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Review article

Root end filling materials: a review

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Abstract – When non-surgical attempts prove unsuccessful or are contraindicated, surgical endodontic therapy is needed to save the tooth. The procedure usually consists of exposure of the involved area, root end resection, root end preparation and insertion of a root end filling material. Numerous materials have been suggested as root end filling materials. This article is a review of the literature on the suitability of various root end filling materials based on their leakage assessment, marginal adaptation, cytotoxicity, and usage test in experimental animals and humans.

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Studies have shown that pulpal and periradicular pathosis develop only when these tissues are exposed to bacterial contamination. To determine the importance of bacteria, Kakehashi et al. (1) as well as Paterson (2) exposed the dental pulps of conventional and germ-free rats to their own oral flora, which resulted in the development of pulpal and periradicular lesions in conventional rats, but failed to create lesions in germ-free rats.

Möller et al. (3) severed the pulps of teeth in monkeys and either sealed aseptically the amputated pulps immediately, or left them open to be contaminated with indigenous oral flora for 1 week and then sealed. Clinical, radiographic, and histological examinations of the teeth that were sealed aseptically showed an absence of any pathological changes in their periradicular tissues. In contrast, teeth with infected root canals had inflammatory reactions in their tissues.

Fabricius et al. (4) inoculated root canals of monkeys with 11 bacterial species separately, or in combinations, and sealed the access cavities for a period of 6 months. Their bacteriological and histological examinations showed that mixed infections have a greqter capacity to cause apical lesions than do monoinfections. Furthermore, they reported that the Bacteroides strain did not survive in the root canals when inoculated as pure cultures. Enterococci survived as pure cultures, and facultative streptococci induced small periradicular lesions.

As a consequence of pathological changes in the dental pulp, the root canal system acquires the capacity to harbour several species of bacteria, their toxins and their by-products. Egress of these irritants from the root canal system into the periapical tissue results in the formation of periradicular lesions which are mediated by nonspecific as well as immune responses (5).

Complete cleaning and shaping of root canals and sealing them in three dimensions should result in resolution of periradicular lesions in all patients who have undergone non-surgical root canal therapy. The degree of success following root canal therapy has been reported as high as 98.7% (6) and as low as 45% (7). Ingle & Glick (8) reported a success rate of 95% of all treated endodontic cases, which compares favourably with other reports of success. In an examination of failed cases from the Washington study Ingle et al. (9) reported that over two thirds of these failures were related to incomplete cleaning and obturation of root canals. Harty et al. (10) have also reported that the majority of non-surgical endodontic procedures which fail do so because of inadequate apical seal.

In addition to the factors cited in the above studies, a number of recent investigations have shown that the exposure of the coronal parts of filled root canals to oral flora results in total contamination of the filled root canals in a few days (11–15). This occurs as a result of either the presence of voids between the dentinal walls and the filling materials used to obturate the root canal system and/or "washing out" of the root canal sealers.

The preferred treatment of failing endodontic cases is non-surgical retreatment. According to Bergenholtz et al. (16) this treatment usually results in successful outcomes. However, because of the complexity of root canal systems, inadequate instrumentation and presence of physical barriers (anatomical, post and core restoration, separated instruments, etc.), ideal goals may be difficult to achieve with a non-surgical approach. Surgical endodontic therapy then becomes the first alternative. Endodontic surgery has a long history. The procedure involves exposing the involved apex, resecting the root-end, preparing a class I cavity, and most often inserting a root-end filling material.

Because most endodontic failures occur as a result of leakage of irritants from pathologically involved root canals the root-end filling material should provide an apical seal to an otherwise unobturated root canal or improve the seal of existing root canal filling materials and be biocompatible with periradicular tissues. To seal the root-end, the operator should remove the apical 2–3 mm of the root-end, prepare a root-end cavity, and place a root-end filling material. A bevelled resected root is crucial to good visibility (17). However, Gilheany et al. (18) demonstrated that as the angle of the bevel increases, the apical leakage also increases due to the permeability of the dentinal tubules. After a root resection, as perpendicular to the long axes of the root as possible, a class I cavity preparation which includes the apical foramen of the root should be prepared with a bur or an ultrasonic instrument. Despite the advantages of ultrasonic tips shown by Wuchenich et al. (19), Abedi et al. (20) have demonstrated that they create more microfractures than burs during root-end cavity preparations. Recently, O'Conner et al. (21) compared the sealing ability of SuperEBA and amalgam with varnish when placed into cavity preparations made with ultrasonic tip or fissure bur. Their results showed no significant difference between the two root-end resections and preparation techniques. However, they showed SuperEBA leaked significantly less than amalgam with varnish.

