

Original

Factors Influencing Bone Healing after Extirpation with Endodontic Microsurgery–Microscopic Apicoectomy for Extensive Radicular Cysts

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Abstract

Background & Aims: The purpose of this study is to identify the clinical and immunohistological factors that inhibit bone healing after extirpation of extensive radicular cysts and endodontic microsurgery-microscopic apicoectomy, and utilize these factors as predictors of treatment outcome. **Methods:** Endodontic microsurgery-microscopic apicoectomy was performed in 26 patients. Outcomes were evaluated based on clinical signs and radiographs. Expression levels of cytokines (IL-1 β , TNF- α , RANKL) in the cyst epithelium and subepithelial connective tissue were also investigated. **Results:** The success rate was 88.5%. The radiological reduction rate was $\geq 75\%$ in 16 patients (good bone healing) and $< 75\%$ in 10 (poor bone healing). In clinical analysis, penetrating bone defects and the site (maxilla) showed significant differences between the two groups ($P < 0.05$). In immunohistochemical analysis, expression of IL-1 β and TNF- α showed significant differences between the two groups ($P < 0.05$). **Conclusions:** Penetrating bone defects, inflammation, and a maxillary site are prognostic factors for a poor outcome after treatment of extensive radicular cysts. Endodontic microsurgery-microscopic apicoectomy is a new technique for treatment of an extensive radicular cyst, and this study is the first to identify factors that influence the outcome after extirpation of the cyst.

Article Information

Key words:

endodontic microsurgery-microscopic apicoectomy,
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Introduction

In surgical treatment of radicular cysts, complete removal of lesions and treatment of the infected root canals of the causative teeth are required.¹ The Partsch II method, whereby cysts are completely removed and the wound is closed, is generally used. The Partsch I method, in which cysts are marsupialized and the cyst epithelium is integrated with the oral mucosal epithelium, or the modified Partsch I method (packed open method), in which the region after complete extirpation of a cyst is left open, may be selected as required in individual cases. Apicoectomy is often concomitantly performed to eliminate the causative teeth.

In the Department of Oral and Maxillofacial Surgery, Gunma University Hospital, radicular cysts extending over three or more teeth are treated using a combination of complete extirpation and endodontic microsurgery-microscopic apicoectomy.^{2,3} This is a new technique for treatment of an extensive radicular cyst, and favorable outcomes have been achieved. However, some cases have delayed healing or a poor prognosis. For a small bone defect after extirpation involving one to two teeth, bone healing related to peripheral bone formation may be achieved by primary closure. However, for an extensive radicular cyst, both bone and scar healing are recognized in many cases. Bone healing of a defect is important for resistance to infection, jaw strength and morphological recovery.

Inflammatory cytokines related to chronic inflammation may inhibit bone healing.⁴⁻⁷ A radicular cyst is

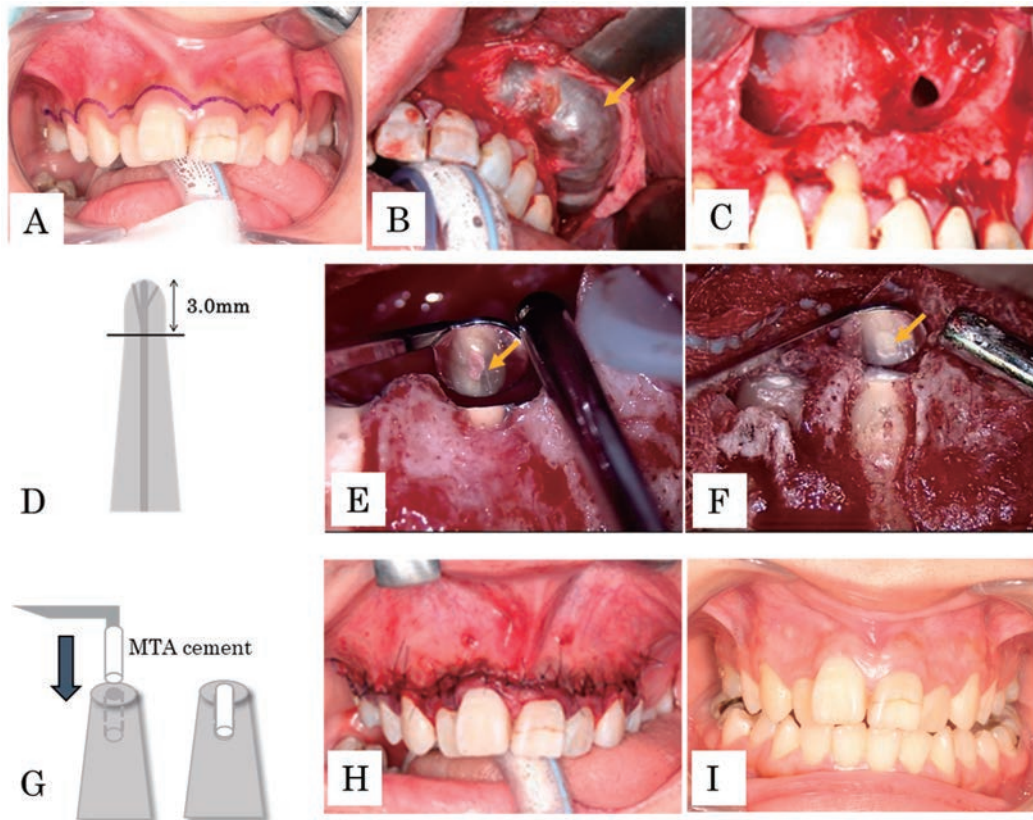


Fig. 1 Surgical procedures

Ochsensbein-Luebke flap (A), manifestation of the cyst (arrow: cyst wall) (B), extirpation of the cyst (C), resection to remove the apical ramification and lateral branches at 3 mm root apex (D), check of cut surface of the causative tooth after microscopic apicoectomy (arrow: cut surface) (E), retrograde filling of the root canal with mineral trioxide aggregate (MTA) cement (arrow: MTA cement) (F and G), complete gingival closure (H), and one year postoperatively (I).

