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Original Article

Histological changes in pulp-dentin complex in tooth subjected to traumatic occlusion and subluxation

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ABSTRACT

Background: This study evaluated the influence of traumatic occlusion in the dentin-pulp complex a molar teeth submitted to subluxation.
Material and methods: Ninety Wistar rats were divided into groups Naïve (N), Subluxation (S) and Subluxation with traumatic occlusion (STO) and submitted to histological analysis after 7 and 21 days. A quantitative analysis was submitted to one-way ANOVA and Tukey's post-hoc test, and Chi-square and Bonferronís post-hoc test.
Results: S and STO showed a significant increase in blood vessels area ($p < 0.0005$), amorphous fundamental substance ($p < 0.0005$) and reactionary dentin formation ($p < 0.0005$), as well as a decrease in the nuclear profile ($p < 0.0005$), odontoblast layer ($p = 0.013$ and $p < 0.0005$) by day 7 when compared with N. These changes normalized by day 21, except for the reactionary dentin ($p < 0.0005$) in both S and STO groups. Interestingly, the STO group exhibited significant changes in the increase of pulp calcification ($p < 0.0005$), presence of tubules with nuclei ($p < 0.0005$), and inflammatory infiltrate ($p < 0.0005$), as well reduction of nuclear profile ($p < 0.0005$), odontoblast layer ($p < 0.0005$) compared with N and S at day 21.
Conclusions: STO impaired the defence response and decreased pulp regeneration capacity by increasing the inflammatory infiltrate and pulp calcification, and decreasing the nucleated cell number in the odontoblast layer and central pulp.

1. Introduction

Subluxation is a mechanical tissue injury that affects the supporting structures of the tooth. Due to the impact, it can affect the pulp and cause damage to the apical vascular-nervous bundle (Pugliesi et al., 2004). Clinically, subluxation presents an increase of tooth mobility, bleeding around the marginal gingiva (Andreasen and Perdersen, 1985), besides increased sensitivity to chewing and percussion tests (Pedrini et al., 2018). The subluxated tooth maintains its original position in the dental arch without vertical and/or horizontal displacement (Andreasen and Pedersen, 1985). Nevertheless, in some cases, apical edema may occur resulting in a slight extrusion of the tooth (Pedrini et al., 2018). Subluxation also causes pulp sequelae by inducing changes in the vascular component, such as increased vascular permeability, hyperemia, hemorrhage or ischemia. Pulp hemorrhage may lead to crown discoloration due to erythrocyte migration and hemoglobin decomposition (Bauss et al., 2009). In addition, dystrophic calcification may occur due to the hemorrhagic consequences combined with odontoblast differentiation (Bauss et al., 2009).

Periodontal ligament healing and neurovascular pulp regenerations are important to determine the outcome of teeth injured by subluxation (Strobl et al., 2005). The risk of pulp being permanently affected by necrosis (Pedrini et al., 2018; Pugliesi et al., 2004) after isolated subluxation (Bauss et al., 2009) may reach 27.1 % (Pedrini et al., 2018; Pugliesi et al., 2004), especially in cases of delayed treatment (Qassem et al., 2014). This occurs because the neurovascular lesion impairs pulp

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defense mechanisms, making the revascularization process difficult (Mjör, 2001).

Traumatic occlusion is another mechanical tissue injury that damage to the periodontal ligament (Amaral et al., 2017), that may be related to the initiation and progression of pulp and periradicular inflammation (Yu, 2004). In these cases, pulp sequelae can occur through neuro-inflammation (Caviedes-Bucheli et al., 2011; Kvinnsland and Heyeraas, 1992), inhibition of pre-dentin formation (Penna and Rode, 2000; Stenvik and Mjör, 1971), vascular component lesions (Stenvik and Mjör, 1971), odontoblast layer disorganization, pulp dystrophies, pulp calcifications, and decreased pulp chamber volume (Penna and Rode, 2000; Popescu et al., 2011).

Traumatic occlusion may exist prior to a subluxation as a result of parafunctional habits, iatrogenics (Fu and Yap, 2007) or malocclusion, especially in children, due to incomplete and temporary eruption of the first permanent molars (Eismann and Reichert, 1990), or it can occur after trauma, due to the periodontal ligament edema or a positional alteration (Pedrini et al., 2018). Under current protocols, the treatment of permanent tooth subluxations requires flexible containment for up to 2 weeks, and a follow-up after 4–8 weeks (Bourguignon et al., 2020). This study evaluated the influence of traumatic occlusion in the dentin-pulp complex of the distobuccal root of the upper first molars submitted to subluxation, to emphasize the importance of occlusal adjustment in addition to the subluxation treatment.

2. Materials and method

2.1. Animals and surgical procedures

All experimental procedures are in agreement with the ARRIVE guidelines and the experimental protocols were approved by the Institutional Animal Use Ethics Committee (number 2016–00970).

Ninety adults female Wistar rats (*Rattus novergicus*) aged 12 weeks were randomically distributed into groups, presenting a 100 % sample power, for a 95 % confidence interval and 5 % margin of error.

The animals were divided into three groups: naive group (n = 30), animals were not subjected to any experimental conditions; subluxation (n = 30), subluxation of the upper right first molar and; subluxation and traumatic occlusion (n = 30), subluxation of the upper right first molar combined with traumatic occlusion using composite resin in the lower right first molar (Sodeyama et al.,1996; Amaral et al., 2017; Pereira et al., 2010) (Supplementary Material 1). The animals were sacrificed by transcardiac perfusion at 7 and 21 days after the experimental procedures. Animals were excluded in the event of death from natural causes or loss of the composite resin occlusal surface of the lower right first molar (Supplementary Material 2).

