Chapter 4

Injury and the Recovery Reaction due to the Penetration of Material into the Mandibular Canal

Chihito Nakamura
Mihoko Tomida
Hiromasa Hasegawa
Toshiyuki Kawakami

《**Abstract**》

A root canal filling material paste, mainly made of calcium hydroxide and iodoform with the addition of silicone oil, was experimentally introduced into the mandibular canals of dogs. The tissue reactions to the paste were examined by radiography, histopathology, and electron microscopy. The examination results showed that the paste was phagocytosed by macrophages and, in general, was gradually resorbed by the passage of time. The paste was also found to cause heterotopic calcification and/or bone formation within the limited area of original penetration. The histopathological tissue changes of the alveolar nerve tissue were also examined. No injury-related histopathological change was observed as long as the injected paste did not contact alveolar nerve tissue, but tissue damage was observed at sites of direct contact. After removal of degenerated nerved debris by macrophages and Schwann cells, the persisting external membrane or "Schwann tubes" appeared to provide a scaffold for axonal regeneration and Schwann cell proliferation.

Introduction

Success following root canal therapy depends on the ability of the periapical connective tissue to undergo repair. As the reason of closure of the apical foemen by osteoid and/or cementoid hard tissue deposition, it is usually referred to as biological filling, mainly composed of calcium hydroxide. Furthermore, the root canal filling material paste, made up of calcium hydroxide (30.3%) and iodoform (40.4%) with the addition of silicone oil (22.4%) and others

(6.9%), is the typical material used throughout the world.

Anatomically, it is extremely adjacent, and sometimes comes in contact with, the position of root canal foemen and the mandibular canal, especially in the molar region. Therefore, during routine infection dental root canal treatment, the canal may be overfilled; soft material may then accidentally penetrate the mandibular canal, especially in the molar region, as one of the pathological conditions caused by accident (Figure 1). Some case reports have been published of paresthesia and other complications of the inferior alveolar nerve caused by such extruded materials [1-8]. When root canal therapy is performed on mandibular teeth posterior to the mental foramen, damage to the inferior alveolar nerve is possible. There are many published papers on this type of accidental case [9-18].

Pogrel [9] reported that most cases have been described in connection with the lower second molars, but cases related to the first molars and to the premolars also have been reported. In that report, three possible mechanisms were envisaged: 1) mechanical trauma from over instrumentation into inferior alveolar canal; 2) a pressure phenomenon from the presence of the endodontic point or sealant within the inferior alveolar canal; and 3) a neurotoxic effect from the medication used to clean the canal or that were in the sealant.

Aesaert [10] mentioned the heat to plasticize and the forces applied to condense that material might have an influence on the periodontal ligament and/or the remaining tooth structure. Furthermore, the overfilling of gutta-percha and more likely the sealer may affect the periodontal tissues. Although rare, mandibular nerve damage and adverse effects on the sinus maxillaries can be the result of inadequate treatment. There is a case report on 4 cases of disabling dysethesia and paresthesia following endodontic treatment of lower molars in which sagittal osteotomy was used to remove the endodontic paste and to perform nerve decompression [11]. Poveda, et al [12] reported the case report on mental nerve anesthesia associated with endodontic paste within the mandibular canal.

Furthermore, damage to the nerve is often attributed to irritants in the paste like paraformaldehyde [1-3]. Brodin [19] has demonstrated experimentally the neurotoxicity of this

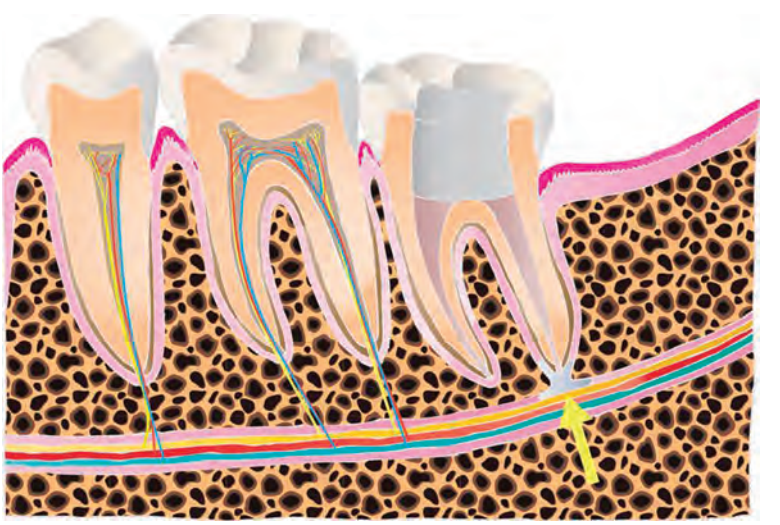

Figure 1 Root canal filling material (yellow arrow) penetrating into the mandibular canal. In these cases, some injuries of the alveolar tissues occasionally occur.

substance and of preparations which contain it. Mechanical pressure exerted by the filling material itself has also been cited as a possible cause [5-8]. Regardless, removal of the foreign material, either surgically [5, 8] or through its gradual resorption [6], may lead to improvement or recovery of nerve function.

Animal experiments

In this chapter, we introduce the results of experimentally extruded Vitapex (Neo Dental Chemical Products Co., Tokyo), a root canal filling paste which has gained widespread acceptance among Japanese dentists over the past decades, through the molar apices and into the mandibular canal of dogs [20, 21]. Whereas the resorption of excess filling material has been documented radiographically in cases of accidental over-filling [6], there have been few histopathological or ultrastructural examinations of the fate of such pastes in the jaws of either human patients or laboratory animals.

In our examination, adult mongrel dogs were used. Under anesthesia, pulpectomy was performed on the 4^{th} premolar and/or 1^{st} molar on each side of the mandible of the animals. The apices of the roots were then perforated with a #50 or #60 reamer. For each tooth, about 0. 5g of Vitapex was injected through the roots into the mandibular canal. Radiographs were taken at the time of injection, and over time. Then, histopathological and electron microscopic examinations were carried out.

Radiography and histology of physiological condition

Radiography revealed that root apices of the 4^{th} premolar and/or 1^{st} molar were mostly in contact with the mandibular canal (Figure 2). The latter was found, upon histology of the periapical region, to be wider than its human counterpart. The canal was bordered by alveolar bone and by peripheral compact bone of the lower mandible, and contained a fibrous capsule surrounding the alveolar nerve tissue, vein, and some loose connective tissues.