Once the root-end preparation has been completed, a suitable root-end filling material must be chosen. According to Gartner & Dorn (22), an ideal material to seal the root-end cavities should prevent leakage of microorganisms and their by-products into the periradicular tissues. It should also be non-toxic, non-carcinogenic, and biocompatible with the host tissues. In addition, it should be insoluble in tissue fluids and dimensionally stable. The presence of moisture should not affect its sealing ability. For practical purposes it should also be easy to use and be radiopaque to be recognized on the radiographs.

Numerous materials have been suggested as root-end filling materials: gutta-percha, amalgam, polycarboxylate cements, zinc phosphate cements, zinc oxide eugenol paste, IRM cement, EBA cement, Cavit, glass ionomers, composite resins, and other materials such as gold foil and leaf, silver points, cyanoacrylates, polyHEMA and hydron, Diaket root canal sealer, titanium screws, and Teflon (23).

The suitability of root-end filling materials has been tested by their leakage assessment, marginal adaptation, cytotoxicity, and usage test in experimental animals and man.

I. Leakage assessment

The quality of apical seal obtained by root-end filling materials has been assessed by the degrees of dye, radioisotope or bacterial penetration, electrochemical means, and fluid filtration technique (24–67).

A. Particle leakage

The results of these studies show that various alloys leak differently and conventional alloy leaks significantly less than other types (27). Amalgam by itself does not prevent penetration of various tracers and its seal improves by addition of varnish (31, 35, 51, 55, 62). In contrast to these findings, King et al. (58) and Olson et al. (59) found no significant difference in leakage of amalgam with and without application of a cavity varnish. Szeremeta-Brower et al. (34) showed good apical seal with apicoectomy alone while Kaplan et al. (30) demonstrated inferior seal with apicoectomy alone compared with heat or cold burnished gutta-percha. However, Bramwell & Hicks (37) reported no significant difference between the amount of leakage of root-end resected teeth when compared with those of hot and cold burnished gutta-percha or root-end cavities filled with amalgam. Becker & Von Fraunhofer (48) showed that the seal of thermoplasticized gutta-percha without a root canal

sealer was worse than that with a sealer and amalgam with varnish. Woo et al. (57) also showed that thermoplasticized gutta-percha with sealer had significantly less leakage than amalgam when used as a root-end filling material. Olson et al. (59) also found that injectable high-temperature gutta-percha without sealer demonstrated significantly more leakage than this material with a sealer, a glass ionomer cement and amalgam with and without varnish. Kaplan et al. (30) reported improved sealability of gutta-percha after cold burnishing of this material, and Minnich et al. (52) noted beneficial effects of cold burnishing for poorly obturated canals. Other investigators (34, 39, 41, 51), have reported no improvement in sealing ability of gutta-percha following cold burnishing. A number of investigators have reported that heat burnishing of gutta-percha does not improve the sealing ability of this substance as a root-end filling material (30, 34, 39, 44, 46). Bramwell & Hicks (37) reported no significant difference between dye leakage of heat or cold burnished gutta-percha in monkeys. In contrast, Abdal & Retief (28) showed that heat sealed gutta-percha provided a better seal than most commonly used root-end filling materials such as amalgam, IRM and SuperEBA. MacPherson et al. (47) as well as Wu et al. (56) and Woo et al. (57) reported obtaining a better seal with thermoplasticized gutta-percha than amalgam with and without varnish. In contrast Escobar et al. (36) and Olson et al. (59) reported equal sealing ability for thermoplasticized gutta-percha and amalgam with and without varnish.

Because amalgam and gutta-percha fail to provide ideal apical seal, other substances such as ZOE based cements, glass ionomers, composites and other substances have been suggested as root-end filling materials. Some investigators (28, 43, 45, 49, 54, 60, 61), showed that glass ionomer cements provide better seal than amalgam. In contrast, King et al. (58) showed the seal provided by Ketac-silver was inferior to those obtained by SuperEBA, and amalgam with and without varnish. In addition, MacNeal & Beatty (40) demonstrated that the seal of two glass ionomers (Ketac and Fuji II) was adversely affected when the root-end cavities were contaminated with moisture at the time of placement of these materials. Abdal & Retief (28), McDonald & Dumsha (39), Thirawat & Edmunds (54) and Danin et al. (63) reported that composite resins provided better seal than that obtained with amalgam. Szeremeta/Brower et al. (34), and Bondra et al. (50) reported that SuperEBA provided a better seal compared with amalgam as a root-end filling material. However, other investigators (44, 51, 54, 58, 66) have shown that SuperEBA provided equal seal to amalgam in conjunction with a cavity varnish. Abdal & Retief (28), Smee et al. (42), and Bondra et al. (50) showed that IRM provided a better seal than amalgam or SuperEBA. Bondra et al. (50) showed that SuperEBA's seal was equal to that of IRM.