2.2. Histological processing and analysis

The samples were impregnated in low melting temperature paraffin and transverse histological semi serial sections (40 μm of interval between adjacent sections) between the cervical and middle portions of the upper right first molar for each animal were analyzed under brightfield microscope (Leica - Aristoplan, Wetzlar, Germany) coupled to an Axiocam MRc digital camera (Carl Zeiss MicroImaging GmbH, Germany).

For the histological analysis of pulp changes, the buccal root pulp of the upper right molar was evaluated by concerning the presence or absence of the following phenomena: pulp hyalinization, pulp nodules, diffuse calcification, vascular congestion, bleeding, thrombosis and dentinal tubules filled with nuclei (Massaro et al., 2009). In addition, four different histomorphological events were qualitatively analyzed (Fiane et al., 2014; Nishioka et al., 1998). (a) the odontoblast layer; (b) deposition of reactionary dentin; (c) pulp necrosis; and (d) inflammatory infiltrate. All of these were defined as: absent; present in a restricted area; partially present; and totally present (Nishioka et al., 1998). To avoid bias, the examiner went through a calibration process and was unaware of which group the images belonged to (Supplementary Material 3).

2.3. Statistical analysis

Data were analyzed using the statistical program GraphPad Prism at $\alpha = 0.05$. After assessing the sample normality standard using the Shapiro Wilk test, the data the continuous variables were submitted to the one-way ANOVA independent sample and Tukey (HSD) post-hoc test for group comparison and expressed as mean ± standard deviation (SD). The group comparison between categorical variables data were by Chi-square and Bonferronís post-hoc test, expressed in percentage.

3. Results

Significant histomorphological changes occurred in subluxated tooth, and the dentin-pulp complex exhibited worst outcomes in conjunction with traumatic occlusion.

3.1. Amorphous fundamental substance and calcification are enhanced in subluxation and subluxation with traumatic occlusion groups

Concerning the distribution and arrangement of amorphous fundamental substance of the pulp, subluxation and subluxation with traumatic occlusion groups showed similar pattern. After 7 days, there was a significant increase in amorphous fundamental substance in subluxation group (Fig. 2) and subluxation with traumatic occlusion (Fig. 3) compared to the naive group (Fig. 1); while these changes were less evident on day 21 (Table 1).

However, these changes were significant only in relation to the naïve group after 21 days (Fig. 3C–D).

3.2. Disruption in odontoblast arrangement occurrence in the in subluxation and subluxation and traumatic occlusion groups

The presence of spherical or elliptical shaped calcifications not associated with pre-dentin, both in the odontoblastic layer and in the cell-poor zone (Fig. 2C–F and Fig. 2E and F), after 7 and 21 days respectively, were not significant in the subluxation group (Table 2). However, it was noticed a significant increase of these calcifications on day 7 and 21 in the subluxation with traumatic occlusion group ($p < 0.0005$), (Fig. 3A–B, Fig. 3E–F) (Table 2).

The dentin and pre-dentin of the buccal vestibule in subluxation and subluxation with traumatic occlusion groups on day 7 showed some irregular dentinal tubules (Fig. 2D and Fig. 3A). In addition, a significant increase in the formation of reactionary dentin in the pulp and a significant reduction in the odontoblast layer was observed in the subluxation and subluxation with traumatic occlusion groups in relation to the naive group (Table 3).

At day 21, subluxation group showed significant presence of reactionary dentin in all samples evaluated (Table 3). However, subluxation with traumatic occlusion group showed reactionary dentin in all samples as well as exhibited significant absence of the odontoblastic layer, presence of odontoblastic nucleus in dentinal tubules, dystrophic calcification (Fig. 2F) (Tables 2 and 3).

3.3. Immune cell infiltration occurs primarily in the subluxation and subluxation with traumatic occlusion groups

Subluxation group did not show inflammation signs when compared to naive group (Tables 2 and 3). However, subluxation and traumatic occlusion group exhibited significant presence of inflammatory cells into the pulp, mainly on day 21 when compared with other experimental groups (Fig. 3D) (Table 3).