The nerve itself was composed of many nerve fibers and fine connective tissue, encapsulated by a layer of loose connective tissue. The nerve fibers appeared as many myelinated axons, each enveloped by a myelin sheath and surrounded by Schwann cell cytoplasm. Electron microscopy shows the axons were seen to have nerofilaments, a small amount of endoplasmic reticulum, and a few mitochondria and microtubules. The myelin sheath consisted of compact myelin lamellae, with its peripheral layer connecting with the Schwann cell plasma membrane. The outer surface of the Schwann cells was covered with an external lamina. Some unmyelinated fibers were also observed as several thin axons enveloped by a single layer of plasma membrane.

Radiography and histopathology of experimental specimens

Radiographically, the injected paste was observed as radiopaque regions with distinct borders in the mandibular canal (Figure 3-a). A decrease in radiopacity, indicating resorption of the paste, was generally observed with the passage of time. In case of moderate extraction, this resorption covered the entire area of the original penetration. By 3 months, a radiopacity due to newly-formed bone-like tissue completely replaced that shown by the paste (Figure 3, b), while maintaining the original outline. When the amount of the extruded paste was greater, filling portions of the mandibular canal and extending into the bone marrow spaces as well (Figure 3-c), similar resorption and formation of new radiopaque tissues were observed in the periphery of the original area, although the center remained densely radiopaque at 3 months (Figure 3-d). In cases where the mandibular canal was completely obturated, however, even peripheral

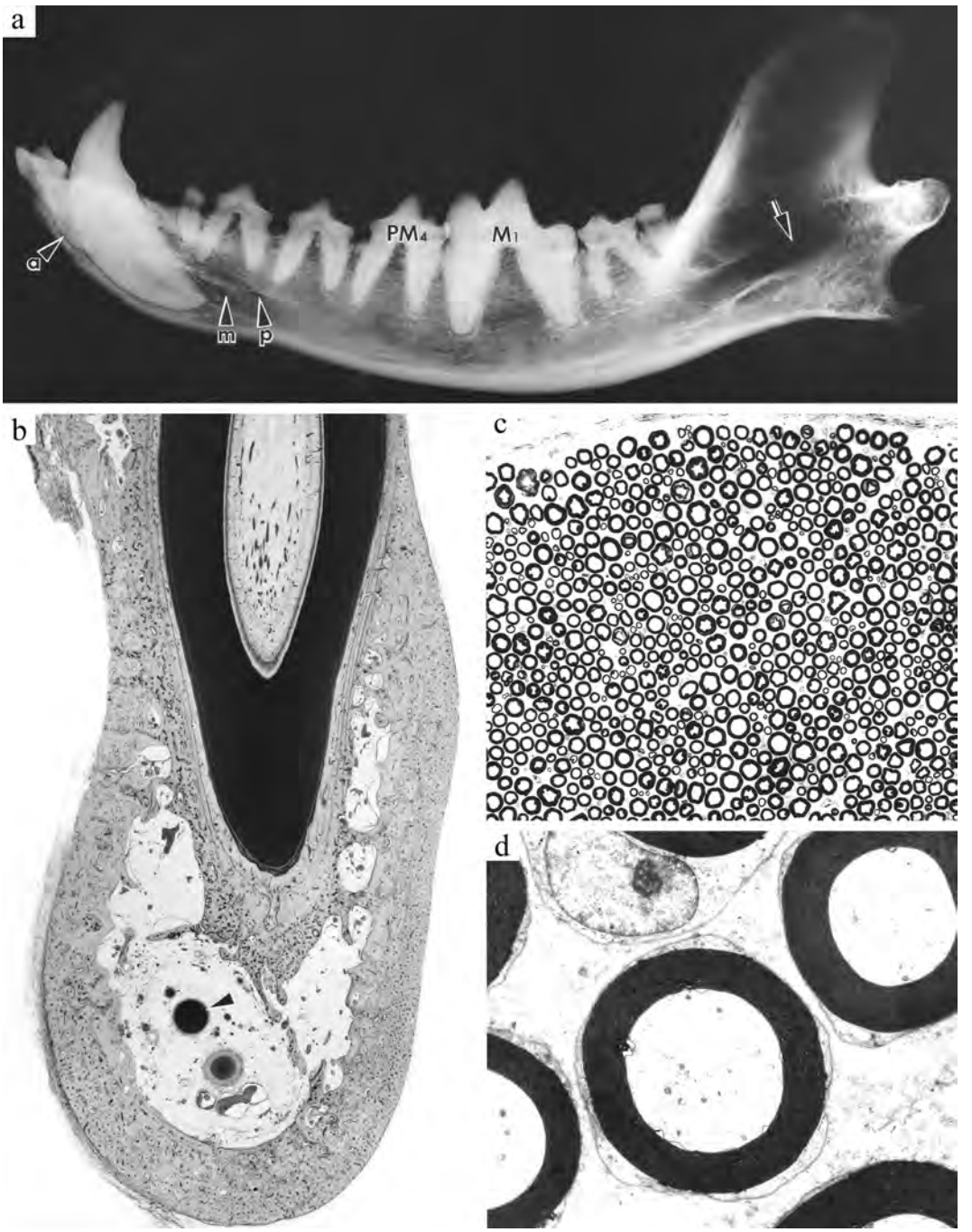

Figure 2 Radiography and histology of dog mandible show the relationship between the apex of the tooth root and alveolar canal (a: radiograph; b: sectional view). Enlarged photograph shows the alveolar nerve structure (c: Toluidine blue stained view; d: electron microscopic view).

