Journal of Hard Tissue Biology 24[4] (2015) 397- 400

CODEN-JHTBFF, ISSN 1341-7649

Technical Note

Pathological Examination of Experimentally Induced Periodontal Polyp in Mice

Saeka Matsuda[1,2], Yukiko Yokoi[2],Keita Moriyama[2], Masahito Shoumura[2,3], Naoto Osuga[2,3], Keisuke Nakano[4] and Toshiyuki Kawakami[1]

[1] *Hard Tissue Pathology Unit, Matsumoto Dental University Graduate School of Oral Medicine, Shiojiri, Japan*
[2] *Department of Pediatric Dentistry, Matsumoto Dental University School of Dentistry, Shiojiri, Japan*
[3] *Oral Health Analysis Unit, Matsumoto Dental University Graduate School of Oral Medicine, Shiojiri, Japan*
[4] *Department of Oral Pathology and Medicine, Okayama University Graduate School of Medicine, Dentistry and Phamaceutical Sciences, Okayama, Japan*
(Accepted for publication, August 10, 2015)

Abstract : The mechanism in the formation of periodontal polyp has been established in several histological studies but details on cell differentiation and/or proliferation have not been elucidated. In the present study, we established a convenient and possible experimental system using ddY mice. Briefly, pentobarbital sodium (Somnopentyl) was injected into the abdominal cavity of the mouse followed by access cavity preparation on maxillary first molar using low speed ½ round bur (Merufa Inc), exposing the pulp and then allowed to perforate the floor of the pulp chamber. Observation was done over time until 6 months using micro CT (m_CT) image photography. Results with transmission image using m_CT showed the expansion in the width of the periodontal ligament in the furcation area. The lesion was excised as one mass and examined histopathologically. The granulation tissue was covered with stratified squamous epithelium. The present experimental technique has been confirmed to be effective in analyzing the formation of periodontal polyp induced by mechanical perforation.

Key words: Periodontal polyp, Micro-CT, Histopathology, Experimental model

Introduction

Various periodontal tissue reactions occur after pulpectomy. Removal of vital pulp under local anesthesia causes a response in periapical tissue due to physical and chemical trauma. In general, the location of lesion is different with the anatomical structure of the root canal. Pulpotomy in deciduous molars is a common treatment in Pediatric Dentistry. However, treatment of deciduous teeth is more complicated than permanent teeth due to the small anatomy and young age. In other words, accidental perforation of the floor of the pulp chamber may occur when cutting the vital pulp of primary teeth. Injury to the periapical tissues is also possible during root canal treatment of permanent teeth by protrusion of instruments like broach into the apical foramen.

Lateral perforation is one of the causes of root canal failure leading to acute traumatic periodontitis. A large perforation causes the growth of chronic granulation tissue. In molars, perforation at the bifurcation of roots causes acute inflammation which then proceeds to chronic inflammation often resulting to proliferative dermatitis in young patients. In this case a polyp is formed which is called periodontal polyp. Following the methodology of Osuga et al.[1], chronic inflammation of molar furcation in mice was induced resulting to periodontal ligament polyp formation. Observation of the progression of the lesion was made possible using micro CT (m_CT)[2,3], and histopathological features were analyzed using a light microscope.

Correspondence to: Dr. Toshiyuki Kawakami, Hard Tissue Pathology Unit, Matsumoto Dental University Graduate School of Oral Medicine, 1780, Hirooka-Gobara, Shiojiri, 399-0781 Japan; Tel & Fax: +81-263-51-2035; E-mail: kawakami@po.mdu.ac.jp

Materials and Methods

A total of eight, 8-week old ddY mice were used in the experiment (Table 1). The mice were acclimatized to the environment for 2 weeks prior to the experiment. The animals were housed in a temperature controlled breeding room with a 12 hour day and night cycle and had free access to sufficient food and water throughout the study. Pentobarbital sodium (Somnopentyl®) was injected into the abdominal cavity of the mice and then fixed on a plate. Access preparation on the maxillary first molar was carried out with low speed ½ round bur allowing the perforation of the floor of the pulp chamber[1]. The study was approved by the Animal Committee of Matsumoto Dental University.