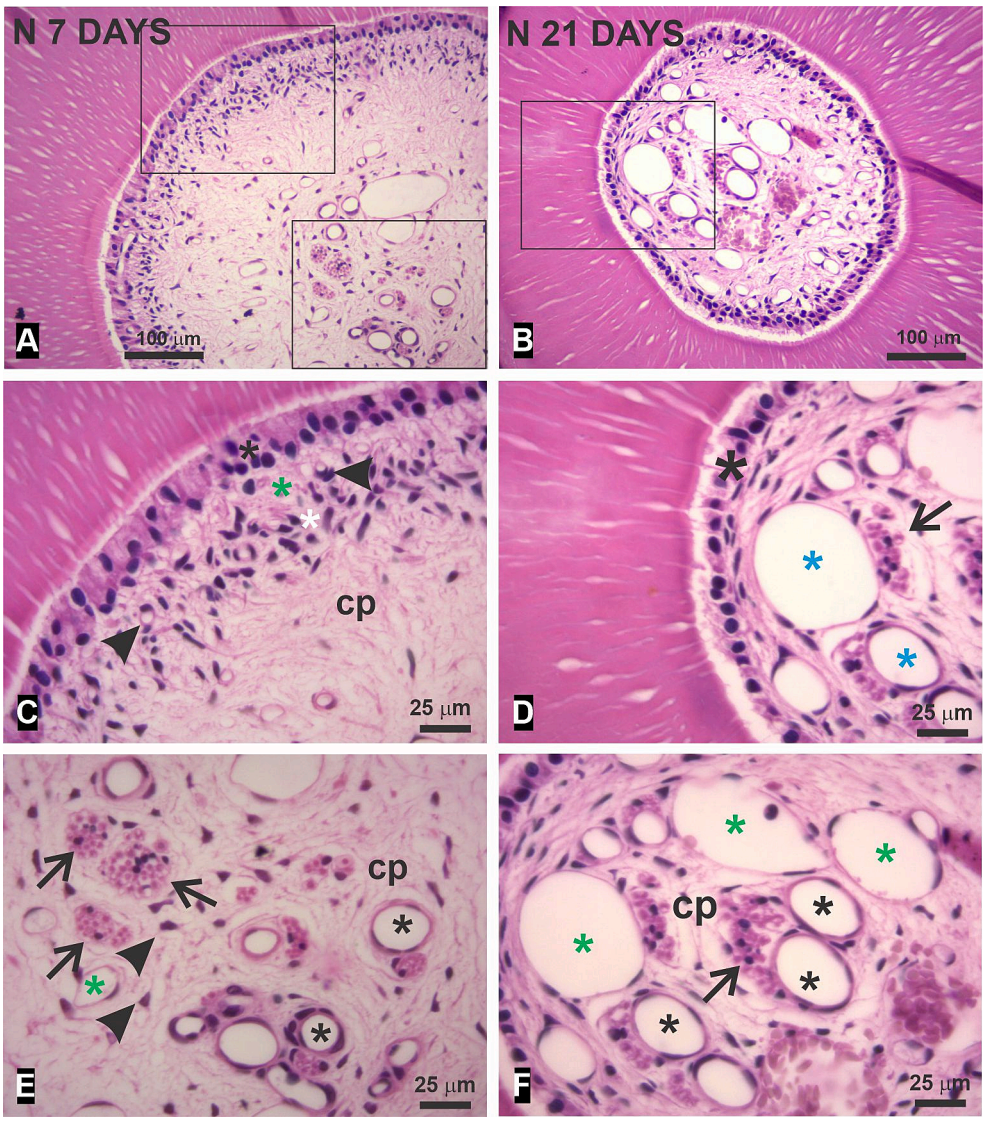

Fig. 1. Light microscopic photomicrographs of horizontal histological sections of the roots of the upper first molar of the naive group, sacrificed 7 days and 21 days, stained with HE method. In 1A and 1B, observe a portion of the pulp-dentin complex of the cervical third of the distobuccal root. In 1C, the different regions of the peripheral pulp, formed by the odontoblast layer (black asterisk), cell poor area or sub odontoblastic layer (green asterisk) and cell rich area (white asterisk), and part of the central pulp (CP) are shown. Note that the odontoblasts have a cylindrical cell body and all tissue layers show normality. Blood capillaries (arrowhead) are also evidenced. 1D shows the peripheral pulp with the cuboidal odontoblasts (black asterisk) and absence of the other tissue layers. 1E and 1F show the central pulp consisting of loose connective tissue with fibroblasts (arrowhead), presence of arterioles (black asterisk), venules (blue asterisk) and nerve bundles (arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Differential temporal changes in blood vessel area and nuclear cell profiles in the subluxation and subluxation with traumatic occlusion groups

Subluxation group exhibited significant increase in blood vessel area and a reduction in the number of nuclear cell profiles on day 7 (Fig. 2C), returning to basal levels by day 21 (Table 1). However, subluxation with traumatic occlusion group showed a significant reduction in the number of nuclear cell profiles on the day 21 when compared to the naïve and subluxation groups (Fig. 3D and F) (Table 1).

4. Discussion

Subluxation is a type of alveolar trauma considered a mild trauma with a favorable prognosis and low risk of causing permanent pulp sequelae (Andreasen and Pedersen, 1985; Pedrini et al., 2018; Pugliesi et al., 2004), permitting to evaluate additional traumatic occlusion on the dentin-pulp complex.

Pulp alterations lead to a doubtful prognosis for teeth subjected to subluxation (Pedrini et al., 2018; Pugliesi et al., 2004), which may be aggravated by factors like delayed treatment (Pedrini et al., 2018), lack of containment (Kahler and Heithersay, 2008), root canal infection (Hermann et al., 2012) and traumatic occlusion (Nakatsu et al., 2014). However, traumatic occlusion is still treated as a background variable, perhaps because it is considered a minor injury in dental trauma (Caviedes-Bucheli et al., 2011; Kvinnsland and Heyeraas, 1992; Penna and Rode, 2000; Popescu et al., 2011).

The method adopted to induce subluxation in these study seek the standardization of direction and intensity of the traumatic mechanical force on the occlusal surface of the teeth, using a tensiometer and a support with adjustable galvanized steel shafts (Amaral et al., 2017; Pereira et al., 2010).

In the present study, subluxation did not cause pulp hyperemia, hemorrhage, thrombosis, inflammation, pulp hyalinization, necrosis,