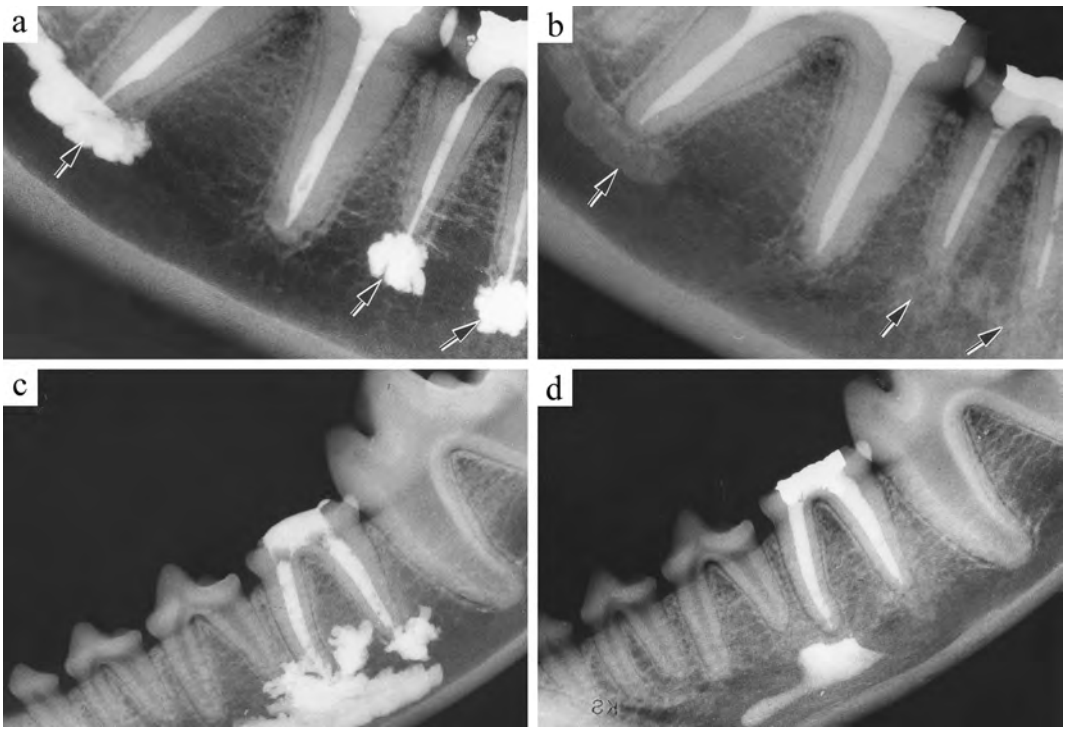

Figure 3 Radiographs showing radiopacity with distinct borders (arrows) at the time of injection (a), bone-like radiopaque structure (arrows) appearing in the injected areas after 3 months (b), extensive extraction of the paste into the mandibular canal at the time of injection (c), and central region of dense radiopacity still remaining after 3 months (d).

resorption was slight, and formation of new calcified tissue was observed.

Tissue injury, reaction and heterotopic bone formation

Histopathologically, one day after the injection, the connective tissue was observed to be compressed by the injected paste. In general, the paste was seen indirectly as lacunae where it had been lost during the processing of the tissue, but some of it appeared as hematoxylin-stained material that had invaded sites of peripheral fatty and collagenous tissues (Figure 4, a). Specimens taken at later times showed granulation tissue gradually forming around the paste. At 2 weeks, components of the paste had been phagocytosed by macrophages, and foreign body giant cells having von Kossa's-positive granules in their cytoplasm appeared in the granulation tissue (Figure 4, b). By 3 months after the injection, the cell-rich granulation tissue had changed to a collagenous one. Irregular calcified and/or ossified tissue formation was also seen to have occurred within the injection site during this period. These tissues then enlarged to completely fill the areas occupied by the paste for a half year (Figure 4, c, d). They were found to contain both irregular trabecular bone and a hematoxylin-stained amorphous structure. No expansion of such calcified or ossified area beyond those originally occupied by the paste was observed, even after the injection.

Electron-microscopic observation of macrophages which appeared within the injection sites at 2 weeks revealed that they had many cytoplasmic projections, along with phagocytosed materials in their cytoplasm. The latter included moderately electron-dense droplets (Figure 5,

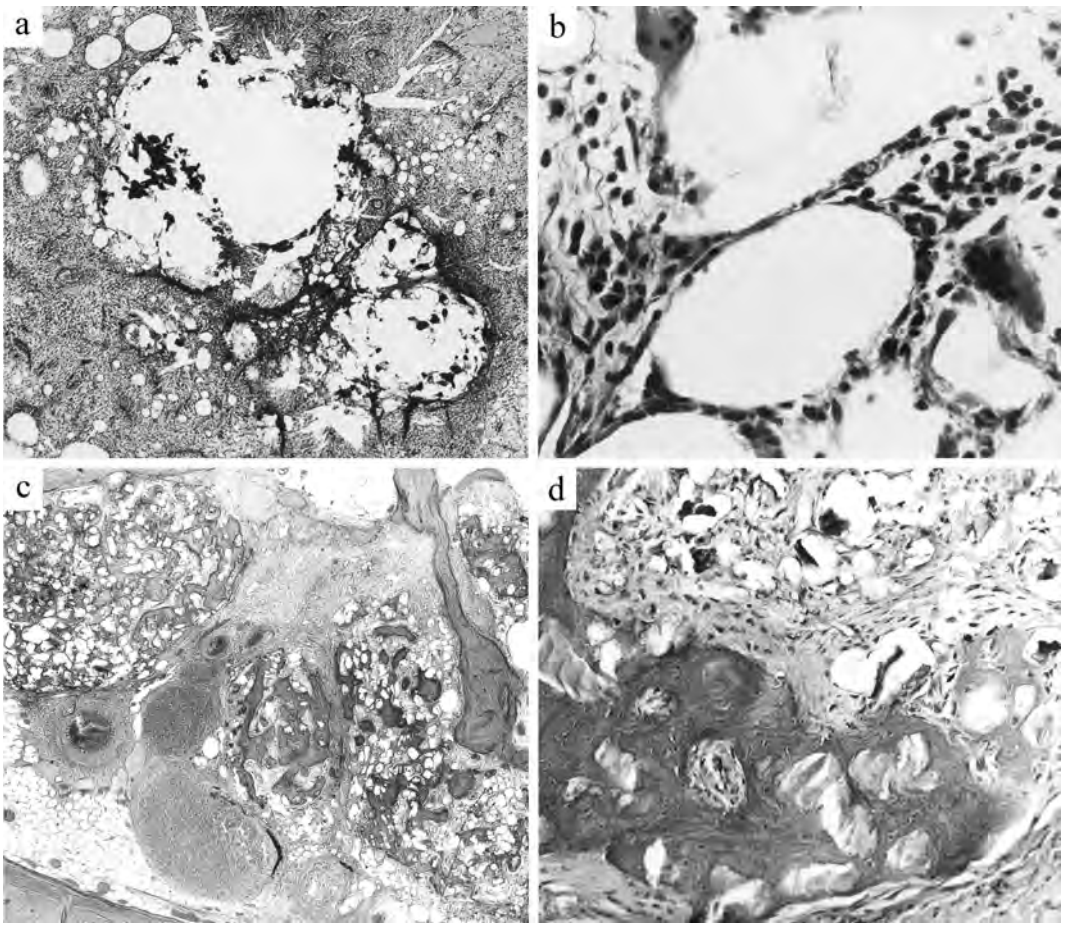

Figure 4 Mandibular canal tissue showing lacunae left by the paste during processing in 1-week specimen (a), foreign body giant cells appearing around lacunae left by the paste during processing in 1-week specimen (b), newly formed bone tissue filling the injection site in 6-month specimen (c), and hematoxylin-stained materials (arrows) around newly formed bone tissue in 6-month specimen (d).