an inflammatory cyst, and many inflammatory cytokines are present in the cyst wall.⁸⁻¹¹ Pericyclic bone resorption depends on the balance between osteoclasts and osteoblasts, and several cytokines are important in osteoclast differentiation, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor κ B (RANK), receptor activator of nuclear factor kappa-B ligand (RANKL), and osteoprotegerin (OPG).⁸⁻¹³

The purpose of this study is to determine the clinical and pathological factors that inhibit bone healing after extirpation of extensive radicular cysts using endodontic microsurgery-microscopic apicoectomy, and to utilize these factors as predictors of treatment outcome.

Treatment Protocol and Surgical Procedure

Initially, a detailed clinical evaluation of the causative tooth and an imaging examination, including dental radiographics, panoramic imaging, and multidetector computed tomography (CT), were performed. Adaptation of apicoectomy should be considered after confirming the periodontal conditions, including the periodontal pocket and furcation area. For detailed imaging, we used a whole-body multidetector CT (Aquilion ONE™; Canon Medical Systems Corp., Otawara, Japan) instead

of a dental cone beam CT. This device is equipped with 320 detectors, the largest number used worldwide, and allows wide-range imaging compared with dental cone-beam CT. This is extremely useful for diagnosis and planning of surgery for lesions in the oral and maxillofacial regions, such as an extensive radicular cyst.

Radicular cyst extirpation was performed using the Partsch II method. Endodontic microsurgery was performed based on the concept described by Kim et al.^{2,3} (Fig. 1). An OME-7000[©] microscope (Olympus Corporation, Tokyo, Japan) for neurosurgery and vascular anastomosis was used, with an assistant working in the same visual field as the operator because of the binocular side-view capacity of the microscope. A papilla base incision¹⁴ and an Ochsensbein-Luebke flap¹⁵ were used depending on the case. An ophthalmological 0° scalpel, which is an excellent tool for gingival incisions because of its size, thinness, flexibility, and blade angle, was used in the procedure. This scalpel is well suited for surgical incisions in a microscopically magnified visual field.

A 3-mm root apex was resected to remove the apical ramification and lateral branches, and a 0° bevel angle of the cut surface was targeted to decrease the exposed dental tubules. After apicoectomy, the presence of residual cyst was checked microscopically and the root

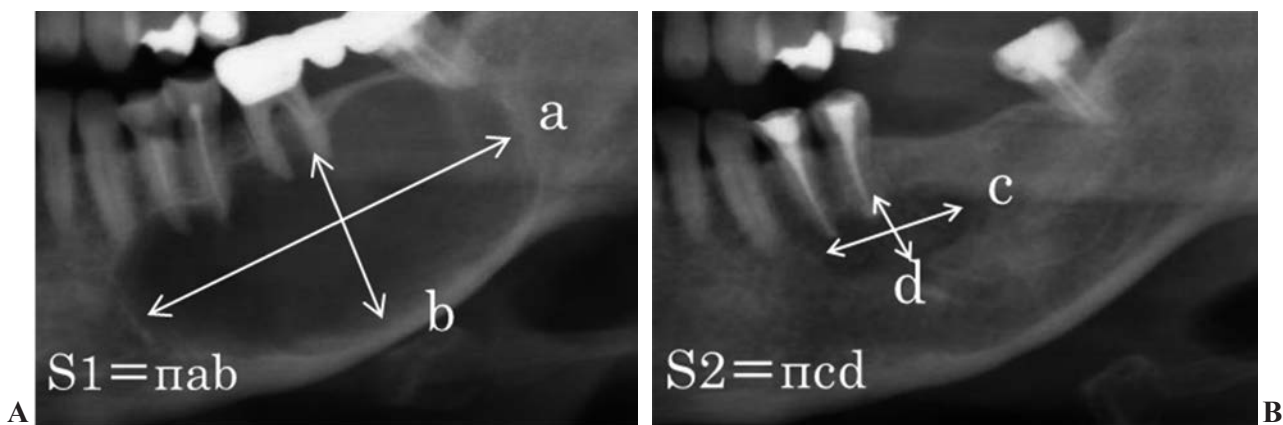


Fig. 2 The rate of reduction area calculated by panoramic X-ray
Radiographic evaluation was performed based on panoramic X-ray films taken preoperatively (A) and postoperatively (B) for one year. The radiolucent area on the X-ray films was calculated as an oval to evaluate the rate of reduction of the bone defect.