Table 1. Number of Experimental Animals

	2 week	12 week	24 week	Total
Experimental	2	2	2	6
Control	2			2

Examination with m_CT

While the animals were under general anesthesia, transmission images were taken using m_CT (Rigaku, Tokyo, Japan) with a tube voltage of 80 kv, tube current of 160.0 μA at a magnification factor of 6.7× and measurement time of 30 s. Under these conditions, the specimens were sectioned at 0.16 mm and photographs were taken preoperatively, immediately after surgery and over time until 6 months.

Histopathological examination

After the observation period, the maxilla was excised en bloc, fixed in 4 % neutral buffered formalin solution and decalcified in 10 % EDTA solution. Then after, specimens were dehydrated in increasing series of alcohol, embedded in paraffin, horizontally sectioned into 4 μm, stained with HE and examined under the microscope.

Results

m_CT image of the control of the first molar revealed intact crown, no defect on enamel and dentin was seen neither on the root. However, transmission image from the pulp cavity to the apex was not clear (Fig. 1-a, b). Histologically, the periodontal ligament fibroblasts were arranged regularly towards the alveolar bone surface from the cementum (Fig. 1-c, d).

At 2 weeks, compared to immediately after surgery, m_CT showed changes in the alveolar bone and periodontal ligament spaces at the apical part (Fig. 2-a, b). Histopathologically, a small abscess was formed just below the floor of the pulp chamber. The fibroblasts were arranged haphazardly and formed granulation tissues. Pulp cavity had disintegrated. The outermost layer was covered with stratified squamous epithelium (Fig. 2-c, d).

At 3 months, the apical part of the root has expanded compared to immediately after surgery. Resorption and destruction of alveolar bone just below the floor of the pulp chamber was clearly observed. Also, the crown has completely collapsed and the mesial and palatal roots were completely separated (Fig. 3-a, b). Histopathologically, granulation tissue with proliferation of fibroblasts was formed at the area of perforation in the bifurcation. Moreover, fibrous tissue formation was evident in the area of bone resorption (Fig. 3-c, d).

At 6 months, alveolar bone below the floor of the pulp chamber has been resorbed and destroyed continuously compared to immediately after surgery (Fig. 4-a, b). Histopathologically, the cellular components of granulation tissue have been reduced and the tissue underwent fibrosis. The alveolar bone at the bifurcation consists of slightly irregular bone trabeculae and chromatophilic remodeling lines stained with hematoxylin as well new bone formation (Fig. 4-c, d).

Discussion

In clinical dentistry, perforation of the floor of the pulp chamber occurs accidentally during cavity preparation. Because of this, various dental materials have been produced to seal the perforation, however results were not necessarily successful. In this regard, it is important to clarify the condition of the periodontal tissue near the furcation specifically the lesion created by accidental perforation of the floor of the pulp chamber. Imaizumi et al [4] performed a histopathological study on the development of lesions after perforation of bifurcation of rat molars. Oka et al [5] also did a similar study. However, the emphasis of the results was on the anatomy of the lesion. On the other hand, researches in the treatment of furcation perforation have also been made by Kudoh et al [6] and Ishida et al.[7].

Periodontal polyps are due to dental caries and faulty root canal treatment such as perforation of the floor of the pulp chamber. Granulation tissue is then formed with sustained stimulation of chronic periodontal ligament infection and sufficient blood supply. Nakamura et al [8], showed a detailed histopathological examination of the changes in the periodontal ligament and growth of granulation tissue. However, the study was limited to histopathological examination.

The present study used experimental animals in an attempt to establish a similar experiment system with Osuga et al.[1], in which no treatment was used. In comparison with the control group, at 2 weeks, the normal physiological sequences in the periodontal ligament have been disturbed; the area was filled granulation tissue and with proliferating fibroblasts. The granulation tissue was covered with stratified squamous epithelium. Histopathological features observed at 2 weeks, 3 months and 6 months were almost similar. Due to the experimental perforation of the floor of the pulp chamber in mice, growth of chronic inflammatory granulation tissue was found to be the main tissue change without causing purulent inflammation. Recently, several studies mentioned that the cellular components of the granulation tissue were derived from the periodontal ligament stem cells (PDLSCs)[9-11].

We utilized bone marrow transplanted GFP mouse to analyze cell differentiation and migration in periodontal tissue. Muraoka et al. [12] showed that macrophages and osteoclasts as well as periodontal ligament fibroblasts were from the bone marrow.

