Scanning Electron Microscopy Reveals Severe External Root Resorption in the Large Periapical Lesion

KENNSUKE OOKUBO, ATSUSHI OOKUBO, MASAKI TSUJIMOTO, KOUJI SUGIMOTO, SHIZUKA YAMADA, AND YOSHIHIKO HAYASHI*

Department of Cariology, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-7-1, Nagasaki 852-8588, Japan

Running title: ROOT RESORPTION ON DIFFERENT CONDITIONS

*Corresponding to: Y. Hayashi; Department of Cariology, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-7-1, Nagasaki 852-8588, Japan. E-mail: hayashi@nagasaki-u.ac.jp
KEY WORDS apicoectomy; computed tomography; periapical root resorption; scanning electron microscopy

ABSTRACT The present study was designed to investigate the relationships between clinicopathological findings and the resorptive conditions of root apices of teeth with periodontitis. The samples included 21 root apices with large periapical radiolucent lesions. The preoperative computed tomography (CT) and intraoperative findings were correlated with the presence, extension, and the progression pattern of periapical resorption using a scanning electron microscope. The subjects' age, gender, chief complaint, type of tooth, percussion test results, size of periapical lesion using CT, and intraoperative findings were recorded. All apicoectomies were performed under an operative microscope for endodontic microsurgery. A significant large size was observed in cystic lesions compared with granulomatous lesions. The cementum surface at the periphery of the lesion was covered with globular structures (2–3 μm in diameter). Cementum resorption started as small defect formations at the surface. As the defect formation progressed, a lamellar structure appeared at the resorption area, and the size of globular structures became smaller than that of globules at the surface. Further resorption produced typical lacuna formation, which was particularly observed in fracture cases. The most morphologically severe destructive pattern of dentin resorption was observed in large cystic lesions. This study is the first report to elucidate the relationships between three clinical types of undesirable periapical lesions: (1) undertreatment, (2) periapical fracture, (3) macro-level resorption, and the microstructure of external root resorption including from small defects at the cementum surface to a significant destructive pattern inside the dentin.

INTRODUCTION

Chronic periapical lesions of teeth represented as granulomatous and cystic tissues are the result of persistent inflammation at the periapical region (Lin et al., 1984; Yanagisawa, 1980). Cystic lesions were reported to account for between 3.2% (Nair, 1987) and 54% (Priebe et al., 1954) of apical lesions, and granuloma lesions for between 45% (Lalonde and Luebke, 1968) and 96.8% (Nair, 1987). These differences may be explained by sampling and histopathological methods. Most of the periapical lesions are granulomas, and cysts demonstrate the largest radiolucent images using digital orthopantomography (Carrillo et al., 2008).

Endodontic microsurgery, particularly apicoectomy, has presented an evolutionary advance for the treatment of large periapical lesions, and is applied using ultrasonic preparation, improved filling material, and a surgical microscope (Kim and Kratchman, 2006). Furthermore, a combination strategy such as a clinical preoperative three-dimensional (3D) diagnosis using computed tomography (CT) is indispensable for a proper and precise operation. These modern endodontic surgical procedures may result in a good success rate (Kim et al., 2008; Maddalone and Gagliani, 2003; Song et al., 2012; Taschieri et al., 2006).

Apicoectomy with both preoperative X-ray imaging using CT and operation under a stereomicroscope was listed in the national health insurance from April 1st, 2014 in Japan. Although the combination of a preoperative 3D evaluation of periapical lesions and microsurgery for apicoectomy has become a popular endodontic procedure worldwide, little is known about the relationships between the preoperative 3D evaluation and the intraoperative findings of periapical lesions, or the progression pattern of external periapical resorption. The present study was designed to investigate the relationships between the clinicopathological findings and the resorptive conditions of extirpated root apices with periapical inflammatory lesions. The preoperative CT findings, clinical symptoms, and the intraoperative findings were correlated with the presence, extension, and the structural changes of periapical external resorption using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Sampling

This study was approved by the ethics committee of Nagasaki University Graduate School of Biomedical Sciences (authorized number: 1501). All patients were informed about the study and provided their written informed consent.

The samples included 21 tooth root apices with large periapical radiolucent lesions from patients who visited the Department of Cariology at Nagasaki University Hospital. The subjects' age, gender, chief complaint, type of tooth, percussion test results, size (x, y, and z directions) of periapical lesion using CT, and intraoperative findings were recorded.

Apicoectomy

All apicoectomies were performed under an operative microscope (Leica M300, Leica Microsystems GmbH, Wetzlar, Germany) for endodontic microsurgery following a dental X-ray and CT (CT High Speed Advantage, GE Healthcare Japan, Tokyo, Japan) examinations, and the root apices were carefully amputated to a length of approximately 3 mm using a #104R diamond point (SHOFU INC., Kyoto, Japan). After apicoectomy, the cavity (about 2-mm in depth) was prepared at the cut surface for retrofilling, and then the cavity was filled with mineral trioxide aggregate (ProRoot MTA, Dentsply Tulsa dental, Tulsa, OK).