$$\text{Rate of reduction} = \frac{S1 - S2}{S1} \times 100(\%)$$

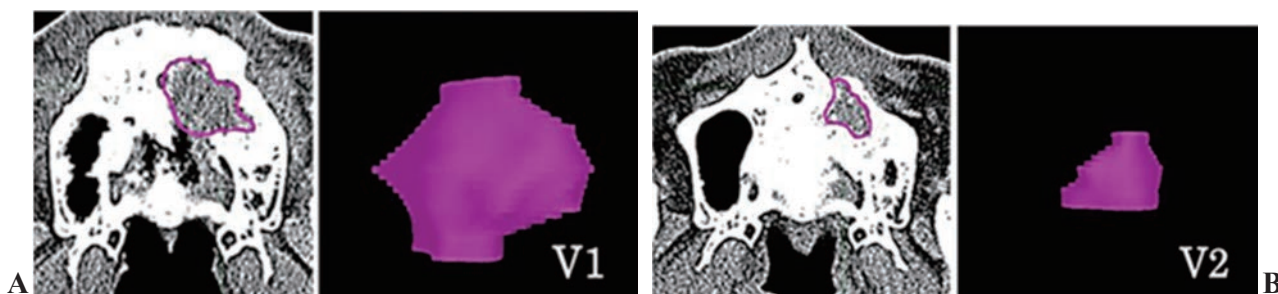


Fig. 3 The rate of reduction area calculated by computed tomography
In CT evaluation, the contour of the bone defect was established on each CT slice and the volume was calculated through the three-dimensional formation of the bone defect (shaded surface display method). The slice width on CT was 0.5 mm, and MIM Maestro ver. 6.6.2[©] was used for image analysis. The reduction rate was calculated from the volumes of the preoperative cyst cavity (A) and the bone defect cavity (B) at one year postoperatively.

$$\text{Rate of reduction} = \frac{V1 - V2}{V1} \times 100(\%)$$

canal crack, accessory root canal, and isthmus were stained using methylene blue. Careful extirpation of large lesions is necessary using a micro-mirror because of the risk of leaving a residual cyst in the back of the root canal. Finally, retrograde filling of the root canal was performed using mineral trioxide aggregate (MTA) cement after ensuring hemostasis and moisture protection. To prevent postoperative gingival trapdoor deformity, concavity and scarring, the wound was tightly sutured with a thin (6-0 to 7-0) nylon monofilament because a buried suture used for skin is not applicable in gingivoperiosteal flaps. The suture was secured under a microscope.

Methods

The Gunma University Ethics Committee approved all aspects of this study (HS2019-144).

Analysis of comprehensive clinical treatment outcome

Endodontic microsurgery-microscopic apicoectomy was performed in 26 patients between 2007 and 2017 in

the Department of Oral and Maxillofacial Surgery, Gunma University Hospital. All patients with radicular cysts pathologically extending over three or more teeth were followed up for one year or longer. The patients included 12 males and 14 females, and were aged 24-69 years, with a median age of 46 years. Outcomes were comprehensively evaluated based on clinical signs and radiographs. The clinical signs included fistulation in the root apex, and pressure and/or percussion pain in the teeth. Radiographic evaluation was performed based on panoramic X-ray films taken preoperatively and postoperatively for one year. The radiolucent area on the X-ray films was calculated as an oval to evaluate the rate of reduction of the bone defect (Fig. 2). CT imaging was used for cases with unclear X-ray images that made evaluation difficult. In CT evaluation, the contour of the bone defect was established on each CT slice and the volume was calculated through the three-dimensional formation of the bone defect (shaded surface display method). The slice width on CT was 0.5 mm, and MIM Maestro ver. 6.6.2[©] (MIM Software Inc., Cleveland, OH, USA) was used for image analysis. The reduction rate