a) and dense, fine granular materials, along with cell debris. Many mitochondria and rough endoplasmic reticulum were also observed in the cytoplasm. Foreign body giant cells appearing at the same time on the periphery of the paste had many small cytoplasmic projections. Their cytoplasm contained irregularly shaped nuclei, mitochondria, and rough endoplasmic reticulum. Clear vacuoles and electron-dense fine granules (Figure 5, b) were also observed. Examination results of the newly-formed bone tissue at a half year revealed a matrix of collagen bundles, and osteoblasts and osteocytes typical of normal physiological bone (figure 5, c, d, e). Irregular amorphous structures composed of fine needle-like or granular material were also present.

There is a large literature on tissue reaction to and the fate of the components of Vitapex embedded subcutaneously in rats [20-28]. The literature suggestes that the embedded paste gradually diffuses and finally disappears approximately 2 months after injection, and the components of the paste are phagocytosed by macrophages and foreign body giant cells [26, 27]. We also placed radioactive travelers in the calcium hydroxide and silicone oil components of the paste, and demonstrated both their movement inside the body and their final excretion via the

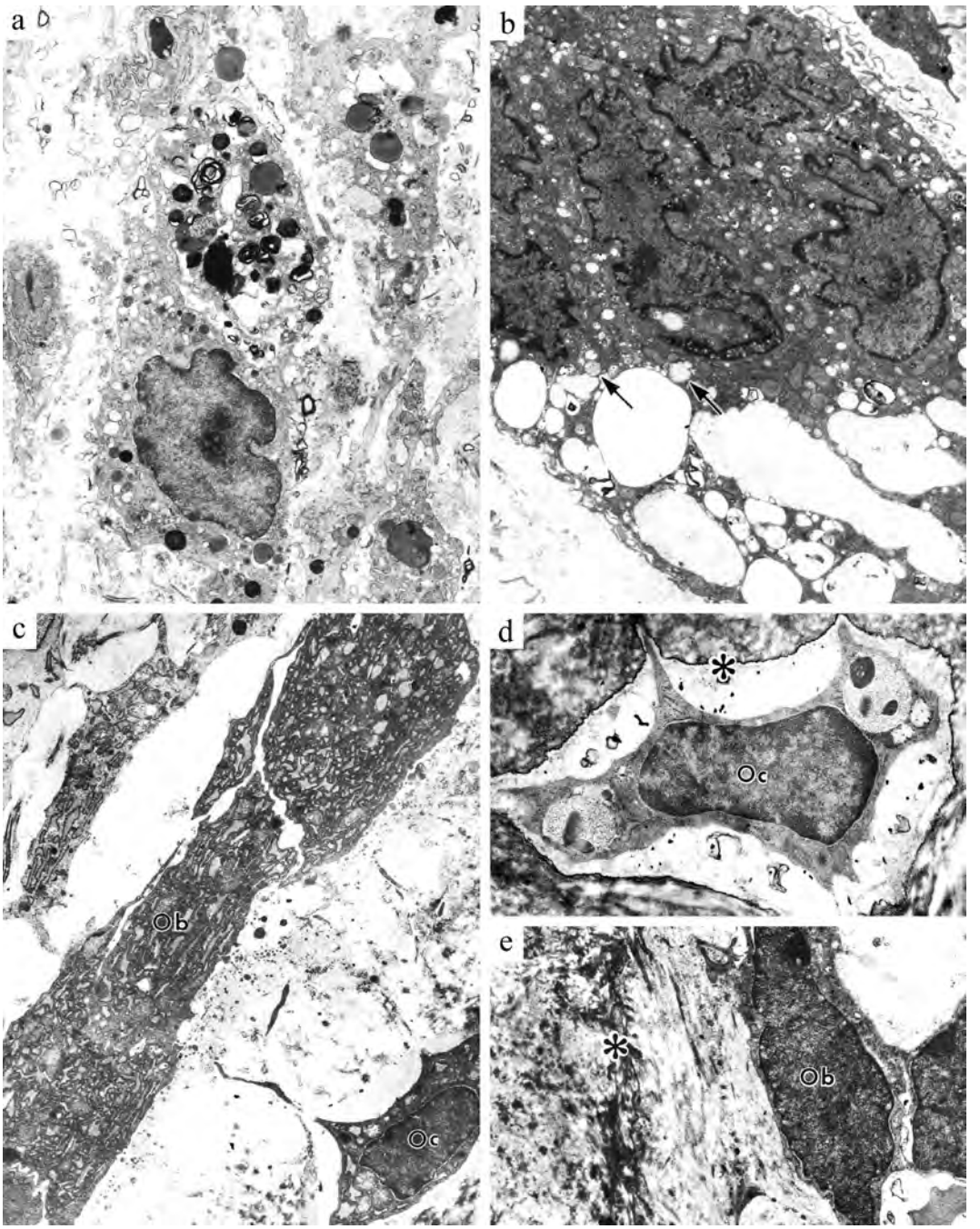

Figure 5 Electron-microscopy showing cell debris and moderately electron-dense droplets (arrows) in 2-week specimen (a), foreign body giant cell containing many vacuoles and electron-dense fine granules in 2-week specimen (b, arrows), organelle-rich osteoblast (Ob) and osteocytes (Oc) seen within the lacunae of the bone tissue in 6-month specimen (c, d, e).

blood system into the feces and urine [23-25, 28]. Additionally, we found two types of heterotopic calcification elicited by the embedded paste: dystrophic calcification, and the matrix vesicle type [22, 27]. Radiolabelled elements derived the calcium hydroxide and the silicone oil components of the paste were also observed in both types of calcification [23, 24]. Furthermore, we observed the formation of osteoid in the root apex in a clinico-pathological study of a patient treated with Vitapex [29]. These data suggest that Vitapex is an excellent biologic root canal filling material which causes little injury to tissues, and that it could moreover induce the differentiation of cells in the periodontal ligament into osteoblasts and/or cementoblasts.