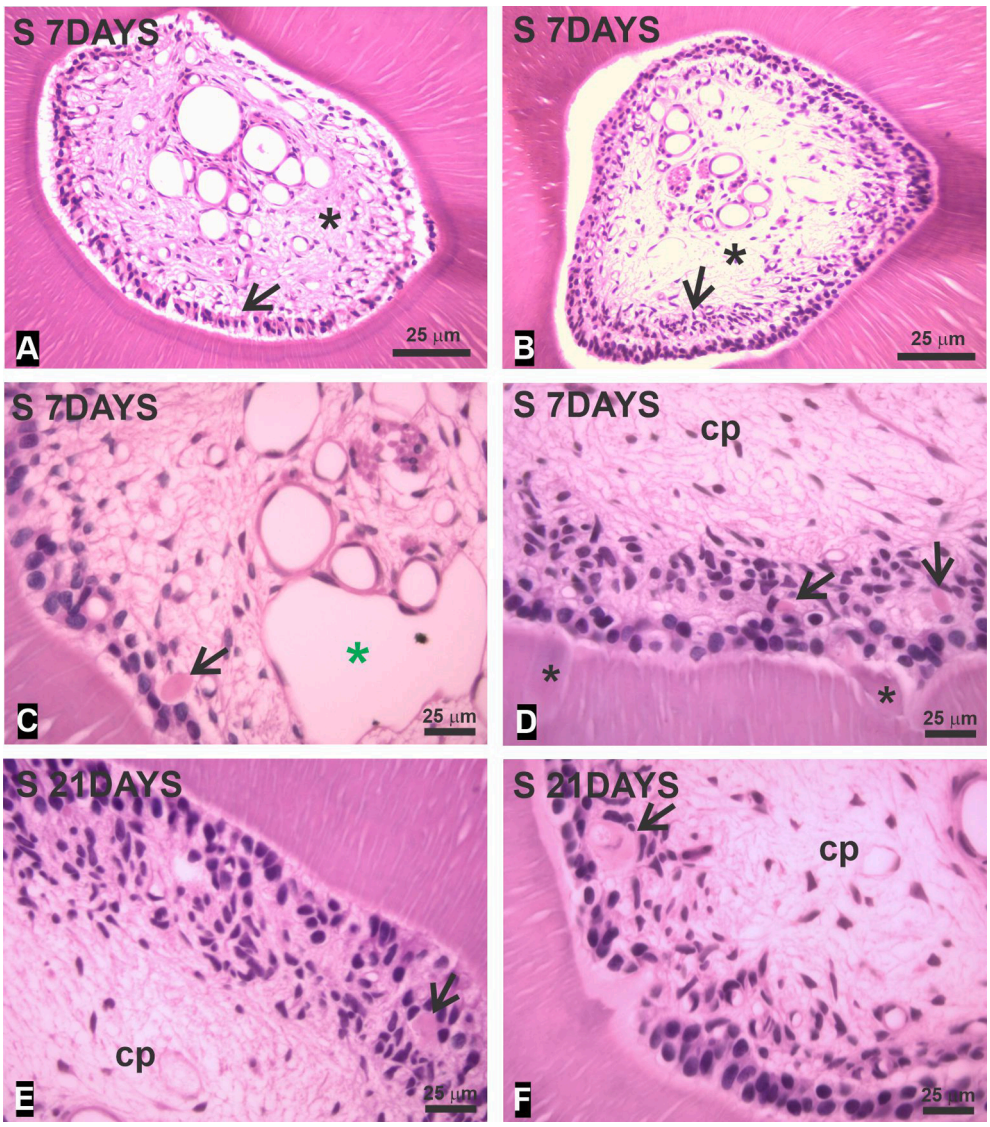

Fig. 2. Light microscopy photomicrographs of horizontal histological sections of the distobuccal root of the first right upper molar stained with HE method of the subluxation group at 7 days (A-D) and 21 days (E-F) HE stained. In 2A, the layer of odontoblasts showing cylindrical cell bodies (arrow) and a greater presence of fibrillar extracellular matrix in the central pulp (asterisk) is evidenced. In 2B it is possible to identify a thickening of the cell-rich area in the peripheral pulp (arrow). However, the central pulp shows a normal picture (asterisk). 2C and 2D show a dilated venule (green asterisk) and a higher concentration of fibrillar extracellular matrix and highlight areas of globular calcifications associated or not with predentin (arrows), as well as changes in the pattern of dentin and predentin organization (asterisks). 2E and 2F show the persistence of these calcifications (arrows) in the peripheral pulp in the animals at 21 days postoperative. Abbreviation CP = central pulp. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nodules or calcifications, as previously reported in the literature (Bauss et al., 2009). These differences noticed in the dentin-pulp complex can be related to the sample nature, standardizing degrees of dental trauma and moment of evaluation. Interestingly was noticed that shortly after subluxation, the histological events observed in the pulp, such as an increased area of amorphous fundamental substance, blood vessels, collagen fibers and reaction dentin, compromised odontoblast layer and reduced nuclear cell profile number due to cell death (Andreasen and Pedersen, 1985; Bauss et al., 2009), which tend to normalize after the 21-day post-trauma, with some reactionary dentin formation and an increase in collagen fibers. Pulp hyperemia, hemorrhage, thrombosis and inflammation were not observed in this study possibly due to the experimental time. Vascular changes and acute inflammation in the pulp can be observed immediately after a traumatic stimulus, so an experimental time of up to 72 h could be interesting to assess whether these conditions are not present after subluxation with constant traumatic occlusion (Bauss et al., 2009). A better understanding of inflammatory reactions by immunohistochemical analysis should be seen as a complementary test to fill the limitation of this study in show the reactions of the dental pulp under mechanical trauma.

In the case of subluxation, when the dental pulp is injured but not ruptured or the blood vessels are compressed, the nutrition of pulp is compromised. This contributes to the aging process through the loss of pulp cells, reduction of cell nutrition, the formation of pulp fibrosis and nodules (Consolaro, 2007). Under low nutrition, the pulp cells encounter intense stress, causing changes in their phenotype to adapt to the new metabolic situation. This change in phenotype and function is called metaplasia, which can lead to obliteration of the pulp chamber and / or root canal, as almost all pulp cells become odontoblast-like (Consolaro, 2007). Simultaneously, there will be random production of dysplastic dentin. Over the years, the pulp may necrotize, including with chronic periapical lesions. This situation promotes the gradual darkening of the traumatized tooth over months to years and is referred to as calcium pulp metamorphosis (Consolaro, 2007).