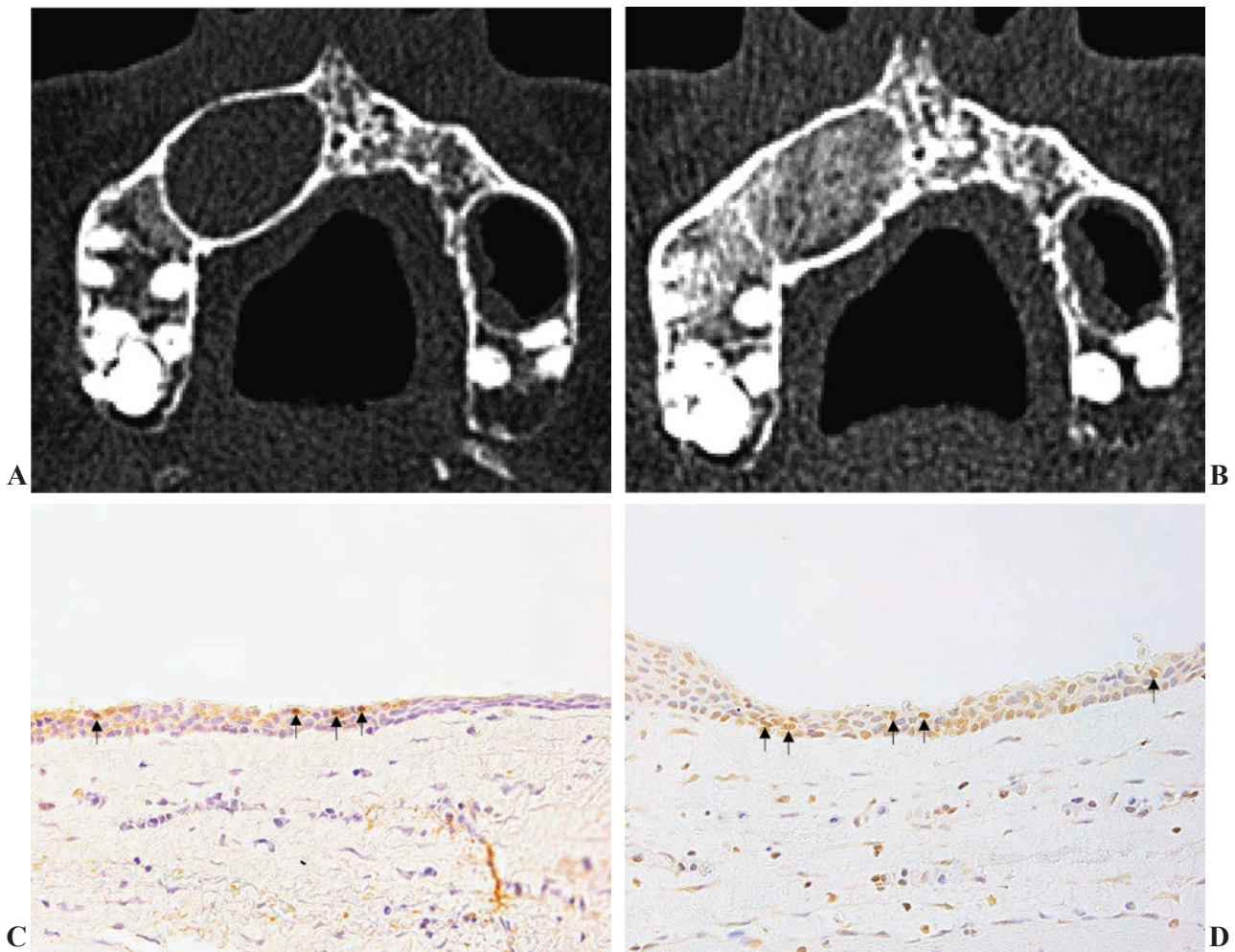


Fig. 4 Case 17

CT imaging at the time of the first medical examination (A), and one year postoperatively (B). The TNF- α (C) and IL-1 β (D) expression rates in the cyst epithelium were $<75\%$ (400-fold magnified viewing field, arrow: positive cell). There were no clinical signs one year after surgery, and the bone defect reduction rate on CT was 90.7%. Therefore, the outcome was evaluated as “successful”.

was calculated from the volumes of the preoperative cyst cavity and the bone defect cavity at one year postoperatively (Fig. 3). Decreases of $\geq 75\%$, 50-75%, and $<50\%$ in the radiolucent area of a cyst were considered to indicate satisfactory healing, a curative tendency, and delayed healing, respectively, as described by Tsiklaskis et al.¹⁶ Cases achieving satisfactory healing or a curative tendency in the absence of clinical signs were assigned a comprehensive outcome of “successful”, and others were defined as a “failure”.

Immunohistochemical analysis

Using radicular cyst samples extirpated in surgery, TNF- α , IL-1 β and RANKL expression was examined immunohistologically in cells comprising the cyst epithelium and subepithelial connective tissue of the cyst wall. The cyst samples were fixed in 10% buffered formalin, embedded in paraffin, cut into 5- μm thick sections, and mounted on slides. The deparaffinized sections were then prepared for antigen retrieval. Immunostaining was carried out using the biotin-streptavidin complex method. For elimination of endogenous

peroxidase, the sections were treated with 3% hydrogen peroxide in water at room temperature for 15 minutes and then incubated overnight at 4°C with antibodies against IL-1 β (1: 200, SC-7884, Poly, Santa Cruz), TNF- α (1: 2,500, ab66579, Poly, Abcam) and RANKL (1:500, SC-377079, Poly, Santa Cruz). After washing, the sections were incubated with a biotin-labeled secondary antibody for 30 minutes at room temperature. After the sections were incubated with peroxidase-labeled streptavidin for 30 minutes at room temperature, they were reacted with 3,3-diaminobenzidine. Finally, the cell nuclei were counterstained with hematoxylin solution, and slide were sealed. Positive cells were counted in a 400-fold magnified viewing field, and positive cell rates of $\geq 75\%$ and $<75\%$ were regarded as high and low expression, respectively.¹⁰

Analysis of factors influencing bone healing

To analyze factors that influence bone healing after cyst extirpation, the 26 patients were divided into groups with $\geq 75\%$ and $<75\%$ reduction rates for the radiolucency area at one year postoperatively, indicating good