Specimen Preparation

Tooth root apices were fixed with 10% formalin in 0.1 M of phosphate buffer at pH 7.4 for 1 week, and then immersed in 3% sodium hypochlorite solution for 15 min, and dehydrated in increasing concentrations of ethanol. Ethanol was replaced with t-butanol and the specimens were freeze-dried and preserved in containers at room temperature until further analysis.

SEM Analysis

The samples were mounted on aluminum holders with adhesive carbon tape, and observed using an operating microscope. Thereafter, the same samples were coated with carbon using a vacuum evaporator (JEE-400; JEOL Ltd., Tokyo, Japan). They were then examined using SEM (S-3500N; Hitachi Ltd., Tokyo, Japan). SEM was conducted using a secondary electron imaging technique, with the instrument operating at 20 kV at a working distance of 15 mm (magnification: 30–2,000×).

The apical samples were classified depending on the presence or absence of external resorption, as well as extension, according to the following criteria. The final score was counted as the score for 1) plus 3), and the score for 2) plus 3), with a maximum total score of 5 at each place.

1. Foraminal resorption: Defined as resorption at the outline of the foramen. Degrees of severity ranging from 0 to 3 were used.

0: Absence of resorption
1: Resorption of up to 1/4 of the circular outline
2: Resorption from 1/4 to 1/2 of the circular outline
3: Resorption of over 1/2 of the circular outline

2. Periforaminal resorption: Defined as the area of resorption outside of the foraminal outline. Degrees of severity ranging from 0 to 3 were used.

0: Absence of resorption
1: Resorption of up to 0.5 mm from the circular outline
2: Resorption from 0.5 to 1mm from the circular outline
3: Resorption of over 1 mm from the circular outline

3: Resorption depth from the surface

0: Absence of resorption
1: Inside the cementum
2: Inside the dentine

Statistical Analyses

The resorption scores were expressed as the means ± SD. The differences about the size of periapical lesions between the two groups were assessed using a one-tailed Student's *t*-test. Significance was set at a level of $P<0.05$.

RESULTS

Size and Degree of Apical Lesions

The clinical and SEM findings in each extirpated apex were summarized in Table 1. Regarding the intraoperative findings of lesions using an operative microscope, a significant large size (x, y, and z directions) in CT examination was observed in the cystic lesion (Fig. 1) compared with the granulomatous lesion (x direction: P<0.01, y and z directions: P<0.05). The patients expressed three chief complaints: swelling (18 cases, 85.7%), occlusal pain (10 cases, 47.6), and tenderness (4 cases, 19.0%). In the present observation, the etiology and/or condition of the apical lesions were classified into three clinical types: undertreatment cases by dental X-ray imaging (Fig. 2), apical fracture cases by the intraoperative and SEM findings (Fig. 3), and external root resorptive cases under dental X-ray imaging (Fig. 4). Regarding these clinical criteria, the type of root resorption presented a significantly larger size of lesion than the fracture type (P<0.05). The z direction of undertreatment was significantly larger than that of the fracture type (P<0.05). The present 18 samples were extirpated from the maxillary alveolar bone. The CT images revealed that the cystic lesion (4 cases) expanded as a concentric circle around the apex.

SEM Findings

The cementum surface at the periphery of the lesion area was covered with globular structures (2–3 lm in diameter), similar to the intact cementum. Regarding the external root resorption scores at periapices, although there were no significant differences between foraminal and periforaminal regions in the cystic lesions (P>0.05), a significantly higher score occurred at the foraminal area compared with the periforaminal area in granulomatous lesions (P<0.05). Furthermore, cystic lesions presented with significantly higher scores in both areas compared with granulomatous lesions (P<0.05). Regarding the three clinical classified types of lesions, although there were no significant differences between the foraminal and periforaminal regions in both undertreatment and root resroption cases (P>0.05), a significantly higher score occurred at the foraminal in the fracture type (P<0.05). Furthermore, among these three clinical types of lesions, although there were no significant differences between the resorption scores at both areas in undertreatment and fracture cases (P>0.05), both areas in the root resorption type presented significantly higher scores compared with the undertreatment and fracture groups [P<0.01, except the foraminal area compared with the fracture type (P<0.05)].

Cementum resorption started as small defects (10–30 μm in width) at the surface of the affected cementum (Fig. 5). As the size of the surface defect increased, a lamellar structure appeared at the resorption area in the cementum, where globular structures were still observed on the lamellae, and the small size of globular structures (～1 μm in diameter) was also recognized at the surface of the lamellae (Fig. 6). Further resorption produced typical lacuna structures which were particularly observed in fracture cases, where orifices of dentinal tubules were identified at some places. The bottom of the lacunae presented relatively smooth surfaces without globular structures (Fig. 7). The most morphologically severe destructive pattern of dentin resorption in the present cases was observed in large cystic cases, where bundle-like structures were obvious at the surface of resorptive dentin (Fig. 8).