Comparing the data obtained from various leakage studies shows considerable variations in the results of these investigations and even within the same group of studies using similar experimental methods. The data generated in most of these studies were collected after longitudinal or cross sectioning, or clearing of the roots, and measuring the linear tracer penetration. These studies provided semi-quantitative data and have a high level of variation. In addition to the fact that dye or isotope penetration studies do not provide the volume of tracers which penetrate through the interface between tooth structures and root-end filling materials, other variables, such as molecular size of tracer, immersion period, pH of tracer solution and entrapped air, have not been standardized.

Kersten & Moorer (68) compared the ability of four obturation methods to prevent leakage of bacteria-sized particles or large protein molecules, and found leakage of the commonly used dye, methylene blue, was comparable with that of a small bacterial metabolic product of similar molecular size. Their findings showed that microleakage of the small molecules could not be prevented, while leakage of bacteria-sized particles and large size protein molecules could be prevented with some of the obturation techniques. Higa et al. (67) evaluated the influence of storage time (0 versus 24 hours) on the amount of dye leakage of amalgam, SuperEBA, or IRM. Their results showed SuperEBA and IRM leaked significantly less than amalgam and storage time had no significant influence on the amount of dye leakage. Although pH of tracer solution may affect the leakage pattern of root-end filling materials, it is interesting to note that most studies did not give the pH of their solutions.

The gap between root-end cavity walls and root-end filling materials may contain air and/or fluid. Goldman et al. (69), Spangberg et al. (70) as well as Oliver & Abbott (71) showed that the use of a vacuum increased the amount of dye penetration. However, recent studies by Peters & Harrison (72) as well as Masters et al. (73) questioned the beneficial effects of this procedure in leakage studies.

The majority of leakage studies have been performed *in vitro* with little or no similarities to conditions *in vivo*. One of their major limitations is the amount of fluid exchange between the apical

root canal walls and the root-end filling material. The amount of tissue fluid present in the apical region is much less *in vivo* compared with *in vitro* tests. In addition, most *in vitro* studies have been performed in dry conditions which do not determine the adverse effects of humidity on these materials. The sealing ability of some of the root-end filling materials, such as glass ionomer cements, can be adversely affected by the presence of moisture as shown by MacNeil & Beatty (40). Dye penetration technique is the most frequently used method to evaluate the sealing ability of various root-end filling materials. Autoradiography is a subjective quantitative technique of measuring the apical leakage. Factors such as type of isotope, distance between radiation source and emulsion, and the length of exposure of the film can affect the results obtained by this technique. Matloff (74) showed that methylene blue dye penetrated further up the canal than ^{45}Ca, ^{14}C-labelled urea, or ^{125}I-labelled albumin, and questioned the validity of the results obtained in radioisotope studies.

Delivanis & Chapman (75) compared the reliability of the electrochemical method, autoradiography and dye penetration for leakage assessment. They concluded that the correlation between these methods was correct at the two extreme ends of the score ranges. They stated that dyes are simpler, cheaper, safer, and easier to handle than radio-isotopes. The leakage assessment does not require a special set-up compared with that needed for the electrochemical method. It is also possible to obtain false leakage other than from the apex with the electrochemical method.

The majority of in vitro apicoectomies have been performed perpendicular to the long axis of the teeth, in contrast to a 45 degree linguo-buccal bevels often used in conditions *in vivo*. Bevelling of the root surface at an angle results in exposure of dentinal tubules. These tubules may provide additional pathways for leakage. Gilheany et al. (18), using a fluid filtration technique, determined the apical leakage of extracted teeth resected at 0, 30, and 45 degress to the long axis of the root and filled the root-end cavity with various increments of a glass ionomer cement. Their results indicate that apical leakage significantly increases as the amount of the bevel increases. In addition, they showed that increasing the thickness of root-end filling materials significantly decreased the apical leakage.

The thickness of root-end filling materials is another uncontrolled variable in leakage studies and has varied from 1 to 5 mm (28, 31, 35, 76–78). Finally, most apical leakage studies have determined the sealing ability of various materials by placing the apical ends in the tracers. Practically, the purpose of placing a root-end filling material is to prevent penetration of irritants from the root canal system into periradicular tissues. In reality, coronal seal of root-end filling materials is probably more important than that of the apical seal.