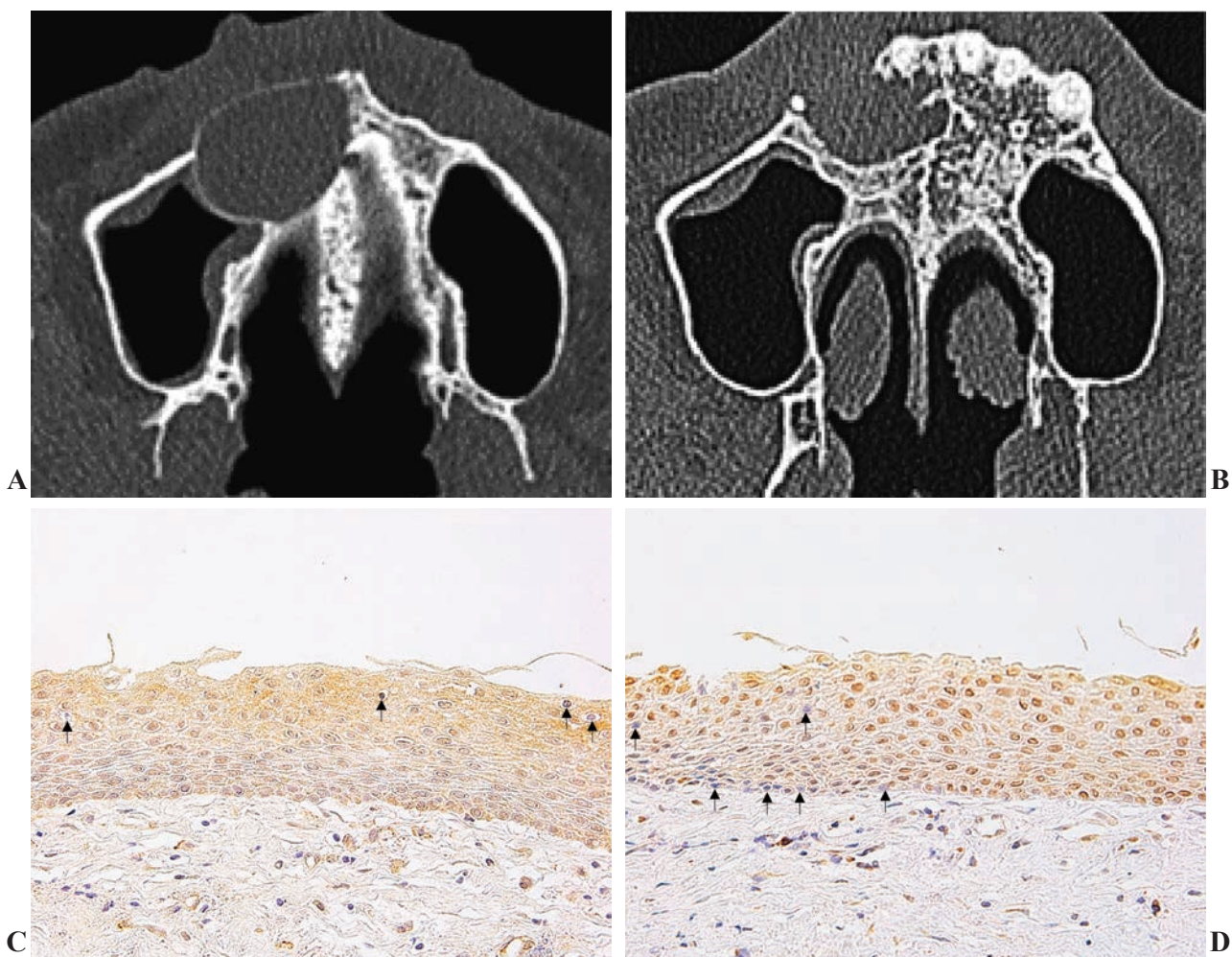


Fig. 5 Case 18

CT imaging at the time of the first medical examination (A), and one year postoperatively (B). The TNF- α (C) and IL-1 β (D) expression rates in the cyst epithelium were $\geq 75\%$ (400-fold magnified viewing field, arrow: negative cell). There were no clinical signs one year after surgery, but the bone defect reduction rate was only 22.4%. Therefore, the outcome was evaluated as a “failure”.

and poor bone healing, respectively. Clinical factors (site, marginal periodontitis: periodontal pocket ≥ 4 mm, fistula, pain, root canal retreatment, preoperative cortical bone defects, and penetrating bone defects) and pathological factors (TNF- α , IL-1 β , and RANKL expression in the cyst epithelium and subepithelial connective tissue) were compared between the groups by univariate analysis using a cross table with a Fisher’s exact test for independence. Next, to extract the preoperative clinical factors influencing bone healing, items with significant differences between the two groups were analyzed using logistic regression analysis with the forward selection method. $P < 0.05$ was considered to denote significance. Statistical analysis was performed using SPSS for Windows, ver. 25 (IBM Corp., Armonk, NY).

Results

Comprehensive treatment outcomes

The overall success rate was 88.5% (maxilla: 85.7%; mandible: 91.7%). The outcomes for two patients with a cyst in the maxilla (Case 2, Case 18) were comprehensively

evaluated as a “failure” because the radiographic reduction rate was $< 50\%$ after one year of follow up (delayed healing), although there were no postoperative clinical signs, definitively. In Case 24 with a cyst in the mandible, a fistula appeared in the buccal gingiva 11 months after surgery, and the outcome was comprehensively evaluated as a “failure” (Table 1).

Analysis of clinical factors influencing bone healing

The radiological reduction rate was $\geq 75\%$ in 16 patients (good bone healing) and $< 75\%$ in 10 patients (poor bone healing). The penetrating bone defects and the site (maxilla) differed significantly between the cases with good and poor bone healing (penetrating bone defect: $P = 0.009$, site: $P = 0.011$) (Table 2). Furthermore, logistic regression analysis with forward selection indicated that the penetrating bone defect was the most important preoperative clinical factor related to poor bone healing with a $< 75\%$ reduction rate in the radiolucent area ($P = 0.011$) (Table 3).

Table 1. Comprehensive outcome of endodontic microsurgery-microscopic apicoectomy in the Department of Oral and Maxillofacial Surgery, Gunma University Hospital