DISCUSSION

This study is the first report to identify the relationships between three types of clinically undesirable periapical lesions and the microstructure of external root resorption using SEM including from small defects at the cementum surface to the significant destructive pattern inside the dentin.

The major advantage of CT is that it is easy to perform searching and extirpation of lesion in operation as a 3D extension of the lesion can be preoperatively confirmed. The lesion size of a cyst is reported to be significantly larger than that of granulomatous tissue (Carrillo et al., 2008; Lalonde, 1970; Mortensen et al., 1970; Ricucci et al., 2006; Trope et al., 1989; White et al., 1994; Zain et al., 1989). Similar results were obtained in the present study. The extension pattern of a cyst in the maxilla may originate from characteristics of the jaw bone. The maxillary bone is generally rich in spongy structures compared with the mandibular compact cortical bone (Abe, 2010). Furthermore, mechanical compression by the significantly large size of a cystic lesion may be relatively stronger than that of granulomatous tissue (Allon et al., 2015). Apicoectomy under an operating microscope is beneficial for dentists. Its main advantage is the ability to perform almost complete curettage of a lesion (Von Arx et al., 2003; Wang et al., 2004; Zuolo et al., 2000). A small bag-like cystic lesion can be precisely and directly confirmed due to direct microscopical confirmation of the lesion nature. Furthermore, after an extirpated cystic lesion, the smooth inside of an alveolar bony defect is also directly confirmed using an operating microscope. Thus, the clinical type of lesions could be classified in this study.

To the best of our knowledge, this is the first report to investigate the relationship between three types of clinically undesirable lesions at the apical-third region associated with endodontic treatment and the structural characteristics of external periapical root resorption. The present scoring system clearly demonstrated that cystic lesions and macro-level resorption represented significantly high scores, which indicate the appearance of severe resorption. Therefore, this system is reasonable and useful for the objective evaluation of periapical root resorption (Vier and Figueiredo, 2002). Unique and characteristic microstructures during several resorption processes could be identified. The small defect at the cementum surface may be produced by local inflammatory dissolution of the cementum globules and osteoclastic activity. Crystal dissolution and deposition occur during cementum resorption, similar to dental caries (Aoba et al., 1981). The small size of the globular structures observed at the progressed resorptive stage of the lamellae indicated typical dissolution of the cementum globules. Although granulomatous lesions (mainly the clinically undertreatment type) produced external root resorption, the depth of resorption demonstrated a shallow tendency. This pattern is likely dependent on the resistance of lamellar structures to acid attacks within the lesion. Furthermore, progressive resorption results in the destruction of the lamellar structure, odontoclastic activity contributes this process.

Typical lacunae appeared at the resorptive region of fracture cases in the dentin. This finding indicates that local inflammation at a fracture site is more severe than that generally observed in periapical lesions, and the fracture space acts as a pathway to spread inflammation, resulting in a poor clinical prognosis. Lacunae with relatively smooth surfaces without globular structures are a differential marker for external root resorption inside the dentin. As resorption displays a honeycomb appearance at low magnification (Felippe et al., 2009; Vier and Figueiredo, 2002), smooth surfaces of the lacunae are important for identifying resorption in dentin. The diameters of these lacunae are equivalent to dozens of micrometers, which is similar to that of odontoclasts (Yawaka, 1993). Cellular resorption together with crystal dissolution occurs at the region corresponding to the cement–dentinal junction after the destruction of the upper part of the cementum.

The greatest morphologically destructive change in the present SEM observation corresponded to clinically cystic root resorptive cases. Many bundle-like structures on the raggedly resorptive surface may be associated with an organic origin, which likely corresponds with the bundle of degenerative collagen fibers. A very similar destructive structure was reported in the root resorption pattern associated with ameloblastoma (Sreeja et al., 2009). In teeth associated with odontogenic tumors, most teeth showed an extensive loss of root length from the resorptive processes (Struthers and Shear, 1976). This aggressive root resorptive pattern reflects the loss of cellular regulatory control with tumor progression, and a rapidly growing lesion (Sreeja et al., 2009). These findings indicate that the final stage of a periapical lesion results from the same mechanism as an odontogenic tumor.

REFERENCES

Abe S. 2010. The difference between maxillary and mandibular bones. J Acad Clin Dent 30:246-247 (in Japanese).

Aoba T, Moriwaki Y, Doi Y, Ozkazaki M, Takahashi J, Yagi T. 1981. The intact surface layer in natural enamel caries and acid-dissolved hydroxyapatite pellets. An X-ray diffraction study. J Oral Pathol 10:32-39.

Allon DM, Allon I, Anavi Y, Kaplan I, Chaushu G. 2015. Decompression of odontogenic cystic lesions in children. J Oral Maxillofac Surg 73:649-654.