King et al. (58), Crooks et al. (64), Gilheany et al. (18), and MacDonald et al. (66) used the fluid filtration technique developed by Derkson et al. (79) to determine the leakage patterns of various root-end filling materials. The main advantages of this technique are: 1) the samples are not destroyed 2) the leakage can be determined at different time intervals and 3) the collected data is quantitative. However, application of solution under pressure does not simulate clinical conditions.

B. Bacterial leakage

Despite their popularity and ease of use, the results and clinical significance of leakage studies have been questioned (14, 80–84).

Mortensen et al. (80) and Krakow et al. (81) showed that microorganism penetration might be more appropriate than dye or isotope penetration for studying leakage *in vivo*. Goldman et al. (82) have pointed out that bacteria give better indication than dye in testing for leakage of hydrophilic materials and that dyes can give a false positive reading if their molecules were small enough. Torabinejad et al. (14) used two species of bacteria, *Staphylococcus epidermidis* and *Proteus vulgaris*, to evaluate the coronal leakage of root canal-filled teeth. Eighty-eight percent of the root canals were completely recontaminated in 30 days following exposures to *Staphylococcus epidermidis* and 85% in 66 days following exposure to *Proteus vulgaris*. Magura et al. (15) evaluated the coronal leakage in obturated root canals using fresh human saliva. They found that salivary leakage was slower than dye penetration. They also reported that salivary penetration at 90 days was significantly greater than that seen after 2, 7, 14, and 28 days. Khayat et al. (83) determined the time needed for bacteria in saliva to contaminate the entire length of obturated root canals and found all root canals were recontaminated in less than 30 days. Wu et al. (84) used the movement of a bacterium (Pseudomonas Aeruginosa) in a capillary glass tube connected to the apex of root filled extracted human teeth as an indicator of leakage and found most obturated root canals do not allow the passage of this bacterium.

Because of inherent inadequacies in dye and radioisotope leakage studies, and a lack of correlation between bacterial leakage and those of dye

and isotope molecules, bacterial leakage studies have been recommended to test the suitability of potential root-end filling materials (85–87).

Kos et al. (85) evaluated the ability of poly-HEMA as a root-end filling material and found that this material prevented leakage of *Proteus vulgaris*, an actively motile gram negative rod and *Streptococcus salivarius*, a gram positive coccus normally found in the human oral microflora. In contrast, cold-burnished gutta-percha, heat-sealed gutta-percha, and zinc-free amalgam showed a high incidence of leakage ranging from 80% to 100% of the specimens tested. Luomanen & Tuompo (86) compared the tightness of titanium screws versus amalgam as root-end filling materials, using *Serratia marcescens* in an *in vitro* model. They found that the bacteria penetrated around the apical titanium screws in 2–7 days, and around the retrograde amalgam fillings on the first day of the experiment. Staining of the teeth with India ink showed that penetration of bacteria had occurred at the tooth/filling margin.

Wong et al. (87) compared the apical seals obtained by placing either amalgam as a root-end filling material or lasing the root apices with the Nd:YAG Laser. After instrumentation and obturation, one group received amalgam as root-end filling material and eight other groups were lased with the Nd:YAG Laser at wattages ranging from 0.75 W to 3.0 W for 20 s. *Streptococcus salivarius* was placed in the coronal reservoir of each root and its apical 10 mm was placed in brain-heart infusion broth and phenol red indicator. Their results showed no significant difference between bacterial leakage in the laser-treated groups and the samples retrofilled with amalgam.

Compared with clinical conditions, models used in bacterial leakage studies to determine sealing ability of various root-end filling materials have several shortcomings. They are static and their bacterial contents are dissimilar to those found in human saliva. In addition, these studies did not determine the ability of root-end filling materials to prevent leakage of bacterial metabolites and their by-products. *In vitro* and *in vivo* animal models simulating clinical conditions are needed to obtain quantitative data and determine the relationship between leakage of bacteria and their by-products and periradicular inflammation.

II. Marginal adaptation

The scanning electron microscope (SEM) has been used in dental research to study normal and inflamed gingival tissues. (88), plaque structure (89), caries formation (90), the effects of etching on marginal adaptation of various restorative materials (91) and the interface between tooth structure and restorative materials such as gold, composite and amalgam (92).