In this chapter, we were able to confirm histopathologically the resorption of the paste in the mandibular tissues of the experimental animals, where it was observed to be phagocytosed by macrophages and foreign body giant cells. We also documented the appearance of osteoblasts, and the formation of heterotopic bone, within the connective tissue at the site of penetration. Whereas such ossification is heterotopic, it did not extended beyond the area of initial penetration; moreover, this could have a beneficial effect when Vitapex is applied clinically by eliciting a layer of osteoid and/or cementoid sealing the root apex. Finally, we observed that the overall damage to surrounding tissues was slight.

These data lead us to believe that if Vitapex should penetrate the mandibular canal at the time of endodontic treatment, the paste will disappear with the passage of time and cause heterotopic calcification and/or ossification within the area of original penetration.

Neural tissue degeneration and recovery

Nerve tissues underwent no change as long as they did not come in contact with the injected paste. Histopathological features showed that the general changes occurred within the mandibular canal upon penetration of the paste; newly-formed trabecular bone could be observed in the area which the paste originally occupied (Figure 6, a). The following is a description of the changes observed in the portion of the alveolar nerve which came in contact with the paste (roughly corresponding to the upper one-third of the nerve tissue visible.

Histopathologically, extensive changes were observed in the nerve fibers over a short initial period of up to 7 days (Figure 6, b, c). Where the paste invaded the endoneurium, the myelin sheaths were seen to lose their circular shape and disintegrate. In severely damaged areas, the myelin stained only slightly with LFB. At 1-2 weeks after injection, degenerated nerve fibers had decreased in number, and many phagocytes bearing LFB-positive granular materials in their cytoplasm were observed (Figure 6, d). After 1 month, these phagocytes had almost disappeared, and lipid debris could be seen as numerous ovoid vacuoles in the endoneurium. A few myelinated nerve fibers, approximately 1/3-1/5 normal diameter, were also visible (Figure 6, e). After 3 months, the fibers increased in number and many clusters of them could be seen (Figure 6, f). Collagen fibers proliferated within the endoneurium at the time, and the myelinated nerve fibers continued to increase in number and size. After 1 year, their diameter and density appeared similar to those of normal fibers.

As observed electron microscopically, just one day after injection the damaged layers of the sheaths of myelinated nerve fibers had become irregular, forming complex fold or undulations. A few days later, the nerve filaments had been destroyed or become irregular; the endoplasmic reticulum and mitochondria were less frequently observed but appeared swollen when visible. Some damage to myelinated nerve tissue then ensued: the axon completely disappeared, and in the area within the remaining external lamina, filamentous ball-like changes in the myelin sheath were seen (Figure 7, a). At this time, electron-dense fine granular structures were scattered among the collagen fibers in the endoneurium.

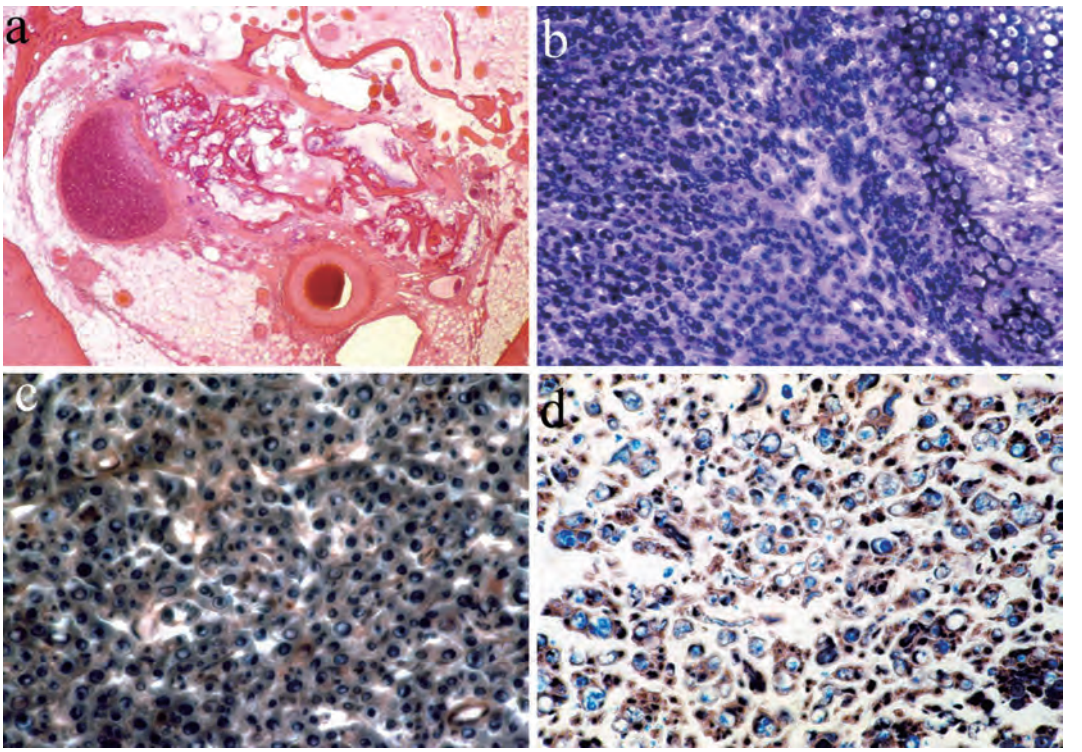

Figure 6 Overview of injection site, with newly-formed trabecular bone and degenerated alveolar nerve tissue visible in 1-year specimen (a), severely damaged alveolar nerve bundle in 1-day-HE-LFB specimen (b), phagocytes containing granular material in 1-week-HE-LFB-specimen (c), and numerous clusters of small myelinated nerve fibers in 3-month-HE-LFB specimen (d).