Carrillo C, Penarrocha M, Ortega B, Marti E, Bagan JV, Vera F. 2008. Correlation of radiographic size and the presence of radiopaque lamina with histological findings in 70 periapical lesions. J Oral Maxillofac Surg 66:1600-1605.

Felippe WT, Ruschel MF, Felippe GS, Pozzobon MH, Felippe MCS. 2009.

SEM evaluation of the apical external root surface of teeth with chronic periapical lesion. Aust Endod J 35:153-157.

Kim S, Kratchman S. 2006. Modern endodontic surgery concepts and practice: a review. J Endod 32:601-623.

Kim E, Song JS, Jung IY, Lee SJ, Kim S. 2008. Prospective clinical srudy evaluating endodontic microsurgery outcomes for cases with lesions of endodntic origin compared with cases with lesions of combined periodontal-endodontic origin. J Endod 34:546-551.

Lalonde ER. 1970. A new rationale for the management of the periapical granulomas and cysts: An evaluation of histopathological and radiographic findings. J Am Dent Assoc 80:10561059.

Lalonde ER, Luebke RG. 1968. The frequency and distribution of periapical cysts and granulomas. Oral Surg Oral Med Oral Pathol 25:861-868.

Lin L, Shovlin F, Skribner J, Langeland K. 1984. Pulp biopsies from the teeth associated with periapical radiolucency. J Endod 10:436-448.

Maddalone M, Gagliani M. 2003. Periapical endodntic surgery: a 3-year follow-up study. Int Endod J 36:193-198.

Mortensen H, Winther JE, Birn H. 1970. Periapical granulomas and cysts: An investigation of 1600 cases. Scand J Dent Res 78:241-250.

Nair P. 1987. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 13:29-39.

Priebe W, Lazansky J, Wuehrmann A. 1954. The value of the roentgenographic film in the differential diagnosis of periapical lesions. Oral Surg Oral Med Oral Pathol 7:979-983.

Ricucci D, Mannocci F, Pit Ford TR. 2006.Astudy of periapical lesions correlating the presence of a radiopaque lamina with histological findings. Oral Surg Oral Med Oral Pathol 101:389-394.

Song M, Chung W, Lee SJ, kim E. 2012. Long-term outcome of the cases classified as successes based on short-term follow-up in endodontic microsurgery. J Endod 38:1192-1196.

Sreeja R, Minal C, Madhuri T, Swati P, Vijay W. 2009. A scanning electron microscopic study of the patterns of external root resorption under different conditions. J Appl Oral Sci 17:481-486.

Struthers P, Shear M. 1976. Root resorption by ameloblastoma and cyst of the jaws. Int J Oral Surg 5:128-132.

Tascieri S, Del Fabbro M, Testori T, Francetti L, Weinstein R. 2006. Endodontic surgery using 2 different magnification devices: preliminary results of randomized controlled study. J Oral Maxillofac Surg 64:235-242.

Trope M, Pettigrew J, Petras J, Bamett F, Tronstad L. 1989. Differentiation of radicular cyst and granulomas using computerized tomography. Endod Dent Traumatol 5:69-72

Vier FV, Figueiredo JAP. 2002. Prevalence of different periapical lesions associated with human teeth and their correlation with the presence and extension of apical external root resorption. Int Endod J 35:710-719.

Von Arx T, Frei C, Bornstein MM. 2003. Periradicular surgery with and without endoscopy: a prospective clinical comparative study. Schweiz

Monatsschr Zahnmed 113:860-865.

Wang N, Knight K, Sweet M. 2004. Treatment outcome in endodontics-The Toront Study. Phases I and II: apical surgery. J Endod 30:751-761.

White SC, Sapp JP, Seto BG, Mankovich NJ. 1994. Absence of radiogmetric differentiation between periapical cysts and granulomas. Oral Surg Oral med Oral Pathol 78.650-654.

Yanagisawa S. 1980. Pathological study of periapical lesion 1. Periapical granulomas: clinical, histopathologic and immunohistopathologic studies. J Oral Pathol 9:288-300.

Yawaka Y. 1993. Observation of odontoclasts in the human deciduoua teeth by tartrate-resistant acid phosphatase activity. Jpn J Oral Biol 35:409-430 (in Japanese).

Zain RB, Roswati N, Ismail K. 1989. Radiographic evaluation of lesion sizes of histologically diagnosed periapical cysts and granulomas. Ann Dent 48:3-5.

Zuolo ML, Ferreira MO, Gutmann JL. 2000. Prognosis in periradicular surgery: a clinical prospective study. Int Endod J 33:91-98.

Figure legends

Fig. 1. CT images of a large cystic lesion (arrows) of upper left lateral incior. A: horizontal plane. B: frontal plane.

Fig. 2. Dental X-ray image of an undertreatment case of upper right second premolar. Note a large radiolucent periapical lesion.

Fig. 3. SEM image of an apical fracture case of upper right lateral incisor.

Fig. 4. Dental X-ray image of a root resorptive case of upper left central incisor. Note a large radiolucent periapical lesion.