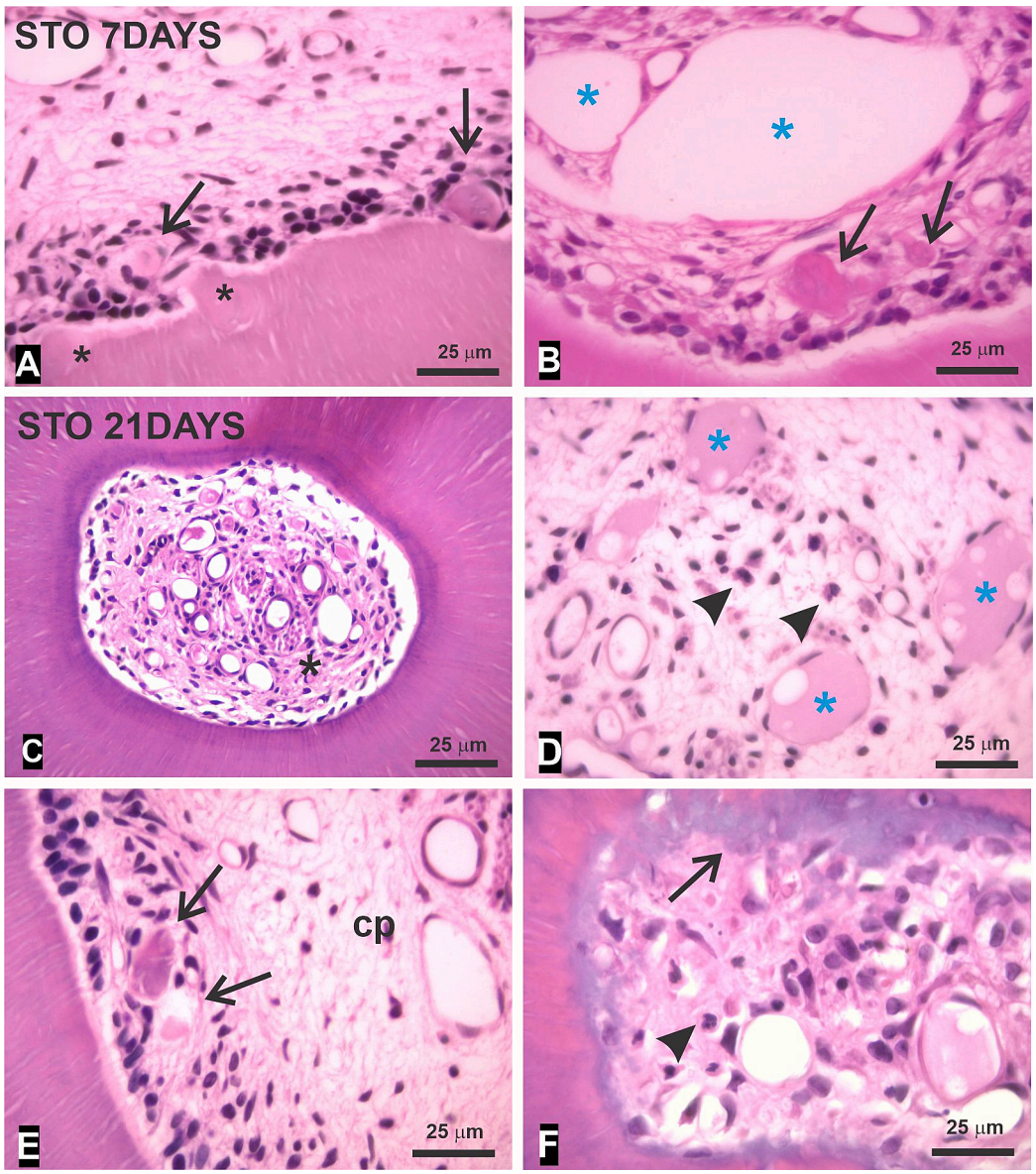

Fig. 3. Light microscopic photomicrographs of horizontal histological sections distobuccal root of the upper first right molar stained with HE method of the subluxation with traumatic occlusion at 7 and 21 days postoperative. 3A show changes in the pattern of dentin and predentin organization (black asterisks). 3B present dilated arterioles and venules (blue asterisk), a more frequent presence of larger globular calcifications in the peripheral pulp (arrows), 3C shows the middle third of the root, demonstrating an increase in the pulp's fibrillar extracellular matrix (black asterisk). 3D shows the central area pulp, with a presence of inflammatory cells (arrowheads) accompanied by numerous lymph vessel (green asterisk). 3E show the peripheral and central pulp, evidencing some inflammatory cells and a presence of globular calcifications in the peripheral pulp (black arrows).3F shows a generalized calcification process (arrow) of the root pulp, associated with inflammatory cells (arrowhead). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Morphological changes in the pulp after subluxation and traumatic occlusion.