In scanning electron microscopy, specimens are considered as a collection of point sources of radiation, each of which transmits information concerning composition and structure. The SEM uses a 2–3 nm spot of electrons that scans the surface of the specimen to generate secondary electrons from the specimen which are then detected by a sensor. The sensor uses this emitted radiation to build up a picture of the complete object. In effect, output signals from the SEM are reflected from an opaque surface. Geometrically, the reflection of electrons from the SEM specimen surface obeys the same laws as the reflection of light from an irregular surface. The result is that SEM images are formed in topographic contrast – the image intensities are related to variations in surface topography. The images appear like those viewed macroscopically or, in other words, three dimensional. In general, electron micrographs represent an overlay in which details from many levels of the specimen appear, in focus, within a single plane (93).

In endodontics, a number of investigators have utilized SEM to investigate the marginal adaptation of root-end filling materials (28, 94–99).

By using SEM and replication technique, Cunningham (94) compared the apical appearance of eight teeth obturated with silver cones and sealer, gutta-percha and sealer with and without root-end resection, and amalgam as root-end filling material. Considerable disruption of the apical seal was seen in the roots apicected after gutta-percha obturation. The smoothest surface and best seal appeared to have been achieved by root filling with a silver point before apical resection. Retrograde amalgam fillings appeared satisfactory, but gutta-percha points used before and specially after the reduction of the apex appeared to have undergone considerable disruption.

Moodnik et al. (95), found defects of 6–150 μm around amalgam retrofillings using the SEM. These deficiencies around amalgam retrofillings questioned the validity of this widely accepted technique. However, the authors did not elaborate on the clinical significant of their findings. Tanzilli et al. (100), compared the marginal adaptation, using the SEM, of retrograde amalgam, heat-sealed gutta-percha, cold-burnished gutta-percha, and apicoectomy only. They concluded cold-burnished gutta-percha was "90% better than any of the other techniques investigated." Retrograde amalgam, heat-sealed gutta-percha, and the apic-

oectomy control showed mean marginal defects from 22 μm to 10 μm, while cold-burnished gutta-percha exhibited a mean defect of only 1.8 μm.

Abdal & Retief (28) used SEM to compare the marginal adaptation obtained by post-resection filling with heat-sealed gutta-percha and when reinforced with 16 retrofilling materials (one sample per material). Most materials tested had gaps. However, no gaps were found in Cavit, Sybraloy (high-copper), polycarboxylate cement, glass ionomer cement, heat-sealed gutta-percha, and Adaptic.

In an SEM study, Stabholz et al. (96), compared the marginal adaptation of root-end filling with Restodent, zinc phosphate cement, Cavit-W, Durelon, and amalgam. Restodent sealed significantly better than the other four materials and demonstrated the best adaptation to cavity walls, while amalgam was significantly inferior to the four other materials in both marginal adaptation and seal. In addition, they showed zinc phosphate did not differ significantly from Cavit or Durelon.

Yoshimura et al. (97), in a coronal and apical microleakage study using a pressurized fluid filtration technique and SEM examination of teeth with retrograde amalgam fillings, showed leakage decreased markedly in the 90 minute to six hour interval after filling. Small changes in leakage were noted between 1 day and 8 weeks. Leakage from the coronal direction was not significantly more than that noted from the apical direction. Gaps as wide as 20 μm were noticed between amalgam and the tooth structure in some selected specimens. They found no correlation between microleakage and the width of the gap appearing on the surface.

Inoue et al. (98) compared the microleakage of amalgam, amalgam with a cavity varnish, a silver-containing glass ionomer (Miracle Mix) and IRM using a fluid filtration technique and SEM observations. Glass ionomer cement and IRM showed significantly less microleakage compared with the amalgam group without varnish. The use of cavity varnish reduced the apical leakage of amalgam significantly. Examination of scanning electron photomicrographs of some selected specimens showed the presence of gaps ranging 5–10 μm between the root-end materials and their surrounding dentinal walls.

Torabinejad et al. (99) in a dye leakage and SEM examination of four retrograde amalgam root-end fillings from four radiographically successful teeth showed presence of methylene blue dye penetration through the interface of amalgam and root-end cavities and varying size gaps between the cavity walls and amalgam. These investigators questioned the correlation between dye leakage, SEM studies and clinical success.

Specimen preparation for examination with the SEM involves several steps, each capable of inducing artificial changes in the specimen. These steps include: fixation, dehydration, drying, heavy metal sputter-coating, and high-pressure vacuuming which can result in the formation of artifacts.

To prevent or reduce the amount of artifacts, different methods have been proposed to produce replicas of the original samples. Investigators have described replication techniques to study the tooth structure-filling material interfaces, and the microvasculature of the pulp (101–103).