After 1 week, comparatively large cells with degenerated cell debris in their cytoplasm appeared in the endoneurium (Figure 7, 8). These cells were also recognized histopathologically in toluidine blue stained specimens (Figure 8, b). They may be divided ultrastructually into two types: one with many pseudopodium-like cytoplasmic projections on its periphery, lysosome-like structures in a comparatively electron-dense cytoplasm, and no external laminal structure; the other with comparatively light cytoplasm showing a smooth surface with an external lamina (Figure 9). Both types of cell phagocytosed the membranous structures — degenerated cell debri — that were scattered through the endoneurium. These cells also penetrated ruptures in the external lamina of the Schwann cells (Figure 8), and were seen with phagocytosed fragments of the myelin sheaths in their cytoplasm. As a result of this phagocytosis, the external lamina appeared in places as "Schwann tubes": empty, folded, and tunnel-like (Figure 8, 9). At this stage, the electron-dense, fine granular structures seen scattered throughout the endoneurium during the early stage were not recognized. A ruptured external lamina was seen at the periphery of these regenerated nerve fibers (Figure 9, b). With the passage of time, these regenerated nerve fibers increased in number and diameter, and finally could not be distinguished from the original ones.

Concerning paresthesia and other complications of the inferior alveolar nerve following penetration of root canal filling material into the mandibular canal, a number of reports have been published [30-31]. In many cases damage to the nerve was specifically attributed to highly irritating components of the paste, such as paraformaldehyde [1-3], the neurotoxicity of which

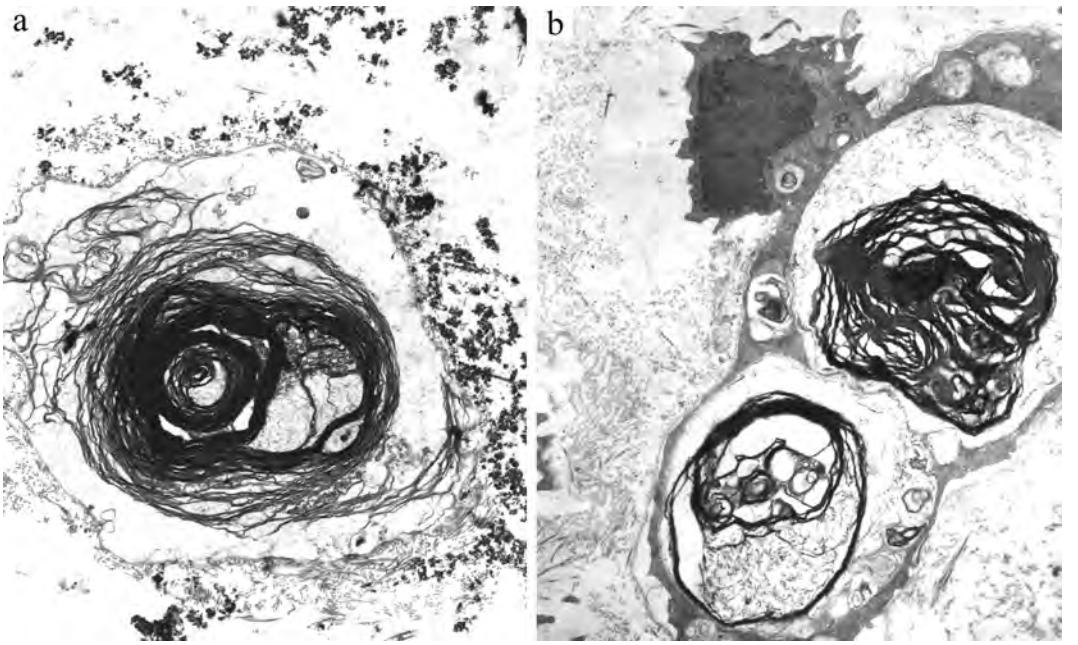

Figure 7 Electron micrograph of damaged nerve fibers in 2-week-specimen (a), and macrophage invading the "Schwann tubes" through a rupture (arrows) in the external lamina in 2-week-specimen (b).

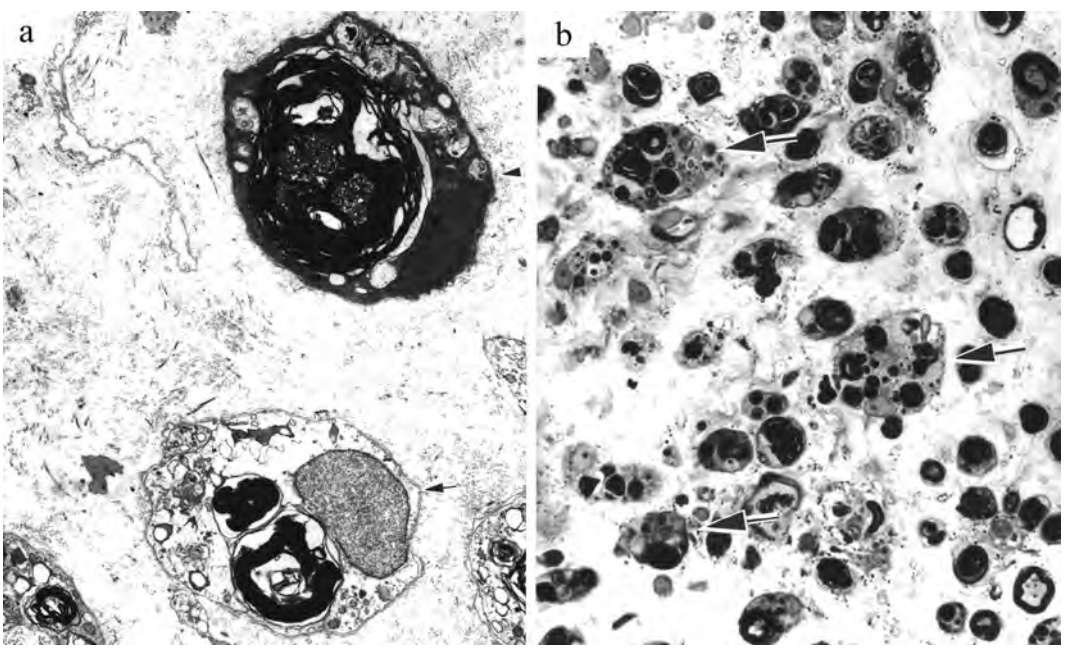

Figure 8 Electron micrograph showing both Schwann cells (arrow) and macrophage (arrowhead) have degenerated nerve tissue in their cytoplasm in 2-week specimen (a), and comparative large cells (arrows) containing some round structures in 2-week toluidine blue-stained specimen (b).