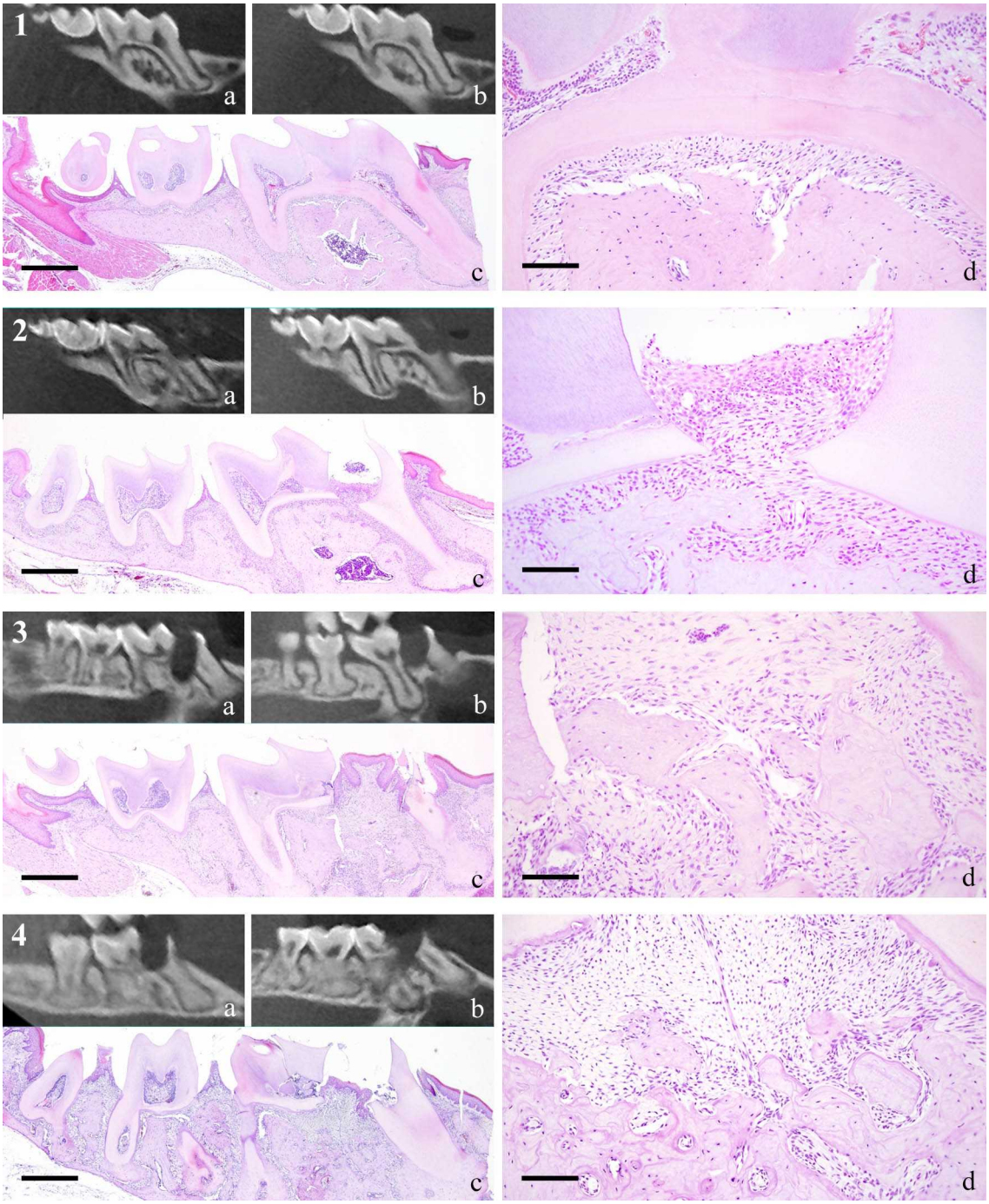

Figure 1. Control specimen. a: m_CT image of non-treatment; b: m_CT image of pre-fixation of 1-month after the taking m_CT (a); c: Histopathological view of the same part of the m_CT image of b, Scale bar=0.5mm; d: Enlarged view of c, Scale bar=100mm.

Figure 2. 2-week-specimen. a: m_CT image of pre-treatment; b: m_CT image of 2-week specimen; c: Histopathological view of the same part of the m_CT image of b, Scale bar=0.5mm; d: Enlarged view of c, Scale bar=100mm.

