$ for lymphocyte infiltrate, $P = 0.198$ for giant cells, $P = 0.12$ for eosinophils and $P = 0.381$ for macrophages, $P = 0.339$ for abscess formation, $P = 1.00$ for hard tissue deposition).

Time of evaluation also significantly influenced the dependent variables (Kruskal–Wallis, $P = 0.000$ for all parameters). For all histological parameters evaluated, after 7 days of analysis, a significantly greater inflammatory response occurred compared with 30 and 60 days (Mann–Whitney *U*, $P = 0.000$ for all parameters and comparisons), except for hard tissue deposition, which scored significantly higher after 30 days (Mann–Whitney *U*, $P = 0.000$). No difference was seen between 30 and 60 days of analysis (Mann–Whitney *U*, $P = 1.00$) for all histological parameters evaluated.

Because significance was detected for both the time of evaluation and the presence and the bacteriological status of the dentine, box-plot graphs of the interaction of these two variables were constructed confirming the influence of the debris presence and status mostly at 7 days of analysis (Fig. 5).

Discussion

The overall result of this study is that the presence of dentine debris (infected or not) did not influence the process of bone repair. The Endodontic scientific community has been systematically assuming that debris extrusion has an impact in apical tissue healing (Boijink *et al.* 2018, Uslu *et al.* 2018), even though, clinically, the determination of the frequency and the specific amount of debris or bacteria extrusion occurring during root canal preparation is yet to be determined. At the same time, the tissue response to different amounts of dentine debris, and the role of infected debris in this process is also unknown.

Numerous laboratory studies have been conducted to determine which intra-canal procedures result in more or less extrusion of hard tissue debris (Bürklein & Schäfer 2012, Bürklein *et al.* 2014, Topçuoğlu *et al.* 2016, Uslu *et al.* 2018) or bacteria (Kuştarci *et al.* 2008, Tinoco *et al.* 2013, Teixeira *et al.* 2015, Aksel *et al.* 2017) through the apical foramen, based on the assumption that apical extrusion of debris is capable of triggering a more severe inflammatory reaction that may impair apical healing. The most relevant finding of the present study is that the amount of dentine debris (infected or not) does not influence the inflammatory process within bone tissue in rats. Thus, associating the amount of extruded debris with the degree of bone inflammatory level may be misleading. At first glance, this may seem as a controversial and peculiar finding since numerous studies have used this very simplistic and biologically plausible rationale to justify the studies attempting to rank root canal preparation systems and techniques according to the amount of extruded apical debris. However, the present result can be taken more conceptually considering the systematic lack of experimental studies specifically designed to evaluate the causality or not between those variables.

In a clinical situation, it is expected that the debris extruded apically remains in intimate contact with periodontal soft and hard tissues. To better mimic the clinical condition, the present study followed the methodology described by Assmann *et al.* (2015), in which sealers are placed in direct contact with bone tissue, using the femur of Wistar rats. The bone tissue reaction to endodontic sealers is frequently described in the literature (Moretton *et al.* 2000, Cintra *et al.* 2006, Sousa *et al.* 2006), and the use of this model to simulate apical extrusion of debris in contact with bone tissues seems to be a suitable experimental model. The effect of endodontic materials or dentinal debris on bone has unquestionable clinical relevance, since a primary goal of endodontics is the maintenance or regeneration of the bone tissue supporting teeth in a healthy state.

Table 1 Median and min-max scores obtained for the evaluated histopathological events, per group, in each time interval

		7 days							30 days							60 days						
		Noninfected debris (mg)			Infected debris (mg)				Noninfected debris (mg)			Infected debris (mg)				Noninfected debris (mg)			Infected debris (mg)			
		5	10	20	5	10	20	No-debris	5	10	20	5	10	20	No-debris	5	10	20	5	10	20	No-debris
Neutrophils	Median	0	2	1	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Min	0	0	0	3	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Max	0	2	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes	Median	2	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Min	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Max	3	3	3	3	3	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Eosinophils	Median	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Min	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Max	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrophages	Median	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Min	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Max	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Giant cells	Median	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Min	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Max	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abscess formation	Median	0	0.5	0.5	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Min	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Max	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hard tissue deposition	Median	1	1	1	1	0	0	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Min	1	1	1	1	0	0	1	2	2	2	3	2	2	3	3	3	3	3	3	3	3
	Max	3	1	1	1	1	0	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3