Fig. 5. Initial defects (arrows) at the cementum surface of upper left first premolar.

Fig. 6. Lamellar and globular structures at the resorptive region of upper right canine. Note different size of globules.

Fig. 7. Typical resorptive region in denti of . A: many lacunae present noneycomb-like structure. Arrows indicate dentinal tubules. B: note the relative smooth surface of enlarged lacumae.

Fig. 8. Severe resorptive region of dentin (upper left central incisor). Note the bundle-like structures.

TABLE 1. Clinical and SEM information in each case

Cases No.	Age Gender	Tooth Type	Size of Lesions (CT, mm) x	y	z	Chief Complaint	Clinicopathological Type	Clinical Type	Resorption Score (Region) Foraminal	Periforaminal
1	65 F	11	6.3	6.6	5.3	Swelling/OP	Granuloma	Undertreatment	4	2
2	54 M	26MB	2.6	2.8	1.3	Swelling/OP	Granuloma	Fracture	2	2
3	82 F	14	4.8	5.8	5.8	Swelling	Granuloma	Undertreatment	2	2
4	74 F	31	5.0	4.6	3.1	Swelling	Granuloma	Fracture	3	2
5	56 F	12	4.2	4.1	3.8	Swelling/OP	Granuloma	Fracture	4	2
6	55 M	16MB	6.9	7.1	6.2	Swelling/OP	Granuloma	Resorption	4	4
7	65 F	24	4.5	5.7	5.8	Swelling	Granuloma	Undertreatment	4	3
8	60 F	22	14.0	12.9	15.8	Swelling	Cyst	Undertreatment	4	4
9	67 F	15	7.4	5.9	4.6	Swelling	Cyst	Undertreatment	4	3
10	67 F	16MB	3.2	3.6	3.5	Swelling	Granuloma	Undertreatment	3	3
11	55 F	34	4.2	4.6	4.0	Swelling/T	Granuloma	Fracture	4	4
12	75 M	24	3.7	3.9	3.2	Swelling/OP/T	Granuloma	Undertreatment	3	2
13	58 F	24	4.0	2.6	2.3	OP	Granuloma	Resorption	5	5
14	58 F	25	3.9	3.2	1.4	OP	Granuloma	Fracture	5	4
15	49 F	21	10.3	10.9	7.4	Swelling	Cyst	Resorption	5	5
16	54 M	44	4.4	5.1	3.5	Swelling/OP	Granuloma	Undertreatment	2	2
17	58 M	26MB	10.9	9.7	7.1	Swelling/OP	Granuloma	Resorption	4	3
18	45 M	11	5.0	5.8	1.9	Swelling/T	Granuloma	Fracture	2	2
19	66 M	13	3.9	1.2	1.4	OP/T	Granuloma	Undertreatment	2	4
20	78 F	22	3.8	4.4	3.5	Swelling/OP	Granuloma	Fracture	3	2
21	28 M	21	10.2	14.8	15.2	Swelling	Cyst	Resorption	4	4

F, female; M, male; Tooth Type is presented using the two-digital tooth numbering system; MB, mesiobuccal root; CT, computed tomography; OP, occlusal pain; T, tenderness.