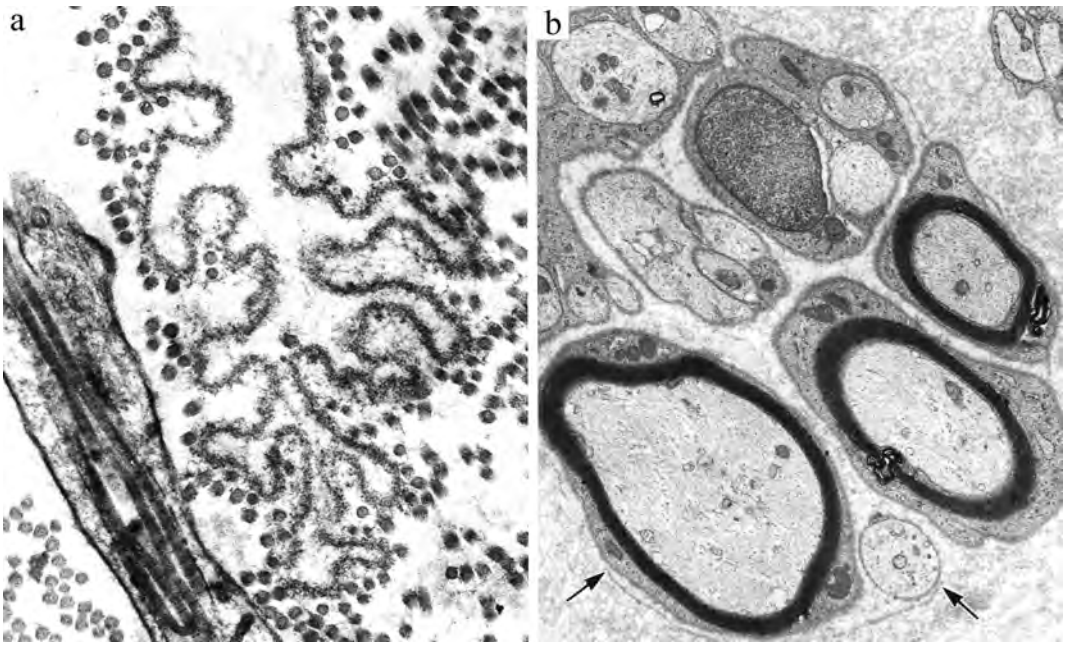

Figure 9 Electron micrograph showing so-called "Schwann tubes" empty, folded, external lamina in 2-week specimen (a), and disrupted external lamina (arrows) surrounding nerve fibers in 1-month specimen (b).

has been experimentally demonstrated by Brodin [19]. For this reason, many researchers have concluded that materials containing paraformaldehyde should not be used [2, 3, 19]. In some cases, however, neural damage has been attributed at least in part to mechanical stimuli, such as over instrumentation [5], or pressure exerted by the filling material itself [8].

According to the data given in this chapter, damage to the alveolar nerve of experimental animal was observed at places where it came in contact with the root canal filling paste Vitapex, which had been intentionally introduced into the mandibular canal. The changes visible in the neural tissue were characteristic of Wallerian degeneration and regeneration: after removal of degenerated nerve tissues by phagocytes, the remaining external lamina or "Schwann tubes" provided a scaffolding for regeneration of axons and proliferation of Schwann cells. These processes have been studied at the light and electron microscopic levels by various authors [38, 39]; our observations differed from those of other reports, however, in that the processes of degeneration and regeneration were both visible at the same time. The two types of phagocytes we observed were considered, because of their ultrastructural features, to be macrophages and Schwann cells.

Vitapex contains no paraformaldehyde but is composed mainly of iodoform, calcium hydroxide, and silicone oil. Since calcium hydroxide is highly alkaline and therefore irritating to tissue [37], it may cause damage to the nerve through chemical stimulation. We suggested, however, that the presence of silicone oil may serve as a prophylactic against some of this alkalinity [26, 29], perhaps because if its low solubility. This appears to be supported by the finding of only slight damage to mandibular tissue, in general (Figure 10). The relatively rapid regeneration which we observed in the alveolar nerve, moreover, despite the residual presence of the paste, led us to believe that the chemical irritation produced by Vitapex was short-lived.

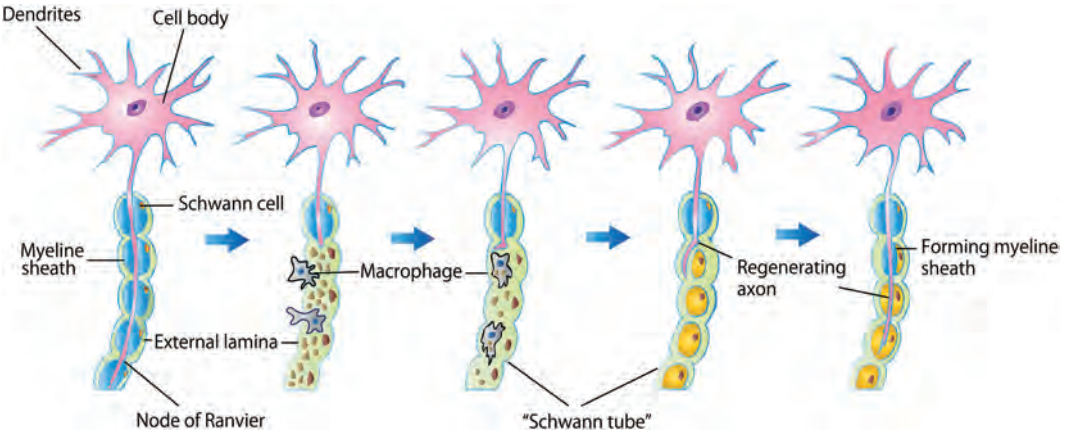

Figure 10 Explanation of regeneration of nerve tissue showing the growth within the "Schwann tube".

Conclusion

We found that Vitapex, an endodontic filling material containing no paraformaldehyde and causing only slight injury to mandibular tissues in general, elicited degeneration of the alveolar nerve tissues on contact. This process was followed by one of gradual regeneration of the nerve.

References

[1] Grossman LI (1978) Paresthesia from N2 or N2 substitute. Report of a case. Oral Surg Oral Med Oral Pathol 45: 114-115.

[2] Grossman LI and Tatoian J (1978) Paresthesia from N2. Report of a case. Oral Surg Oral Med Oral Pathol 45: 700-701.