Figure 3. 3-month-specimen. a: m_CT image of pre-treatment; b: m_CT image of 3-month-specimen; c: Histopathological view of the same part of the m_CT image of b, Scale bar=0.5mm; d: Enlarged view of c, Scale bar=100mm.

Figure 4. 6-month-specimen. a: m_CT image of pre-treatment; b: m_CT image of 6-month-specimen; c: Histopathological view of the same part of the m_CT image of b, Scale bar=0.5mm; d: Enlarged view of c, Scale bar=100mm.

Kaneko et al. [13] also showed that the periodontal ligament fibroblasts were derived from the bone marrow. On the other hand Tsujigiwa et al. [14] showed that bone marrow mesenchymal cells could also differentiate into odontogenic cells. Hence, further analysis to clarify the nature of cells making up the periodontal membrane polyp is necessary.

Acknowledgement

This study was supported in part by Grant-in-Aid for Scientific Research (C) #25463204, (C) # 26463104 and (C) #26463031 from the Japan Society for the Promotion of Science.

References

1. Osuga N, Matsuda S, Shoumura M, Moriyama K, Yokoi Y, Nakano K and Kawakami T. Establishment of experimental periapical inflammatory lesions in mice. J Hard Tissue Biol 22: 517-520, 2013
2. Arai Y, Honda K, Iwai K and Shinoda K. Practical model "3DX" of limited c one-beam X-ray CT for dental use. Car' 01 Computer assisted radiology and surgery. Amsterudam: Elsevier 671-675, 2001
3. Arai Y, Yamada A, Ninomiya T, Kato T and Masuda Y. New micro computed tomography (R_mCT) developed for in vivo animal experiment. Oral Radiol 21: 14-18, 2005
4. Imaizumi I, Iwama A, Shibata N, Kumazawa M, Yamasaki M, Nakamura H, and Kameyama Y. Experimental studies on lesion progression following perforation of the furcation area of rat molars. Aichi Gakuin Dent J 34: 717-723, 1996
5. Oka Y, Yoshikawa M, Takemura M, Yamamoto K and Toda T. Histological examination on periodontal tissue reaction of rat molar following perforation of the chamber floor. Jpn J Conserv Dent 34: 1574-1579, 1991
6. Kudoh J. A Study on the treatment of the furcation perforation using germfree rat. Jpn J Conserv Dent 32: 201-213, 1989
7. Ishida T, Tachibana T, Sato K, Sawada M, Watanabe K, Endo M, Saito H, Takeda Y and Ishibashi M. Experimental study of endodontic treatment for furcation perforation. Jpn J Conserv Dent 28: 1372-1382, 1985
8. Nakamura C, Hayashi T, Iso K and Nakamura F. A case of periondontal polyp, clinically resembled to pulp polyp. J Matsumoto Dent Univ Sci 5: 89-93, 1979
9. Park JC, Kim JM, Jung IH, Kim JC, Choi SH, Cho KS and Kim CS. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: *in vitro* and i*n vivo* evaluations. J Clin Perindontol 38: 721-731, 2011
10. Nakajima R, Ono M, Hara ES, Oida Y, Shinkawa S, Pham HT, Akiyama K, Sonoyama W, Maekawa K and Kuboki T. Mesenchymal stem/progenitor cell isolation from tooth extraction sockets. J Dent Res 93: 1133-1140, 2014
11. Li C, Wang X, Tan J, Wang T and Wang Q. The immunomodulatory properties of periodontal ligament stem cells isolated from inflamed periodontal granulation. Cells Tissues Organs. 199: 256-65, 2014
12. Muraoka R, Tsujigiwa H, Nakano K, Katase N, Tamamura R, Tomida M, Okahuji N, Nagatsuka H and Kawakami T. Transplanted bone marrow-derived Cell migration into periodontal tissues and cell differentiation. J Hard Tissue Biol 20: 301-306, 2011
13. Kaneko K, Matsuda S, Muraoka R, Nakano K, Iwasaki T, Tomida M, Tsujigiwa H, Nagatsuka H and Kawakami T. Histological evaluation of periodontal ligament in response to orthodontic mechanical stress in mice. Int J Med Sci 12: 689-694, 2015
14. Tsujigiwa H, Katase N, Sathi GA, Buery RR, Hirata Y, Kubota M, Nakano K, Kawakami T and Nagatsuka H. Transplanted bone marrow derived cells differentiated to tooth, bone and connective tissues in mice. J Hard Tissue Biol 20: 147-152, 2011