Fig1

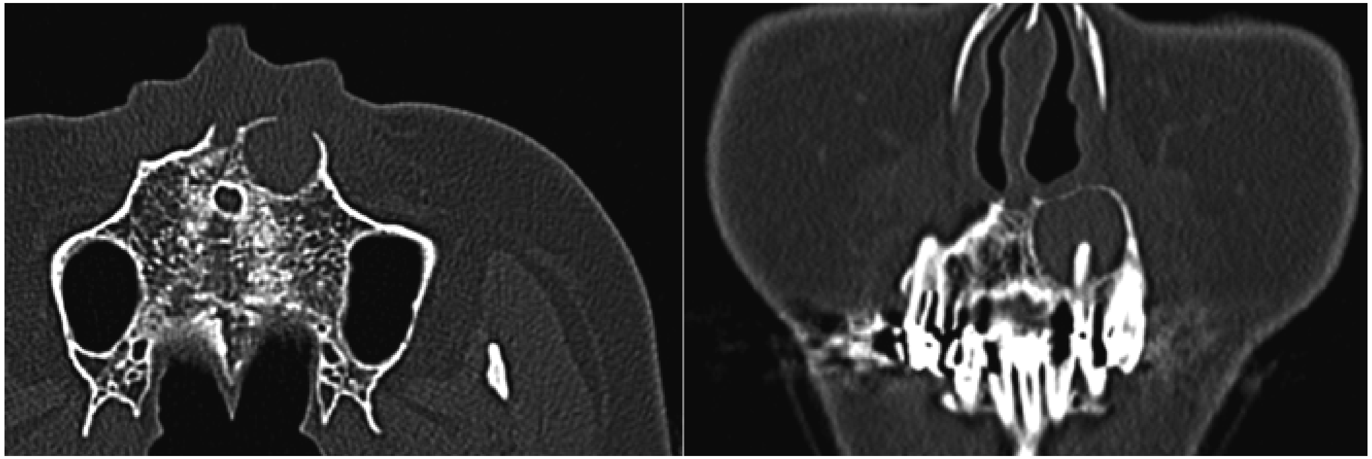


Fig2

Fig3

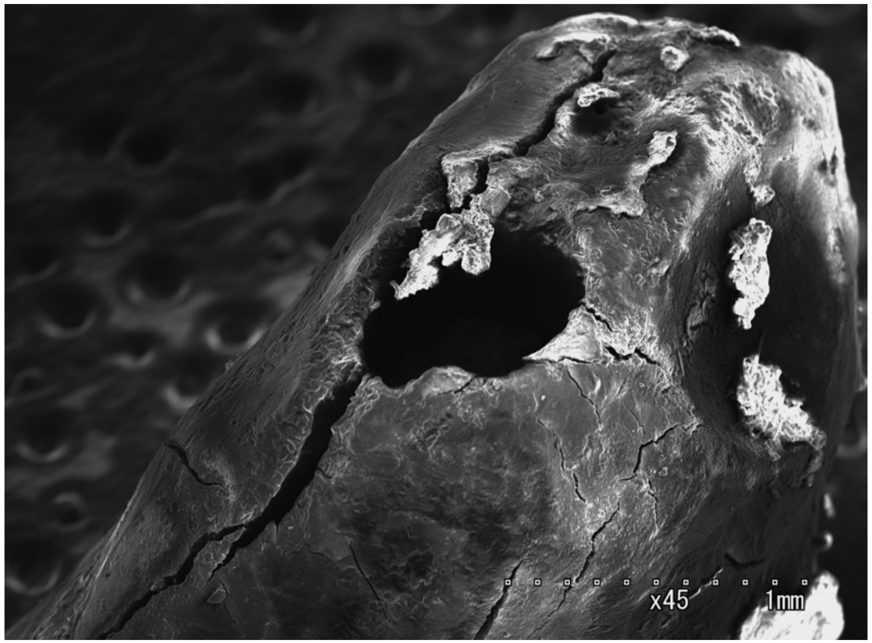


Fig4

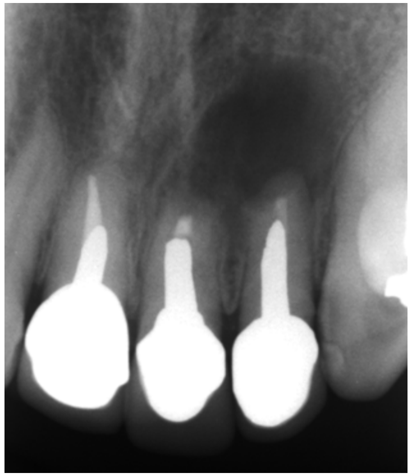