Moodnik et al. (95), and Torabinejad et al. (99) in their case reports revealed the presence of large defects at the amalgam-tooth interface. These studies with their small sample sizes as well as the study by Tanzelli et al. (100) did not utilize any replication technique to minimize the introduction of artifacts. Abdal & Retief (28) employed a negative/positive replication technique to evaluate only a single sample of each of the 16 root-end filling materials. However, in this study, the teeth were sectioned longitudinally with a diamond saw, which may have affected the findings. Stabholz et al. (96) used a negative replica technique and sectioned their samples which might have affected the results of this investigation. Yoshimura et al. (97) and Inoue et al. (98) also reported presence of gaps between root-end cavity walls and amalgam in some selected cases in their leakage and SEM investigations.

Despite its shortcomings (introduction of artifacts during sample preparation, showing only 1 surface which may not represent adaptation of 2 surfaces in 3 dimensions and a lack of correlation between marginal adaptation and sealability), SEM examination of marginal adaptation of various root-end filling materials to their surrounding structures can provide information which could be used as an indicator of the sealing ability of these materials.

III. Biocompatibility

The materials used in endodontics are frequently placed in intimate contact with the hard and soft tissues of the periodontium. This is particularly true for the substances used as root-end filling materials. Therefore, it is essential that a potential root-end filling material be non-toxic and biocompatible with its surrounding host tissues. Currently, there are three recommended tests to evaluate biocompatibility of dental materials. An initial test which provides general toxicity profile

of potential materials, secondary tests, which evaluate local toxicity, and usage tests in which the potential substances are used in the teeth of experimental animals according to clinical protocols.

A. Cytotoxicity tests

Cytotoxicity is one of the most commonly used *in vitro* tests to measure biocompatibility. It is a simple, rapid, and inexpensive screening test, and gives a valuable indication as to which materials should be discarded or subjected to further testing. Many methods have been used to determine the cytotoxicity of various dental materials. These methods involve either observing the inhibition of cell growth or recording cellular injury and/or death. The three most commonly used cytotoxicity tests include agar overlay technique, millipore filter method and radiochromium release test.

1. Agar overlay technique

In this test the cells are first cultivated to a confluent monolayer in 24 hours. After removal of the culture medium, the cells are covered with Eagle's Minimum Essential Medium (MEM) supplemented with 1% calf serum and 1% agar and stained with 0.01% Neutral Red solution. The test material is then placed on the agar surface and incubated for 24 hours. The cell monolayers are then examined under an inverted microscope and the plates are visually analyzed for cellular lysis. The dead cells lose their Neutral Red staining and will provide a clear zone subjacent to the test material. The toxicity of the material can then be registered according to a lysis index from 0 to 5 (104).

2. Millipore filter method

Wennberg et al. (105) introduced the "Millipore filter method" in which Millipore filter disks are placed in tissue culture disks and covered with cell suspensions containing human epithelial cells, HeLa cells or mouse fibroblasts (L929). After establishment of a cell monolayer on the filters and removal of culture medium, the filters are covered with Eagle's MEM and 1.5% agar. The nutrient agar is allowed to solidify and then the agar filter is placed in the Petri dish upside down. The test material is placed on the filters and allowed to influence the cells for 2–24 hours. The staining intensity of monolayer cells for succinate dehydrogenase activity is used as the indicator of cell vitality around the test materials. The relative degree of toxicity of the material can be registered from 0 to 3 indicating the extent of the zone with reduced or inhibited enzyme activity (105).

3. Radiochromium release test

According to Spångberg (106) the radiochromium release test is the third commonly used method to asses cytotoxicity of various dental materials. In this method, the test material is first placed in the bottom of the wells of tissue culture plates. It is then covered and inoculated with grown, harvested and prelabelled cells with $Na^{51}CrO_4$ for 1–24 hours depending on the experimental design. After appropriate incubation period, a standardized amount (1.0 mL) of the culture medium is withdrawn from each well and the amount of ^{51}Cr released into the medium is measured in a gamma particle counter. A comparison between the amount of the original incorporated radio-chromium in the cells with that released from cells after incubation with the test material as well as the positive and negative controls is used as an indicator of toxicity of a dental material (106).

The cytotoxicity of potential root-end filling materials have been determined by several investigators using various techniques (107–117).

Spångberg et al. (107), used radiochromium-labelled HeLa cells to test the biological effects of some potential root-end filling materials and reported that freshly prepared IRM produced total cell lysis, while after setting for 1–4 weeks the toxicity decreased slightly. In addition, they showed relatively low cytotoxicity of polycarboxylates as compared with ZOE and IRM.