| Case | Age | Sex | Site | Radiograph | Reduction rate of radiolucent area of postoperative 1 year | Clinical sign | | Comprehensive Outcome |
|------|-----|-----|--------------|-----------------|--|---------------|-------------|-----------------------|
| | | | | | | Fistula | Pain | |
| | | | | | | Pre./ Post. | Pre./ Post. | |
| 1 | 53 | M | Mx. Incisor | Panoramic X-ray | 78.3 | - / - | + / - | Successful |
| 2 | 49 | M | Mx. Incisor | CT | 25.8 | - / - | - / - | Failure |
| 3 | 43 | F | Mx. Incisor | Panoramic X-ray | 63.9 | - / - | - / - | Successful |
| 4 | 28 | M | Mx. Incisor | Panoramic X-ray | 53.8 | - / - | + / - | Successful |
| 5 | 24 | F | Mx. Incisor | Panoramic X-ray | 84.3 | - / - | - / - | Successful |
| 6 | 43 | F | Mx. Incisor | Panoramic X-ray | 66.1 | + / - | - / - | Successful |
| 7 | 39 | F | Mx. Incisor | CT | 52.2 | + / - | + / - | Successful |
| 8 | 41 | F | Mx. Incisor | Panoramic X-ray | 67.5 | + / - | - / - | Successful |
| 9 | 47 | M | Mx. Incisor | Panoramic X-ray | 59.2 | + / - | - / - | Successful |
| 10 | 64 | F | Mnd. Incisor | Panoramic X-ray | 83.2 | - / - | - / - | Successful |
| 11 | 64 | F | Mnd. Molar | Panoramic X-ray | 76.6 | - / - | - / - | Successful |
| 12 | 55 | F | Mnd. Molar | Panoramic X-ray | 76.5 | - / - | - / - | Successful |
| 13 | 66 | M | Mnd. Molar | Panoramic X-ray | 77.3 | - / - | - / - | Successful |
| 14 | 44 | M | Mnd. Molar | Panoramic X-ray | 87.5 | - / - | - / - | Successful |
| 15 | 33 | M | Mx. Molar | Panoramic X-ray | 77.2 | - / - | - / - | Successful |
| 16 | 39 | M | Mnd. Molar | Panoramic X-ray | 87.4 | - / - | - / - | Successful |
| 17 | 65 | F | Mx. Incisor | CT | 90.7 | - / - | + / - | Successful |
| 18 | 59 | M | Mx. Incisor | CT | 22.4 | - / - | + / - | Failure |
| 19 | 46 | M | Mx. Molar | Panoramic X-ray | 76.1 | - / - | - / - | Successful |
| 20 | 44 | F | Mx. Molar | Panoramic X-ray | 60.5 | - / - | - / - | Successful |
| 21 | 60 | M | Mnd. Incisor | Panoramic X-ray | 55.0 | - / - | - / - | Successful |
| 22 | 53 | F | Mnd. Incisor | Panoramic X-ray | 82.0 | - / - | - / - | Successful |
| 23 | 44 | M | Mnd. Incisor | Panoramic X-ray | 79.8 | + / - | - / - | Successful |
| 24 | 46 | F | Mnd. Incisor | Panoramic X-ray | 89.0 | - / + | - / - | Failure |
| 25 | 69 | F | Mnd. Incisor | Panoramic X-ray | 89.0 | - / - | + / - | Successful |
| 26 | 24 | F | Mx. Incisor | Panoramic X-ray | 80.0 | + / - | - / - | Successful |

Mx.: Maxilla, Mnd.: Mandible Pre.: Preoperative, Post.: Postoperative

The overall success rate was 88.5% (maxilla: 85.7%; mandible: 91.7%). The outcomes for two patients with a cyst in the maxilla were comprehensively evaluated as a "failure" because the radiographic reduction rate was <50% after one year of follow up (delayed healing), although there were no clinical signs. In Case 24, a fistula appeared in the buccal gingiva 11 months after surgery, and the outcome was comprehensively evaluated as a "failure".

Immunohistochemical analysis of pathological factors influencing bone healing

In the good bone healing group, expression of IL-1 β , TNF- α , and RANKL was lower in the epithelium of the cyst wall. Especially, expression levels of IL-1 β and TNF- α were significantly lower compared to those in the poor bone healing group (IL-1 β : P=0.014 and TNF- α : P=0.017) (Table 2). Neutrophils, macrophages, plasma cells, and fibroblasts infiltrating the sub-epithelial connective tissue of the cyst wall exhibited low expression for IL-1 β , TNF- α , and RANKL in the good bone healing group, as for the epithelium. There were no significant differences in any other parameters between the two groups (Table 2).

Case presentation

Case 17

A 65-year-old Japanese female with swelling of the right maxillary gingiva consulted with the Department of Oral and Maxillofacial Surgery, Gunma University Hospital. Panoramic X-ray showed a continuous radiolucent area involving the root apex of the right upper central and lateral incisors. Under a diagnosis of a right maxillary radicular cyst, cyst extirpation and endodontic microsurgery-microscopic apicoectomy for the right upper central and lateral incisors, as causative teeth, were performed. There were no penetrating bone defects. The TNF- α and IL-1 β expression rates in the

cyst epithelium were <75%. There were no clinical signs one year after surgery, and the bone defect reduction rate on CT was 90.7%. Therefore, the outcome was comprehensively evaluated as "successful" (Fig. 4, Table 1).

Case 18

A 59-year-old Japanese male with swelling of the right maxillary gingiva consulted with the Department of Oral and Maxillofacial Surgery, Gunma University Hospital. Panoramic X-ray showed a continuous radiolucent area involving the root apex of the right upper lateral incisor. Under a diagnosis of a right maxillary radicular cyst, cyst extirpation and endodontic microsurgery-microscopic apicoectomy for the right upper lateral incisor, as the causative tooth, were performed. A bone defect was observed at the palatal side after cyst extirpation, resulting in penetration. The TNF- α and IL-1 β expression rates in the cyst epithelium were \geq 75%. There were no clinical signs one year after surgery, but the bone defect reduction rate was only 22.4%. Therefore, the outcome was comprehensively evaluated as a "failure" (Fig. 5, Table 1).