PERIOD	VARIABLES	GROUPS						
		N		S		STO		P value
		Mean	SD	Mean	SD	Mean	SD	
7 days	Blood vessel area (%)	8.2^{A}	3.3	21.2^{B}	7.1	17.5^{B}	6.5	<0.0005
	AFS area (%)	23.1^{A}	5.1	43.0^{B}	9.0	37.6^{B}	4.7	<0.0005
	Nuclear profile (n)	33.1^{A}	7.1	15.6^{B}	4.9	16.8^{B}	4.4	<0.0005
21 days	Blood vessel area (%)	6.7^{A}	3.5	7.1^{A}	3.6	4.9^{A}	4.1	0.286
	AFS area (%)	17.6^{A}	4.5	24^{A}	4.0	21.2^{A}	9.3	0.081
	Nuclear profile (n)	34.6^{A}	7.0	28.5^{A}	3.9	14.1^{B}	8.8	<0.0005

The statistically significant difference between groups was determined by the One-Way ANOVA and Tukeýs (HSD) post-hoc test for continuous variables. Different letters indicate a statistically significant difference between the groups in each event. N-Naive group, S-Subluxation group, STO-Subluxation with traumatic occlusion, and AFS-Amorphous fundamental substance.

Table 2
Presence of microscopically observed pulp alterations in the experimental groups according to score proposed by Massaro et al., 2009.

PERIOD	PRESENCE OF PULP ALTERATIONS	GROUPS			
		N (%)	S (%)	STO (%)	P value
7 days	Diffuse calcification	0^{A}	26.7^{A}	73.3^{B}	<0.0005
	Tubules with nuclei	0^{A}	0^{A}	20^{A}	0.040
21 days	Diffuse calcification	0^{A}	40^{A}	100^{B}	<0.0005
	Tubules with nuclei	0^{A}	0^{A}	64.3^{B}	<0.0005

The statistically significant difference between groups with categorical variables was determined by the Chi-square and Bonferroni's post-hoc test. Different letters indicate a statistically significant difference between the groups in each event. N-Naive group, S-Subluxation group, and STO-Subluxation with traumatic occlusion.

Subluxation with traumatic occlusion group did not show the same pattern of development as just subluxation group. In addition to the pulp not recovering and normalizing its histomorphological characteristics, traumatic occlusion induced consequences like the presence of inflammatory cells, presence of odontoblastic nucleus into dentinal tubules, and the formation of pulp calcifications. Traumatic occlusion is considered an additional, continuous and more intense trauma (Bourguignon et al., 2020; Kindelan et al., 2008), since it exerts an intrusive force capable of not only prolonging the injury caused by subluxation, but also impairing the repair process that should occur after about 10 days' post-trauma (Giovanini et al., 2011). Pulp defense reactions may be more intense and cause inflammatory or degenerative pulp changes, depending on the type, frequency and intensity of the traumatic agent. If these insults are not removed, the pulp will remain in an aging process and consequently present root canal calcification or pulpitis and pulp necrosis (Giovanini et al., 2011). Although necrosis did not occur until the 21-day post-trauma in the present study, in view of the other alterations present it can be considered that traumatic occlusion associated with previous subluxation can cause, in the long term, aseptic pulp necrosis by accelerating the pulp aging process and even dental darkening through pulp obliteration resulting from metaplasia (Consolaro, 2007; Giovanini et al., 2011).

These results show that traumatic occlusion associated with previous subluxation caused a significant proliferation of inflammatory cells, showing a relation with the onset and progression of a pulp and peri radicular inflammation (Yu, 2004) and further contributing to the occurrence of pulp sequelae such as odontoblast layer disorganization, pulp dystrophy, proliferation of collagen fibers, pulp calcification and a decreased volume of the pulp chamber (Penna and Rode, 2000; Popescu et al., 2011). The occurrence of injury to the apical neurovascular bundle following trauma promotes protein denaturation, characterized by water loss, where the pulp cells are kept in place in their framework, but coagulated and lifeless (Consolaro, 2007; Giovanini et al., 2011). Pulp cells are poor in lysosomes and organelles loaded with proteolytic enzymes. In traumatized teeth, structurally sound but with a ruptured neurovascular bundle, without the exposure of dentin and / or pulp to the oral environment and without periodontal involvement, coagulation necrosis represents pulp infarction - also known as aseptic necrosis (Consolaro, 2007; Giovanini et al., 2011). The restriction of the pulp neurovascular supply is related to pulp space obliteration, by an increased deposition of dentin after healing. (Andreasen et al., 1987; de Cleen, 2002).

Endodontal tissue obliterations following subluxation occur in 8 %–11 % of teeth, and a delayed complication after pulp obliteration is necrosis (Andreasen et al., 1987; de Cleen, 2002). It is not yet clear how pulp necrosis arises in such cases, but cavities, restorative treatments and a second trauma may be likely causal factors. It can be assumed that the limited neurovascular supply due to a narrow apical foramen makes the pulp more vulnerable to such damage (Andreasen et al., 1987; de Cleen, 2002). In this context, traumatic occlusion may have significant impact as a secondary trauma affecting already vulnerable pulp.

Once these results showed that the presence of traumatic occlusion associated with previus subluxation impair pulp repair and prolong cellular stress, what aggravates the process of pulp aging and metaplasia, may lead to necrosis. Therefore, it shows the importance of a traumatic occlusion verification and of an occlusal adjustment while proceeding a subluxated teeth treatment protocol.

5. Conclusion

We conclude that traumatic occlusion in teeth undergoing subluxation intensified the short-term pulp defense response and generated lower long-term pulp regeneration capacity by decreasing the odontoblast layer and cell number, and increasing collagen fibers,

Table 3
Presence and intensity of microscopically observed pulp changes in the experimental groups according to the scores proposed by Nishioka et al., 1998.

