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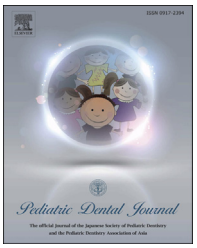

Research Paper

Healing after experimental luxation and intraalveolar root fracture in immature rat teeth

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ABSTRACT

Aims: The purpose of this study was to clarify the histopathological reactions, growth and complications on immature rat molar with intraalveolar root fracture over 3–4weeks.
Methods: The upper first molars of 4-week old male Crlj:WI rats weighing 93.5–107.0 g were pushed horizontally toward the palate to cause a constant amount of dislocation by our previously developed experimental luxation model. The molars were then scanned using two types of 3-dimensional (3D) X-ray micro-computed tomography (μCT), and a 3D analysis was performed. Decalcified sections were also prepared and observed.
Results: In experimental group, root fracture occurred in four mesial roots out of eight teeth, and reparative dentin and cementum formed on the root fracture line over 3–4 weeks. On the periodontal side of the fracture, the surface of the dentin was covered with cementum, and partially covered with a large amount of cellular cementum. No distinct differences in root length were identified between the control group and the experimental group. All molars in the experimental group defined external root resorption. On the palatal area (cervical compression side), the depth and range of cervical external root resorption lacunae increased to 4 weeks. In experimental group, the width of periodontium tended to be larger than that in the control group, mesial tooth sockets had many blood vessels.

1. Introduction

Root fractures are comprising 0.5%–7% of the injuries affecting the immature permanent dentition [1–8]. According to some clinical reports, healing with calcified tissue and interposition of connective tissue of intraalveolar horizontal root fractures were achieved by correct repositioning and ridged fixation [1,9–12]. Many follow-up examinations can disclose complications, such as pulp necrosis or narrowing of the pulp chamber [13–15].

The nature of early histologic events after root fractures has been studied experimentally [16–18]. These studies,

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however, have not clarified the pathogenesis is of the complications developed after root fracture.

Therefore, it is difficult to predict the long-term reactions of immature teeth with root fractures in traumatic dental injuries. In the former studies, we developed the experimental method to induce luxation and intraalveolar root fractures in immature molars of the rat upper jaw without infection. Using this experimental method, it was reported the repair process of root fractures after 1 h, 1 day, 3 days [16,18], 7 days, and 2 weeks [17]. These histopathological finding showed as follows. At postoperative 1 h, transformation and exudation of pulp were seen especially near the root fracture area. Acute inflammation and degenerated cells were seen after 1 day. Irregular odontoblastic layer and wide pulpal blood vessel were observed at postoperative 3 days. Rare cell area and degenerated periodontium appeared in the palatal region after 1 day. Within 3 days after the operation, the rare cell area, degenerated periodontium and odontoclasts appeared in the palatal side. On the surface of the tooth socket, direct or undermining alveolar bone resorption occurred [16,18].

Newly differentiated odontoblast-like cells had formed immature reparative dentin on the pulpal side of the fracture line at 1 postoperative week, and deposited reparative dentin with tubular structure at 2 weeks. While surface resorption and granulation tissue formation occurred on the periodontal side of the fracture line, the narrow fracture line remained unchanged, and the surface resorption lacunae were partly covered with cementum at postoperative 2 weeks [17].

The healing with hard tissue or connective tissue or interposition of granulation tissue were described in the case reports of root fractures [13–15] and experimental studies of root fractures with immature root formation [19–23]. Unfortunately, few studies have examined the repair process of intraalveolar root fractures incurred due to trauma for relatively long period, and many aspects of this repair process remain unclear. But these observations were considered to be in the process of repair or complications. On the other hand, injuries to immature developing teeth might influence the growth of jaw. Thus, the purpose of the present study is to investigate the repair processes of immature teeth and maxillae after luxation and intraalveolar root fracture in rat upper molars over 3–4 weeks, using histopathological techniques and 3-dimensional (3D) X-ray micro-computed tomography (μCT) imaging by two kinds of μCT (in vivo CT and industrial CT).

2. Materials and methods

In this study, 15 4-week old male Crlj:WI rats (ORIENTAL YEAST CO., LTD. Tokyo, Japan) were used. Eight rats were used for experimental group and others saved as control. The animals' body weights ranged from 93.5 to 107.0 g before the operation. All animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (approval number: 0170222A).

All rats were anesthetized via intraperitoneal injection of chloral hydrate. Butorphanol tartrate was then injected subcutaneously into their back. After the injection, the rats in the experimental group were immobilized on the rat molar luxation device described previously [17,24,25]. The rats were secured on the rat fixing apparatus, then the bilateral maxillary first molars were dislocated horizontally toward the palate with modified Hoe's pliers at rotate angle of the movable handle 3° weighted with 900 g within 20 s. The control animals were subjected to the same procedure except for the luxation of the maxillary molars.

Head of all rats was scanned using in vivo 3D X-ray μCT for experimental animals (R_mCT2, Rigaku Corporation, Tokyo, Japan) at postoperative 30 min, 2, 3 or 4 weeks. They were killed by excess CO_2 exposure at 3 to 4 postoperative weeks. The maxillae were dissected from the rats and fixed in 10% buffered formalin at 4 °C for 16–55 days. After fixation, these left first molars were scanned with industrial μCT (inspeXio SMX-100CT, Shimadzu, Kyoto, Japan).

The maxillae were decalcified with Plank-Rychlo solution and embedded in paraffin using a standard histopathological procedure. Frontal sections of 5-μm thickness were stained with hematoxylin and eosin and observed with an optical microscope. Histomorphometric measurement were performed in photomicrographs by scale.

The μCT data were converted into 3D images (INTAGE Volume Editor™ and INTAGE Station Expert INTAGE™, Cybernet Systems Co., Ltd., Tokyo, Japan) to enable visualization of the 3D structure of each molar.

The measurement values of each group were statistically analyzed using Student's t-test.

3. Results

3.1. *Overview of root fractures using histopathological findings and X-ray μCT*

One rat died and two rats damaged during the experimental procedure in control group (Table 1).

Table 1 shows the number of rats imaged by using in vivo X-ray μCT.

On histopathological examination, the roots in the control group have smooth outline (Fig. 1a) and was elongated by cellular cementum deposition after 3–4 weeks.

In the experimental group root fractures were observed in the mesial roots of four out of eight teeth (four locations). These fractures consisted of irregular reparative dentin formation in dentin defects (Fig. 1b,c,f and g). At the same fracture sites irregular linear deformations were observed on experimental appearance of 3D X-ray industrial μCT images.

Table 1 – The number of rats scanned by in vivo μCT at every observation period.

Group	Control	Experimental
Observation period		
30 minutes	6	8
2 weeks	6	8
3 weeks	6	8
4 weeks	3	4

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