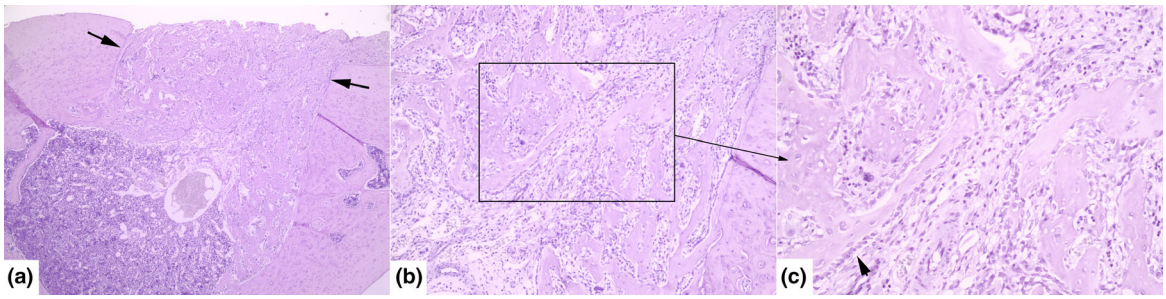

Figure 2 Histopathological parameters assessed within the Control group at 7 days. Haematoxylin and eosin staining. (a) 40×. Intense immature bone tissue deposition, closing the surgical cavity; the arrows indicate the limits of surgical cavity; (b) 100X magnification of (a), presenting mild chronic inflammatory infiltrate surrounded by bone tissue deposition; (c) 200×magnification of (a), the arrows evidence the presence of active osteoblasts.

Studies carried out in rats on bone tissue reaction, are usually performed with a maximum experimental period of thirty, sixty, or ninety days (Cintra *et al.* 2006, Rahimi *et al.* 2012, Assmann *et al.* 2015, Quintana *et al.* 2018). The present study lasted 60 days, which is consistent with the majority of studies. The experimental periods chosen were adequate to validate the differences between groups, which occurred at 7 days. Moreover, there was no need for longer periods of follow-up, since at 60 days all the samples revealed absence of inflammation and complete hard tissue barrier deposition. This has also a relevant ethical aspect, since there will be a reduction in the number of animals needed for the experiment.

The choice regarding the amount of debris to be included in surgical cavities (5, 10 and 20 mg) was based on previous laboratory studies, which reported