Dahl & Tronstad (108) determined the cytotoxicity of a glass ionomer (ASPA III) cement and a conventional silicate cement using the radio-chromium method. Based on their findings, it appeared that the silicate cement was less toxic than the glass ionomer cement and the toxicity of the latter decreased with setting time.

Antrim (109) used radioactive Cr released from KB carcinoma cells as a measure of cell lysis in response to Groosman's sealer, N2 (permanent), Rickert's sealer, and Cavit. He reported that all the materials tested possessed lasting tissue toxicity; Grossman's sealer was the most toxic material, followed by N2, Rickert's, and Cavit. Cavit showed a low initial toxicity (24 hours), but this increased with time and remained highly toxic thereafter. The response to Cavit was somewhat erratic and was attributed to the deterioration of the material.

Tronstad & Wennberg (110) compared the toxicity of dental amalgams, silicate cement, methyl

methacrylate resin, composite resins, zinc phosphate cement, polycarboxylate cement, ZOE paste and calcium hydroxide GR-isotonic paste on mouse fibroblasts by means of Millipore technique. The conventional amalgams and a high copper amalgam (Dispersalloy) showed similar responses. The conventional amalgam and high copper amalgam were initially somewhat toxic with a decrease in toxicity after 24 hours. However, the old style high copper alloys (30% copper) were more toxic. Zinc phosphate and silicate cements as well as methyl methacrylate resin were highly toxic when freshly mixed. The wet and dry varieties of ZOE paste were moderately toxic and calcium hydroxide paste proved to have a strong and lasting toxic effect.

Wennberg & Hasselgren (111) evaluated the cytotoxicity of a number of temporary filling materials including Cavit, IRM, and ZOE cement by the Millipore filter method and showed that all the cements had some degree of cytotoxicity through the experimental period; ZOE and IRM showed a continuing decrease in their cytotoxicity with time.

Meryon et al. (112) compared the cytotoxicity of two glass ionomer cements (ASPA and Chem-Bond) on fibroblasts and macrophages. Both materials were found to be initially cytotoxic to fibroblasts; this was evident by the inhibition of succinic dehydrogenase staining in cells grown on Millipore filters. These substances were also cytotoxic to macrophages assessed by a reduction in cell numbers and by enzyme staining kinetics and quantitative enzyme analysis. The cytotoxicity of both materials decreased considerably after 24 hours.

Milleding et al. (113), compared the relative cytotoxic effect of corroded and non-corroded amalgams of different types using a cell culture and Millipore filter method. Corrosion was produced by storing the amalgam in distilled water or artificial saliva with a pH of 4, 5, or 7 for up to 28 weeks. The set, non-corroded amalgam did not show any cytotoxic effects, whereas corroded amalgam surfaces showed various degress of cytotoxicity.

Al-Nazhan et al. (114), compared the cytotoxicity of a composite resin (Restodent) with those of Cavit and Dispersalloy using the radiochromium labelling technique. Their results showed that Restodent was more toxic than amalgam; Cavit had no toxic effect. They concluded that amalgam was still the material of choice due to its low cytotoxicity, and that composite was the most toxic material tested and should not be used.

Safavi et al. (115), attempted to grow fibroblasts on root-ends filled with amalgam or composite resin. Study of the cell attachment to the substrate under SEM showed cell attachment to the composite surface was remarkably less than that to amalgam.

Pissiotis et al. (116), compared the cytotoxicity of silver glass ionomer (SGI) cement and amalgam using ^{51}Cr release test and found that SGI plus varnish was less cytotoxic than amalgam. They concluded that SGI should be considered as an alternative root-end filling material.

Bruce et al. (117) measured the cytotoxicity of several dentine bonding systems, SuperEBA and amalgam using the agar overlay test at 24 hours, 7, 15, and 30 days. Except for amalgam and Tenure Visar Seal, the rest of the materials were initially cytotoxic. SuperEBA, Caulk-filled resin, and Gluma sealer & Gluma bond were initially toxic and they lost their toxicity 7 days after incubation with the monkey kidney cells (VERO). Cytotoxicity of amalgam increased as it aged. Based on their results they concluded that some dentine bonding agents are cytotoxic; however, they lose their cytotoxicity as they age.

Based on the results of these studies it appears that ZOE-based materials in the freshly mixed and unset state are very cytotoxic and they lose their cytotoxic effect as they age (107, 109, 111–117). Conflicting reports have been made regarding cytotoxicity of Cavit, glass ionomer cements and composites. Despite the ease of control of experimental factors in cytotoxicity tests there are several disadvantages with performing these procedures. The results of these tests are relative, cannot be compared with one another, and are dependent on cell types and degree of diffusibility of test materials. In addition, these investigations cannot study the complex interaction between material and host tissue and the measurements obtained by themselves have little relevance to clinical circumstances (106).