VARIABLES	SCORES	GRUPOS							
		7 days			P Value	21 days			P Value
		N (%)	S (%)	STO (%)		N (%)	S (%)	STO (%)	
ODONTOBLAST LAYER	1.Absent	0^{A}	6.7^{A}	0^{A}		$0^{A, B}$	0^{B}	42.9^{A}	
	2. Present in restricted area	0^{A}	13.3^{A}	0^{A}		0^{A}	0^{A}	0^{A}	
	3. Partially present	0^{A}	$20^{A, B}$	46.7^{B}		$0^{A, B}$	0^{B}	35.7^{A}	
	4.Totally present	100^{A}	60^{B}	53.3^{B}		100^{A}	100^{A}	21.4^{B}	
	GROUP COMPARISON				0.013				<0.0005
REACTIONAL DENTIN	1.Absent	100^{A}	0^{B}	0^{B}		100^{A}	0^{B}	0^{B}	
	2. Present in restricted area	0^{A}	26.7^{A}	13.3^{A}		0^{A}	6.7^{A}	0^{A}	
	3. Partially present	0^{A}	46.7^{B}	46.7^{B}		0^{A}	26.7^{A}	0^{A}	
	4.Totally present	0^{A}	$26.7^{A, B}$	40^{B}		0^{A}	66.7^{B}	100^{B}	
	GROUP COMPARISON				<0.0005				<0.0005
NECROSIS	1.Absent	100	100	100		100	100	100	
	2. Present in restricted area	0	0	0		0	0	0	
	3. Partially present	0	0	0		0	0	0	
	4.Totally present	0	0	0		0	0	0	
	GROUP COMPARISON	It is not possible to perform statistical analysis, identical scores.							
INFLAMMATORY INFILTRATE	1. Absent	100^{A}	93.3^{A}	100^{A}		100^{A}	100^{A}	0^{B}	
	2. Present in restricted area	0^{A}	6.7^{A}	0^{A}		0^{A}	0^{A}	57.1^{B}	
	3. Partially present	0^{A}	0^{A}	0^{A}		0^{A}	0^{A}	7.1^{A}	
	4.Totally present	0^{A}	0^{A}	0^{A}		$0^{A, B}$	0^{B}	35.7^{A}	
	GROUP COMPARISON				0.360				<0.0005

Statistical significance between the groups with categorical variables was determined by the Chi-square and Bonferroni's post-hoc test. Different letters indicate a statistically significant difference between the groups in each event. N-Naive group, S-Subluxation group, STO- Subluxation with traumatic occlusion.

inflammatory infiltrate and calcifications.

CRediT authorship contribution statement

Marina Fuzette Amaral: Conceptualization, Methodology, Investigation. **Cláudio Aparecido Casatti:** Funding acquisition. **Afsar Raza Naqvi:** Writing – review & editing. **Caio Vinicius Lourenço Debortoli:** Formal analysis. **Daniela Atili Brandini:** Conceptualization, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sdentj.2023.11.012.

References

Amaral, M.F., Poi, W.R., Debortoli, C.V.L., Panzarini, S.R., Brandini, D.A., 2017. The influence of traumatic occlusion on the repair process for teeth following subluxation. Dent. Traumatol. 33, 245–254. https://doi.org/10.1111/edt.12330. Epub 2017 Apr 10 PMID: 28208234.

Andreasen, F.M., Pedersen, B.V., 1985. Prognosis of luxated permanent teeth–the development of pulp necrosis. Endod Dent. Traumatol. 1, 207–220. https://doi.org/10.1111/j.1600-9657.1985.tb00583.x. PMID: 3867505.

Andreasen, F.M., Zhijie, Y., Thomsen, B.L., Andersen, P.K., 1987. Occurrence of pulp canal obliteration after luxation injuries in the permanent dentition. Endod. Dent. Traumatol. 3, 103–115. https://doi.org/10.1111/j.1600-9657.1987.tb00611.x. PMID: 3476298.

Bauss, O., Röhling, J., Meyer, K., Kiliaridis, S., 2009. Pulp vitality in teeth suffering trauma during orthodontic therapy. Angle Orthod. 79, 166–171. https://doi.org/10.2319/010708-7.1. PMID: 19123692.

Bourguignon, C., Cohenca, N., Lauridsen, E., Flores, M.T., O'Connell, A.C., Day, P.F., Tsilingaridis, G., Abbott, P.V., Fouad, A.F., Hicks, L., Andreasen, J.O., Cehreli, Z.C., Harlamb, S., Kahler, B., Oginni, A., Semper, M., Levin, L., 2020. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and Luxations. Dent. Traumatol. 36, 314–330. https://doi.org/10.1111/edt.12578. Epub 2020 Jul 17 PMID: 32475015.

Caviedes-Bucheli, J., Azuero-Holguin, M.M., Correa-Ortiz, J.A., Aguilar-Mora, M.V., Pedroza-Flores, J.D., Ulate, E., Lombana, N., Munoz, H.R., 2011. Effect of experimentally induced occlusal trauma on substance p expression in human dental pulp and periodontal ligament. J. Endod. 37, 627–630. https://doi.org/10.1016/j.joen.2011.02.013. PMID: 21496661.

Consolaro, A., 2007. Pulp changes induced by orthodontic treatment: dogmas and lack of information. Rev. Dent. Press Ortodon. Ortop. Facial. 12, 15–17.

de Cleen, M., 2002. Obliteration of pulp canal space after concussion and subluxation: endodontic considerations. Quintessence Int. 33, 661–669.

Eismann, D., Reichert, M., 1990. Individual overbite behavior between the deciduous and permanent dentition. Fortschr Kieferorthop. 51, 213–217.

Fiane, J.E., Breivik, M., Vandevska-Radunovic, V., 2014. A histomorphometric and radiographic study of replanted human premolars. Eur. J. Orthod. 36, 641–648. https://doi.org/10.1093/ejo/cjt088. Epub 2013 Dec 19.

Fu, J.H., Yap, A.U., 2007. Occlusion and periodontal disease–where is the link? Singapore Dent J. 29, 22–33.