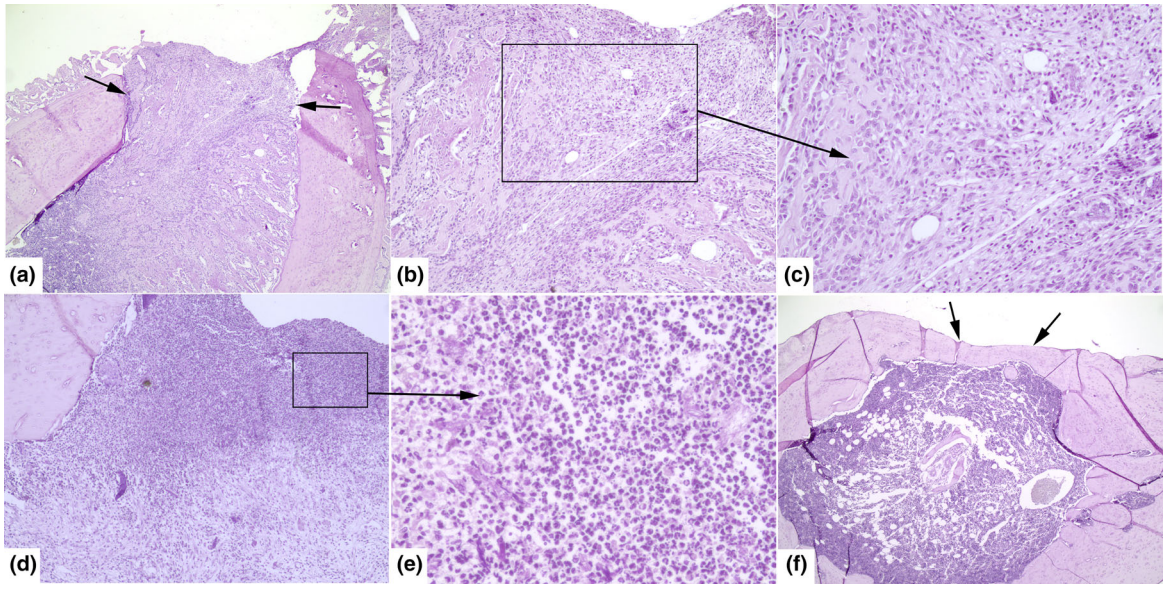

Figure 3 Histopathological parameters assessed within the group containing noninfected debris. Haematoxylin and eosin staining. (a, b, c) Tissue reaction at 7 days to 5 mg of noninfected debris; (a) 40×magnification, marginal bone tissue deposition without abscess areas; the arrows indicate the limits of surgical cavity; (b) 100×magnification of (a) showing immature bone tissue deposition; (c) 200×magnification of (a), showing the deposition of bone trabecular tissue and mild chronic inflammatory infiltrate; (d, e) 100×and 400×magnification; 7 days reaction to 10 mg of noninfected debris; (d) the surgical cavity is still not closed; (e) presence of intense acute inflammatory infiltrate, within an abscess area; (f) 40×magnification; 30 days experimental period; absence of inflammation in contact with 20 mg noninfected debris; the arrows indicate the complete closure of the surgical cavity.

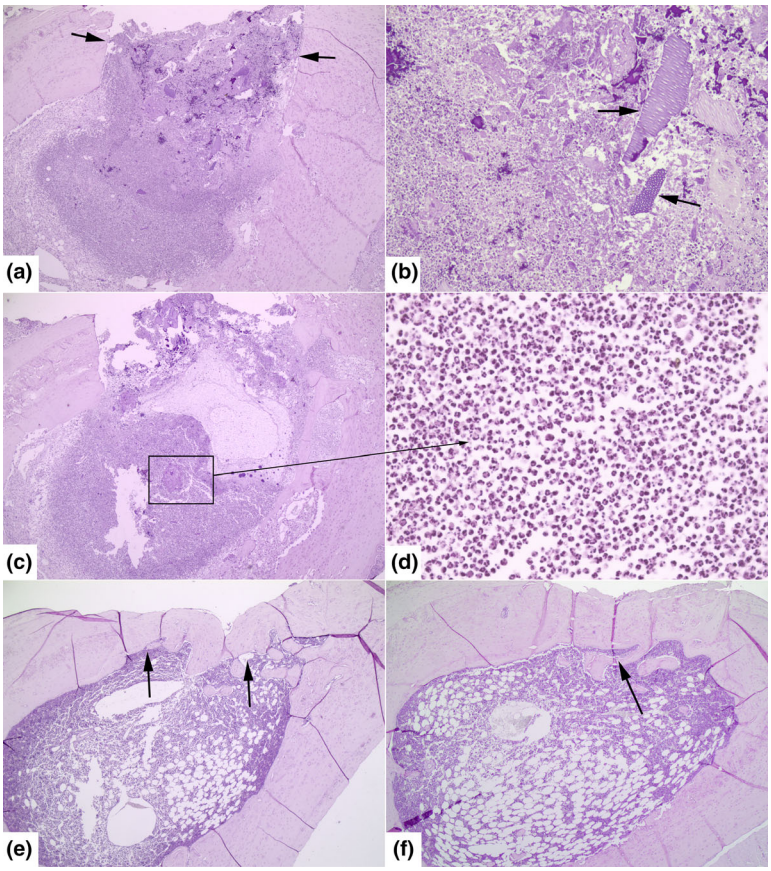

Figure 4 Histopathological parameters assessed within the group containing different amounts of infected debris. Haematoxylin and eosin staining. (a) 40×magnification, 7 days in contact with 20 mg of infected debris. Intense neutrophilic inflammatory infiltrate, occurrence of abscess and absence of bone tissue deposition; (b) 200×magnification of (a), the arrows indicate the presence of dentine debris; (c) 10 mg of infected debris at 7 days; extensive area of tissue necrosis within the surgical cavity; absence of bone tissue neoformation; (d) 400×magnification of (c), showing intense neutrophilic infiltrate; (e, f) 40×magnification, 30 and 60 days, respectively; both areas demonstrating the complete hard tissue deposition (arrows), with complete healing of surgical area.