Discussion

Microscopic apicoectomy was first reported by Reuben et al.¹⁷ in 1984. In 1997, this procedure was established as endodontic microsurgery by Kim et al.^{2,3}

Table 2. Factors influencing bone healing (Results of the univariate analysis with a Fisher's exact test for independence)

| Factor | | Reduction rate of radiolucent area of postoperative 1 year | | Total | Odds ratio | p Value |
|---|-----------|--|--------------|-------|------------|---------|
| | | ≥ 75% (n=16) | < 75% (n=10) | | | |
| Clinical factor | | | | | | |
| Penetrating bone defect | + | 4 | 8 | 12 | 0.083 | 0.009 |
| | - | 12 | 2 | 14 | | |
| Cortical bone defect | + | 9 | 7 | 16 | 0.391 | |
| | - | 7 | 3 | 10 | | |
| Site | Maxilla | 6 | 9 | 15 | 0.067 | 0.011 |
| | Mandibule | 10 | 1 | 11 | | |
| Periodontal pocket | ≥4mm | 0 | 2 | 2 | 0.138 | |
| | <4mm | 16 | 8 | 24 | | |
| Fistula | + | 2 | 4 | 6 | 0.128 | |
| | - | 14 | 6 | 20 | | |
| Pain | + | 3 | 3 | 6 | 0.420 | |
| | - | 13 | 7 | 20 | | |
| Root canal retreatment | + | 5 | 6 | 11 | 0.150 | |
| | - | 11 | 4 | 15 | | |
| Pathological factor | | | | | | |
| IL-1β (Epithelium) | High | 0 | 4 | 4 | — | 0.014 |
| | Low | 16 | 6 | 22 | | |
| TNF-α (Epithelium) | High | 2 | 6 | 8 | 0.095 | 0.017 |
| | Low | 14 | 4 | 18 | | |
| RANKL (Epithelium) | High | 0 | 2 | 2 | 0.138 | |
| | Low | 16 | 8 | 24 | | |
| IL-1β (Subepithelial Connective tissue) | High | 0 | 2 | 2 | 0.138 | |
| | Low | 16 | 8 | 24 | | |
| TNF-α (Subepithelial Connective tissue) | High | 1 | 0 | 1 | 0.615 | |
| | Low | 15 | 10 | 25 | | |
| RANKL (Subepithelial Connective tissue) | High | 0 | 1 | 1 | 0.385 | |
| | Low | 16 | 9 | 25 | | |

The radiological reduction rate was ≥ 75% in 16 patients (good bone healing) and < 75% in 10 patients (poor bone healing). The penetrating bone defects and the site (maxilla) differed significantly between the cases with good and poor bone healing. In immunohistochemical analysis, expression of IL-1 β and TNF- α in the cyst epithelium differed significantly between two groups.

Table 3. Clinical factor influencing bone healing (Results of the logistic regression analysis with forward selection analysis)

| Clinical factor | | Reduction rate of radiolucent area of | | Total | β (Regression coefficient) | SE (β) (Standard error) | Odds ratio | 95% CI | p Value |
|-------------------------|---|---------------------------------------|-------------|-------|----------------------------|-------------------------|------------|---------------|---------|
| | | ≥ 75% (n=16) | < 75%(n=10) | | | | | | |
| Penetrating bone defect | + | 4 | 8 | 12 | - 2.485 | 0.979 | 0.083 | 0.012 — 0.568 | 0.011 |
| | - | 12 | 2 | 14 | | | | | |

The logistic regression analysis with forward selection analysis indicated that the presence of a penetrating bone defect was the most important preoperative clinical factor related to the poor bone healing with < 75% reduction rate radiolucent area.

The efficacy of endodontic microsurgery-microscopic apicoectomy can be attributed to advantages of (1) the amount of root apex to be resected can be set; (2) exposure of the dental tubule can be minimized; (3) the root canal crack, accessory root canal, and isthmus can be identified; (4) the root canal can be tightly sealed off with root canal filling; and (5) lesions around the roots can be reliably removed.^{2,3}

By resecting a 3-mm root apex, 98% of apical ramifications and 93% of lateral branches are removed. The bevel angle of the cut surface minimizing exposure of the dental tubule is 0°. In conventional macroscopic apicoectomy, the bevel angle of the cut surface when preparing the retrograde cavity is 45-60°. In contrast, using the microscopic procedure, a cavity with a bevel angle close to 0° can be prepared with use of an ultra-

sonic retrotip. The use of retrotips has the following benefits: 1) perforation of the root canal wall can be avoided by preparing a cavity parallel to the longitudinal axis of the tooth; and 2) a 3-mm or deeper cavity, needed for avoiding loss of any retrograde root canal-filling agent, can be prepared. We used biocompatible MTA cement,¹⁸ a retrograde root canal-filling agent with good cavity-sealing characteristics, because it contains inorganic oxides, such as CaCO₃ and SiO₂. Moreover, it has a favorable sealing ability, even when exposed to blood,¹⁹ and is reported to induce formation of cementoblasts.²⁰ Therefore, MTA cement may be the ideal post-apicoectomy retrograde root canal-filling material.

We used endodontic microsurgery for causative teeth of extensive radicular cysts over three or more teeth, and obtained favorable results (success rates: max-

illa, 85.7%; mandible, 91.7%). The success rates of conventional macroscopic apicoectomy for intractable apical periodontitis, including radicular cyst, have remained at about 40-60%.²¹⁻²⁴ However, these success rates were obtained for lesions located in one or two teeth, with various evaluation criteria, observation periods, and methods. These results cannot be simply compared with our outcomes, especially because our procedure was applied to severe lesions extending over three or more teeth. The results of our study suggest that this procedure is useful for extensive radicular cysts. However, two patients with maxillary radicular cysts had delayed bone healing (Case 2, Case 18). Neither case had clinical signs or serial changes at the bone defect, suggesting a cicatrization-related delay in bone healing. Scar healing of a bone defect after cyst extirpation may cause problems such as reduced bone strength, susceptibility to infection, and gingival excavation.