[3] Kaupman AY and Rosenberg L (1980) Paresthesia caused by Endomethasone. J Endod 6: 529-531.

[4] Shochat S, Garfunken D and Laufer D (1973) Neurologic complications arising from overfilled root canals. Oral Surg Oral Med Oral Pathol 35: 684-688.

[5] Spelman A, Gutman D and Laufer D (1981) Anesthesia following endodontic overfilling with AH26. Oral Surg Oral Med Oral Pathol 52: 554-556.

[6] Tamse A, Kaffe I, Littner NM and Kozlovsky A (1982) Paresthesia following overextension of AH-26: Report of two cases and review of the literature. J Endod 8: 88-90.

[7] Pyner DA (1980) Paresthesia of inferior alveolar nerve caused by Hydron: a case report. J Endod 6: 527-528.

[8] Forman GH and Rood JP (1977) Successful retrieval of endodontic material from the inferior alveolar nerve. J Endod 5: 47-50.

[9] Porgel MA (2007) Damage to the inferior alveolar nerve as the result of root canal therapy. J Am Dent Assoc 138: 65-69.

[10] Asaert G (2000) Introgenic rists during the obturation of the root canal system. Rev Belge Med Dent 55 (2): 104-123.

[11] Acolozzi P, Lombardi T and Jaques B (2004) Successful inferior alveolar nerve decompression for dyseathesia following endodontic treatment: report of 4 cases treated by mandibular sagittal osteotomy. Oral Surg Oral Med Oral Pathol 97: 625-631.

[12] Poveda R, Bagan JV, Fernandez JM and Sanchis JM (2006) Mental nerve paresthesia associated with endodontic paste within the mandibular canal: report of a case. Oral Surg Oral Med Oral Pathol 102 (5): e46-49.

[13] Koseoglu BG, Tanrikulu S, Subay RK and Sencer S (2006) Anesthesia following of a root canal sealer into the mandibular canal: a case report. Oral Surg Oral Med Oral Pathol 101: 803-806.

[14] Scarano A, Carlo di F, Quaranta A and Piatteli A (2007) Injury of the inferior alveolar nerve after overfilling of the root canal with endodontic cement: a case report. Oral Surg Oral Med Oral Pathol 104 (1): e56-59.

[15] Brkic A, Gurkan-Koseoglu B and Olgac V (2009) Surgical approach to iatrogenic complications of endodontic therapy: a report of 2 cases. Oral Surg Oral Med Oral Pathol 107 (5): e50-53.
[16] Grotz KA, Al-Nawas B, Aguiar de EQ, Schulz A and Wagner W (1998) Treatment of injuries to the inferior alveolar nerve after endodontic procedures. Clin Oral Investg 2: 73-76.
[17] Kockapan C (1993) Overfilling into the mandibular canal as an endodontic complication. A review. Schweiz Monatsschr Zahnmed 103: 20-28.
[18] Morse DR (1997) Endodontic-related inferior alveolar nerve and mental foramen paresthesia. Compend Contin Educ Dent 18: 963-968, 970-973, 976-978.
[19] Brodin P (1988) Nerotoxic and analgesic effects of root canal cements and pulp-protecting dental materials. End Dent Traumatol 4: 1-11.
[20] Kawakami T, Nakamura C and Eda S (1991) Effects of the penetration of a root canal filling material into the mandibular canal. 1. Tissue reaction to the material. Endod Dent Traumatol 7: 36-41.
[21] Kawakami T, Nakamura C and Eda S (1991) Effects of the penetration a root canal filling material into the mandibular canal. 2. Changes in the alveolar nerve tissue. Endod Dent Traumatol 7: 42-47.
[22] Kawakami T, Nakamura C, Hasegawa H, Akahane S and Eda S (1987) Ultrastructural study of initial calcification in the rat subcutaneous tissues elicited by a root canal filling material. Oral Surg Oral Med Oral Pathol 63: 360-365.
[23] Kawakami T, Nakamura C, Hasegawa H and Eda S (1987) Fate of ^{45}Ca-labeled calcium hydroxide in a root canal filling paste embedded in rat subcutaneous tissues. J Endod 13: 220-223.
[24] Kawakami T, Nakamura C, Hasegawa H and Eda S (1987) Fate of ^{14}C-labelled dimethylpolysiloxane (silicon oil) in a root canal filling material embedded in rat subcutaneous tissues. Dent Mater 3: 256-260.
[25] Kawakami T and Eda S (1988) Excretion of silicon oil embedded in rat subcutaneous tissue. Med Sci Res 16: 837.
[26] Kawakami T, Nakamura C, Hayashi T, Eda S and Akahane S (1979) Studies on tissue reaction to the paste of calcium hydroxide added iodoform (root canal filling material: Vitapex), First report, a histopathological study. J Matsumoto Dent Coll Soc 5: 35-44.
[27] Kawakami T, Nakamura C, Haysshi T, Eda S and Akahane S (1979) Studies on tissue reaction to the paste of calcium hydroxide added iodoform (root canal filling material: Vitapex), Second report, and electron microscopic study. J Matsumoto Dent Coll Soc 5: 161-170.
[28] Kawakami T, Nakamura C, Uji H, Hasegawa H and Eda S (1989) Fate of silicone oil component in a root canal filling material in rat subcutaneous tissue. J Matsumoto Dent Coll Soc 15: 167-172.
[29] Eda S, Kawakami T, Hasegawa H, Watanabe I and Kato K (1985) Clinico-pathologial studies on the healing of periapical tissues in aged patients by root canal filling using paste of calcium hydroxide added iodoform. Gerodontics 1: 98-104.
[30] Efrmann EH (1964) Treatment with N2 root canal sealer. Br Dent J 117: 409-410.
[31] Orlay H (1966) Overfilling in root canal treatment, two accidents with N2. Br Dent J 120: 376.
[32] Holtman E and Novikoff AB (1965) Lysosomes in the rat sciatic nerve following crush. J Cell Biol 27: 651-668.
[33] Liu HM (1974) Schwann cell properties. II. The identity of phagocytes in the degenerating nerve. Am J Pathol 75: 395-416.

Address for Correspondence: Professor Toshiyuki Kawakami, Hard Tissue Pathology Unit, Department of Hard Tissue Research, Matsumoto Dental University Graduate School of Oral Medicine, Shiojiri, 399-0781 Japan
Phone and Fax：+ 81-263-51-2035, E-mail：kawakami@po.mdu.ac.jp