that the average weight of debris extruded during root canal preparation ranged from 0.19 to 11.5 mg (Tanalp *et al.* 2006, De-Deus *et al.* 2010, Taşdemir *et al.* 2010, Bürklein & Schäfer 2012, Koçak *et al.* 2013, 2015, Bürklein *et al.* 2014, Capar *et al.* 2014, Surakanti *et al.* 2014, Ehsani *et al.* 2016, Boijink *et al.* 2018, Uslu *et al.* 2018). However, if instead of the average, the maximum amount of debris reported is considered, it is possible to find reports of up to 30.8 mg of debris extruded apically (Uslu *et al.* 2018). Nevertheless, it is important to highlight that periapical tissues may serve as a natural barrier providing a physical back pressure, thereby limiting the apical extrusion of debris and irrigants (Bonaccorso *et al.* 2009).

Similarly to the weight of debris extruded (quantitative factor), it is commonly assumed that there is a direct correlation between the virulence of the bacteria and the severity of the periapical inflammation (qualitative factor) (Kalra *et al.* 2017). In fact, bacteria may extrude along with debris through the apical foramen (Naidorf 1985, Siqueira 2003). However, the present study was not focused on classifying the intensity of the inflammatory reaction based on the virulence of different microorganisms, but rather to observe whether the presence of debris infected with anaerobic bacteria would negatively influence the tissue reaction. At 7 days, independently of the amount, infected debris induced an intense acute inflammatory infiltrate and abscess formation in every sample, with