In bone healing after cyst extirpation, periosteal bone formation of the inner wall of the defect begins and matures with organization of blood clots. Subsequently, bone formation at the periosteum of the cortical bone begins, leading to filling of the defect by new bone.²⁵ However, after extensive radicular cysts are extirpated, this healing process does not always occur due to factors such as the defect size, a difference in bone quality between the maxilla and mandible, residual lesions, and leakage of root canal bacteria. These problems may be reduced by microscope-facilitated accurate lesion extirpation and close sealing of the canal system. It is necessary to treat the bone defect in order for the treatment known as crypt control to be successful.²⁶

In many cases of large radicular cysts extending over three or more teeth, the periosteum at the bone defect is absorbed. If the wound is simply closed with gingiva after cyst extirpation, the surface of the wound may be covered by subepithelial tissue (mesenchymal tissue). The main cells in this tissue are fibroblasts, which have a shorter division cycle and a higher proliferative activity than those of osteoblasts.²⁷ Therefore, fibroblasts invade the bone defect early after surgery, leading to fibrous healing; that is, scar healing. A large bone defect enables fibroblast invasion, and the penetrating bone defect provides an environment for fibroblasts to invade the defect more readily. Thus, favorable bone healing may require prevention of early scar healing related to fibroblast invasion.

Undifferentiated mesenchymal cells in bone marrow can differentiate into fibroblasts or osteoblasts, depending on the peripheral environment.²⁸ Therefore, to promote bone healing, it is also important to establish a peripheral environment that is advantageous for bone formation. Dahlin et al.²⁹ described the method of guided bone regeneration (GBR), in which bone formation is induced using Teflon film around the alveolar bone in dental implants. This method can be used selectively to induce bone formation by preventing fibroblast invasion. Application of GBR for surgical endodontics, especially in apicoectomy, has recently been reported.³⁰⁻³⁴ Pecora et al.³² performed apicoectomy for

teeth with apical lesions of >10 mm on X-ray, and found that application of Teflon film for bone defect led to early bone healing. Abramovitz et al.³³ combined apicoectomy with Teflon film application for teeth with apical lesions involving alveolar bone defects, and noted bone regeneration. These studies suggest that a bone defect after extensive radicular cyst extirpation should be covered with Teflon film to achieve bone formation.

On the other hand, it is reported that inflammatory cytokines related to chronic inflammation inhibit bone healing.⁴⁻⁷ Radicular cysts are inflammatory cysts, and a large number of regulatory molecules are present in the cyst wall.⁹⁻¹¹ Pericyclic bone resorption depends on the balance between osteoclasts and osteoblasts, and participation of regulatory molecules in differentiation to osteoclasts has been suggested. In particular, TNF- α , IL-1 β , M-CSF, RANK, RANKL, and OPG are important in this process,⁸⁻¹² and many studies have investigated RANKL, TNF- α , and IL-1 β as key factors.^{4-12,35} RANKL (receptor activator of NF- κ B ligand) is a membrane protein expressed on the osteoblast surface. RANKL binding to an osteoclast precursor cell receptor induces NFATc1, a master transcription factor that integrates signals, and differentiation from an osteoblast to an osteoclast is promoted. In the process of radicular cyst formation, IL-1 β and TNF- α , which are mainly produced by macrophages, act directly on osteoclast precursor cells in the apical inflammatory region, which promotes differentiation to osteoclasts by direct action on osteoblasts and induction of RANKL expression. In short, IL-1 β and TNF- α induce osteoclast differentiation via the RANK/RANKL system. IL-1 β and TNF- α also inhibit Wnt signals for differentiation of osteoblasts and suppress proliferation of osteoblasts to osteocytes; in particular, TNF- α induces expression of Dickkopf-1 and sclerostin, both of which inhibit Wnt signals.¹³

In this study, IL-1 β and TNF- α expression showed significant differences between cases with good and poor bone healing, but RANKL did not differ between these cases. This suggests that the mechanism of bone absorption in cyst formation and inhibition of bone formation after cyst extirpation may dominantly occur via a route that is dependent on the non-RANK/RANKL system.³⁶⁻⁴⁰ We examined expression of cytokines in the cells of subepithelial connective tissue, but none were found that inhibit bone formation. Production of cytokines in the cyst epithelium markedly influences bone formation.

Recently, the suppression of inflammatory cytokine production or anti-cytokine therapy has been proposed as a new treatment. In treatment of topical bone destruction related to rheumatoid arthritis, targeting of IL-1 β and TNF- α using biologics has efficacy, and low-molecular-weight compounds for oral administration have also been developed.⁴¹⁻⁴³ In patients with high expression of IL-1 β and TNF- α in the cyst wall, anti-cytokine therapy may eliminate bone healing inhibitors and act as a bone healing mechanism.

This is the first study to analyze the factors influencing bone healing after extirpation of extensive radicular

cyst with endodontic microsurgery-microscopic apicoectomy. However, the number of cases undergoing this surgical procedure is small, since this procedure is very new, even at an international level. Additional cases, and also laboratory research on molecular mechanisms of bone resorption caused by inflammatory cytokines are needed to establish reliable surgical and medical therapy (including cytokine therapy) of extensive radicular cysts.

Conclusion

Penetrating bone defects after cyst extirpation, a maxillary site, and inflammation are prognostic factors for a poor outcome of this procedure. For successful treatment (i.e., bone healing of an extirpation cavity (bone defect)), it is necessary to establish advantageous environmental conditions that include prevention of fibroblast invasion and blocking of inflammation.

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