Fig5

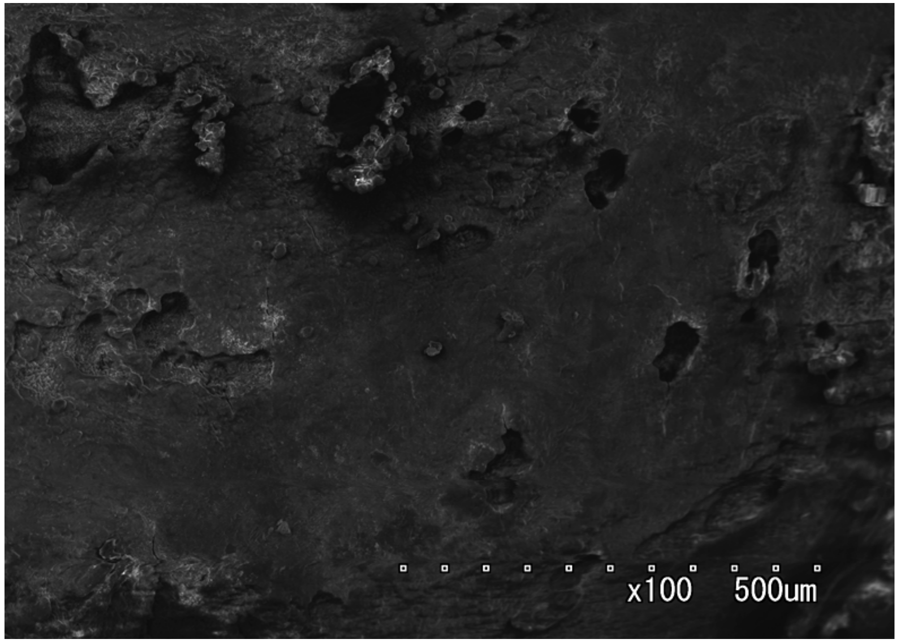


Fig6

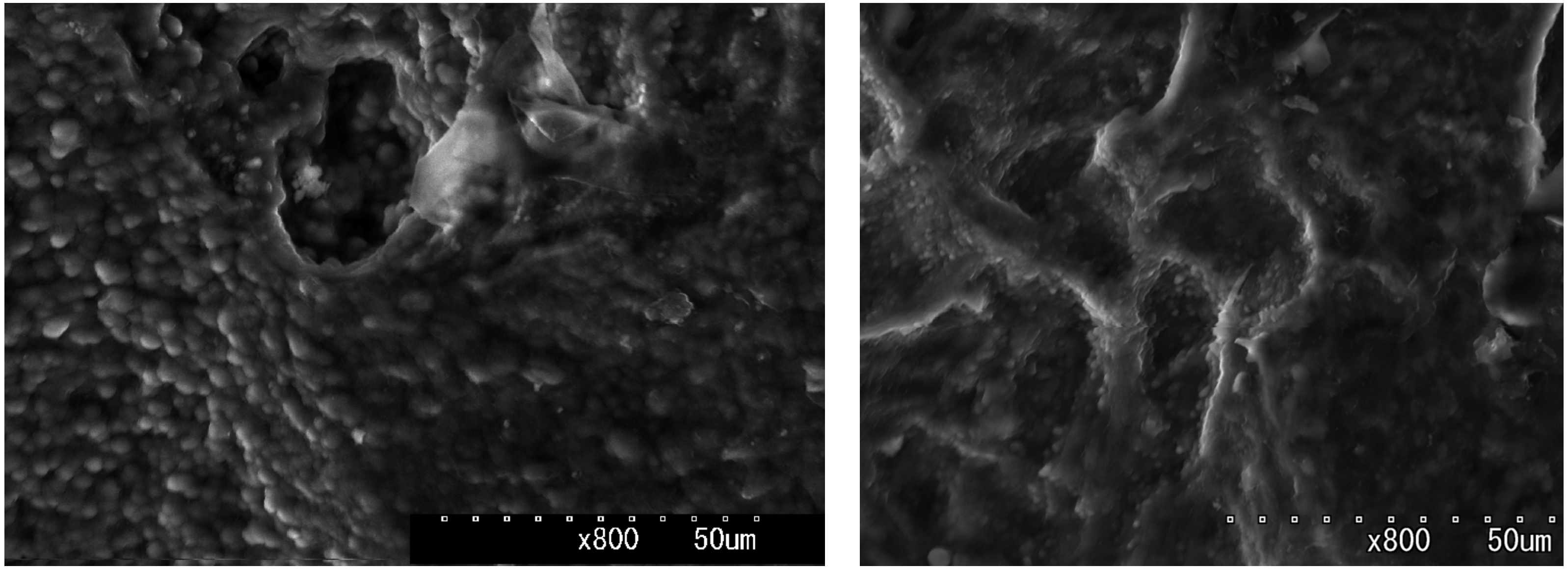


Fig7