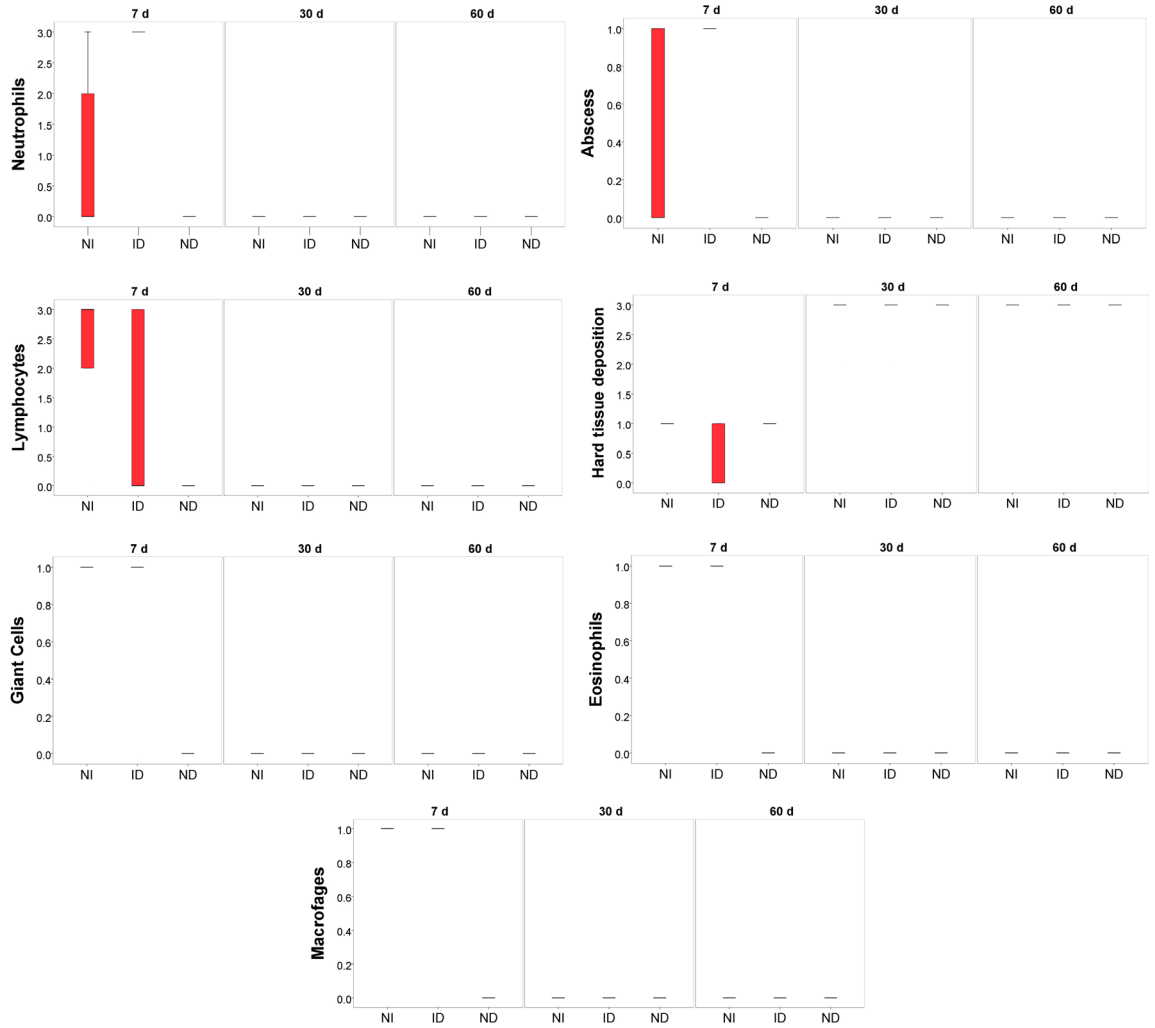

Figure 5 Box-plot graphs of the interaction of the time of evaluation and the presence and the bacteriological status of the dentine on the evaluated inflammatory parameters and hard tissue deposition. (ID, infected debris; ND, no debris; NI, noninfected debris).

high scores for neutrophils, and as a consequence, being more irritating to bone tissue than noninfected debris. These findings are in accordance with the literature, which described that pathogenic bacterial content of debris is associated with acute inflammatory reactions, via greater neuropeptide expression in the periradicular tissues (Caviedes-Bucheli *et al.* 2010, 2013, 2016).

Even though infected debris increased the frequency and the severity of inflammatory infiltrate and abscess formation at the initial period of evaluation, after 30 and 60 days, all the groups were similar regarding these cellular events. Moreover, the hard tissue barrier was complete in all samples from experimental and control groups, at 60 days. The longitudinal character of the present histological evaluation is of interest, since it helps to increase the understanding of the overall role of extruded debris. It highlights the fact that, even though infected debris extrusion may be initially pro-inflammatory, after a short term the initial negative impact is mitigated and tissue healing occurs invariably. Nevertheless, it is important to note that, although bone cavities were created, these defects may not entirely translate to periapical tissues that might already be impaired by the presence of apical periodontitis. Accordingly, it remains to be studied

whether the amount and bacteriological status of dentine debris would affect bone healing of an already diseased, immunologically challenged periapical environment.

Although the present results are derived from the bone tissue of rats, it challenges the overall role of the presence of dentine debris in bone tissue, and, as such, the provocative character of the current animal study invites reflection on the generally accepted biological rational used to rank instruments and instrumentation techniques based on the occurrence of debris extrusion. Because no *in vivo* human study has been designed to test this hypothesis, it is reasonable to say that the endodontic scientific community has been actually assigning an unproven role for the phenomenon of the studies on apical extrusion of debris. In other words, the core rationale used by laboratory-based apical extrusion studies has been accepted based on a nonexperimentally tested concept. Actually, this is a common behaviour amongst the endodontic scientific community, to assume surrogates tested in laboratory as being of *in vivo* relevance based on simply logical or biological plausibility. Speculative biological plausibility has been largely employed as rational to formulate working hypotheses based on which several *in vitro* studies are conducted. However, it is fair to say that a given working hypothesis without experimentation is an unsupported speculation; thus, as far as the real role of extruded debris is concerned, there is a clear need of gathering *in vivo* experimental evidence and, in this sense, the current study fits very well into this background, by adding a primary outcome on the behaviour of animal tissue response to dentine debris. Hopefully, the present results will trigger further *in vivo* human investigations aiming to achieve more clinically applicable conclusions, helping to clarify the actual clinical role of dentine debris extrusion on apical bone tissue repair.

Conclusion

The generally accepted assumption that the amount of extruded dentine may negatively affect the inflammatory response of bone tissue was not validated in the present *in vivo* animal study. Infected dentine triggered some inflammatory parameters, most specifically, neutrophils and abscess, especially over the first 7 days of contact with simulated bone defects in rats; however, in the long run these negative effects diminished and complete healing was achieved.

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Conflict of Interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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