Giovanini, A.F., Leonardi, D.P., Baratto-Filho, F., Vlença, P.C., Moresca, R.C., Moro, A., Schramm, C.A., 2011. An endodontic sealer induces a pathological condition when associated with persistent tissue toxicity and presence of myofibroblasts. Braz. Dent. J. 22, 369–376. https://doi.org/10.1590/s0103-64402011000500004.

Hermann, N.V., Lauridsen, E., Ahrensburg, S.S., Gerds, T.A., Andreasen, J.O., 2012. Periodontal healing complications following concussion and subluxation injuries in the permanent dentition: a longitudinal cohort study. Dent. Traumatol. 28, 386–393. https://doi.org/10.1111/j.1600-9657.2012.01165.x.

Kahler, B., Heithersay, G.S., 2008. An evidence-based appraisal of splinting luxated, avulsed and root-fractured teeth. Dent. Traumatol. 24, 2–10. https://doi.org/10.1111/j.1600-9657.2006.00480.x.

Kindelan, S.A., Day, P.F., Kindelan, J.D., Spencer, J.R., Duggal, M.S., 2008. Dental trauma: an overview of its influence on the management of orthodontic treatment. Part 1. J. Orthod. 35, 68–78. https://doi.org/10.1179/146531207225022482.

Kvinnsland, I., Heyeraas, K.J., 1992. Effect of traumatic occlusion on CGRP and SP immunoreactive nerve fibre morphology in rat molar pulp and periodontium. Histochemistry. 97, 111–120. https://doi.org/10.1007/BF00267300.

Massaro, C.S., Consolaro, R.B., Santamaria, M.J., Consolaro, M.F., Consolaro, A., 2009. Analysis of the dentin-pulp complex in teeth submitted to orthodontic movement in rats. J. Appl. Oral Sci. 17 (Suppl), 35–42. https://doi.org/10.1590/s1678-77572009000700007.

Mjör, I.A., 2001. Pulp-dentin biology in restorative dentistry. Part 5: Clinical management and tissue changes associated with wear and trauma. Quintessence Int. 32, 771–788.

Nakatsu, S., Yoshinaga, Y., Kuramoto, A., Nagano, F., Ichimura, I., Oshino, K., Yoshimura, A., Yano, Y., Hara, Y., 2014. Occlusal trauma accelerates attachment loss at the onset of experimental periodontitis in rats. J. Periodontal Res. 49, 314–322. https://doi.org/10.1111/jre.12109.

Nishioka, M., Shiiya, T., Ueno, K., Suda, H., 1998. Tooth replantation in germ-free and conventional rats. Endod. Dent. Traumatol. 14, 163–173. https://doi.org/10.1111/j.1600-9657.1998.tb00832.x.

Pedrini, D., Panzarini, S.R., Tiveron, A.R.F., Abreu, V.M., Sonoda, C.K., Poi, W.R., Brandini, D.A., 2018. Evaluation of cases of concussion and subluxation in the permanent dentition: a retrospective study. J. Appl. Oral. Sci. 26, e20170287 https://doi.org/10.1590/1678-7757-2017-0287.

Penna, L.A., Rode, S.M., 2000. Morphological study of the pulp of Wistar rats molars under experimental occlusal interference. Pesqui Odontol. Bras. 14, 159–164.

Pereira, A.L., de Mendonça, M.R., Sonoda, C.K., Cuoghi, O.A., Poi, W.R., 2010. Histological evaluation of experimentally induced subluxation in rat molars and its implications on the management of orthodontic treatment. Dent. Traumatol. 26, 37–42. https://doi.org/10.1111/j.1600-9657.2009.00837.x.

Popescu, M.R., Deva, V., Dragomir, L.P., Searpe, M., Vătu, M., Stefârţă, A., Rauten, A.M., 2011. Study on the histopathological modifications of the dental pulp in occlusal trauma. Rom. J. Morphol. Embryol. 52, 425–430.

Pugliesi, D.M., Cunha, R.F., Delbem, A.C., Sundefeld, M.L., 2004. Influence of the type of dental trauma on the pulp vitality and the time elapsed until treatment: a study in patients aged 0–3 years. Dent. Traumatol. 20, 139–142. https://doi.org/10.1111/j.1600-4469.2004.00242.x.

Qassem, A., Goettems, M., Torriani, D.D., Pappen, F.G., 2014. Radicular maturity level of primary teeth and its association with trauma sequelae. Dent. Traumatol. 30, 227–231. https://doi.org/10.1111/edt.12072.

Sodeyama, T., Maeda, T., Takano, Y., Hara, K., 1996. Responses of periodontal nerve terminals to experimentally induced occlusal trauma in rat molars: an immunohistochemical study using PGP 9.5 antibody. J. Periodontal Res. 31, 235–248. https://doi.org/10.1111/j.1600-0765.1996.tb00488.x.

Stenvik, A., Mjör, I.A., 1971. The effect of experimental tooth intrusion on pulp and dentine. Oral Surg. Oral Med. Oral Pathol. 32, 639–648. https://doi.org/10.1016/0030-4220(71)90331-8.

Strobl, H., Moschen, I., Emshoff, I., Emshoff, R., 2005. Effect of luxation type on pulpal blood flow measurements: a long-term follow-up of luxated permanent maxillary incisors. J. Oral Rehabil. 32, 260–265. https://doi.org/10.1111/j.1365-2842.2005.01497.x.

Yu, C.Y., 2004. Role of occlusion in endodontic management: report of two cases. Aust. Endod. J. 30, 110–115. https://doi.org/10.1111/j.1747-4477.2004.tb00423.x.