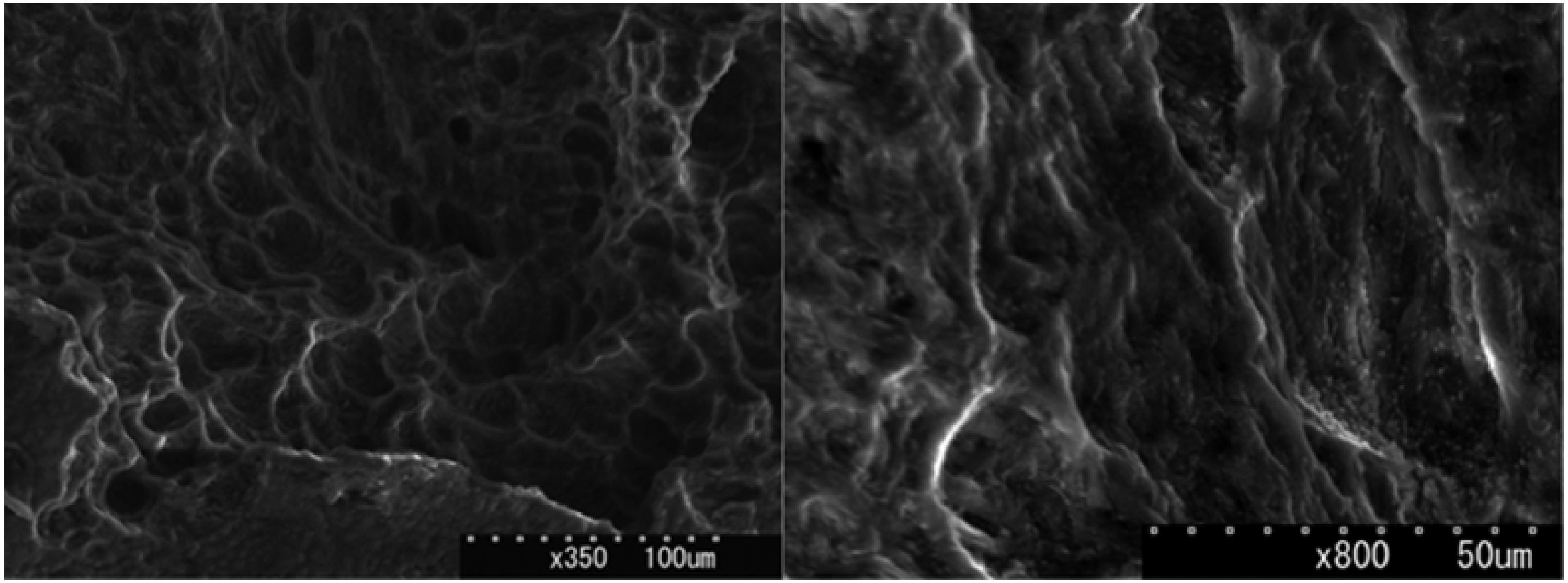
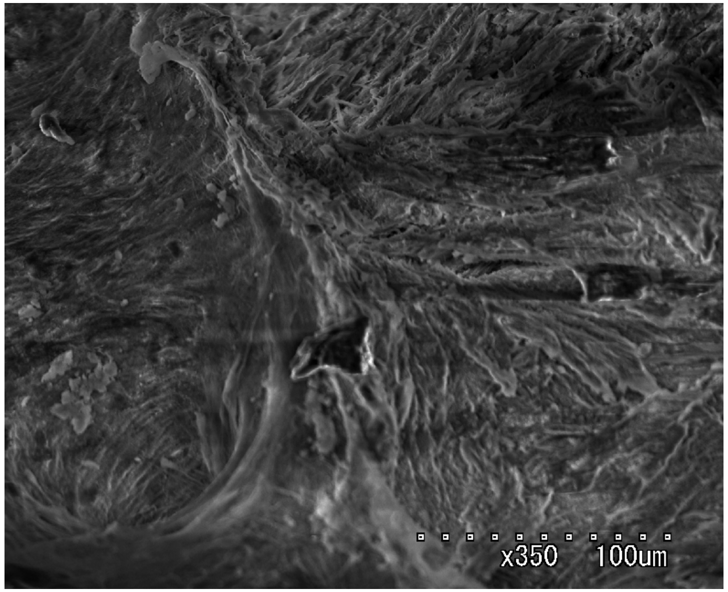
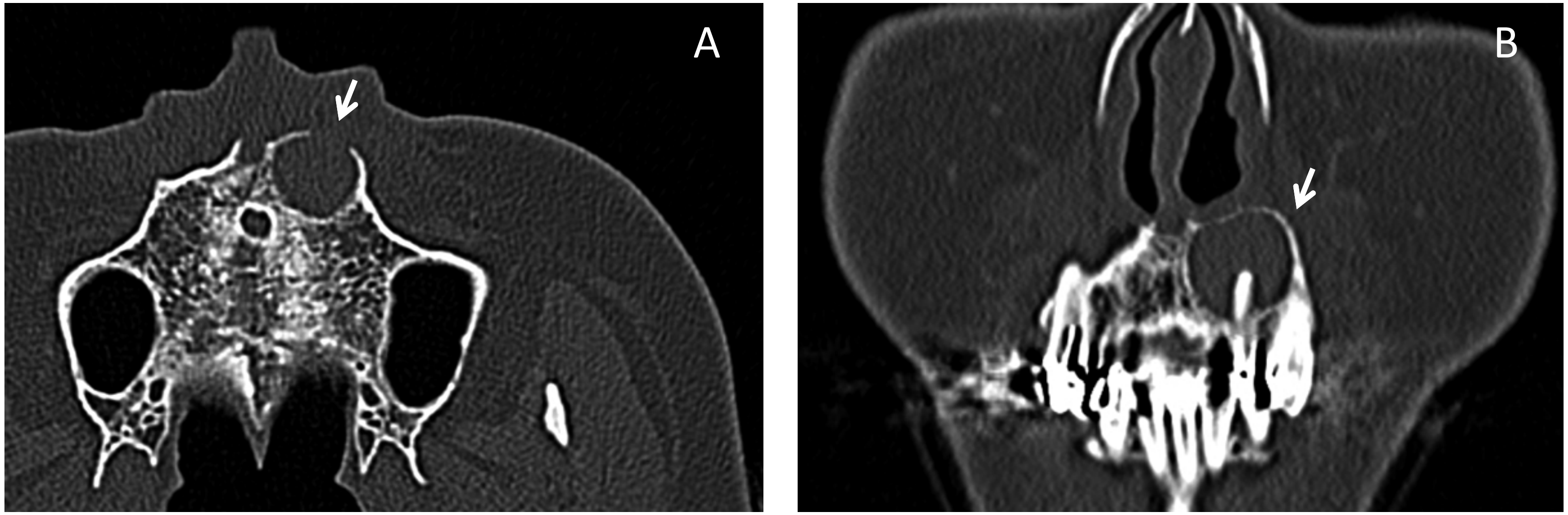


Fig8




A
B