B. Implantation tests

Because of limitation of cytotoxicity tests, *in vivo* subcutaneous and intraosseous implantation techniques in small laboratory animals have been recommended (107, 118–121). Subcutaneous and intraosseous implantation techniques are considered suitable secondary tests to evaluate the biocompatibility of various dental materials. Early implantation studies involved placement of pellets of dental materials in various soft tissues and bones. The implantation method was refined when Friend and Browne (118) used Teflon tubes as a vehicle to place small standardized surfaces of fresh or set dental materials in contact with desig-

nated tissues. According to Spångberg (106) these tests can provide valuable information without excessive cost and unnecessary animal sacrifice.

Potential root-end filling materials have been implanted subcutaneously and intraosseously in small laboratory animals (122–133).

Wolfson & Seltzer (122) used an injection technique and implanted several brands of gutta-percha, natural gutta-percha, latex, an experimental formulation containing gutta percha (20%) and calcium hydroxide (80%), and Kloroperka N-˝O in the subcutaneous tissue of rats. Histological examination of specimens showed an initial acute response followed by fibrous tissue encapsulation. Kloroperka N-˝O caused severe tissue destruction and inflammatory cellular infiltrate containing PMN leukocytes, macrophages, lymphocytes, plasma cells and giants cells. The gutta-percha formulation with calcium hydroxide elicited a foreign-body giant cell response.

Flanders et al. (123) implanted zinc-free amalgam and Cavit subcutaneously and next to the bone in rats and evaluated them histologically after 10, 30, 90 and 180 days. Cavit produced more of a foreign body reaction than amalgam in both tissues. Cavit produced osteocytic death in the adjacent bone and a thicker fibrous capsule next to connective tissue. The inflammatory response to both materials decreased with time.

In a bone implantation study, Zartner, et al. (124) placed freshly mixed amalgam and zinc polycarboxylate (ZPC) cement into the tibias of rabbits and evaluated them histologically at 2 weeks, 2 and 4 months. Comparison of the bone adjacent to ZPC cement and amalgam showed identical tissue responses. There was evidence of viable osteocytes, healthy vascular and connective tissue, and a lack of inflammation.

Martin et al. (125), evaluated the rat connective tissue response to zinc containing amalgam, zinc-free amalgam, and zinc carbonate crystals and evaluated them histologically at 2, 14 and 30 days. There was no significant difference in inflammatory responses to zinc containing or zinc-free amalgams at any time interval. In contrast, zinc carbonate showed a severe foreign body giant cell and macrophage response. No zinc carbonate was formed in any of the zinc containing specimens. However, the specimens were not placed in contact with another metal to allow for the electrolysis required for zinc carbonate formation.

Liggett et al. (126) placed freshly mixed zinc and non-zinc amalgam in the tibias of rats and examined them via light microscopy, SEM, and microprobe analysis. Over a period of 12 weeks they found both types of amalgam to be well tolerated by the rat osseous tissue. However, the microprobe analysis showed evidence of tin and sulphur in the bone adjacent to the implants with both amalgam types. They attributed this to the formation of corrosion products and breakdown of the alloy.

Zmener & Dominguez (127) compared the biocompatibility of zinc phosphate cement with a glass ionomer cement by implantation in dog tibias. Initially, the glass ionomer cement caused less inflammatory response than the zinc phosphate. However, at the end of 90 days the tissue response to both tested materials appeared to be similar, with resolution of inflammation and progressive new bone formation. Based on their findings, the authors recommended that glass ionomer should be considered as a replacement for the zinc phosphate cement as a luting material.

Blackman et al. (128) placed freshly mixed pellets of glass ionomer-silver cement lightly coated with a cavity varnish and IRM into the connective tissues and bones of rats and examined them histologically after 14, 30 and 80 days. Despite presence of mild inflammation up to 80 days, each material appeared to be well tolerated. Bone apposition occurred adjacent to glass ionomer-silver, while fibrosis was observed next to IRM.

Leonardo et al. (129) studied histologically the subcutaneous connective tissue responses of rats to the placement of three different formulations of gutta-percha: Obtura, Ultrafil, and gutta-percha points recommended for use with the McSpadden compactor system. Obtura gutta-percha showed a severe inflammatory response from the 7th to the 120th day similar to that seen with gutta-percha recommended for the McSpadden technique. The Ultrafil gutta percha caused a severe response initially which became moderate and mild in subsequent observation periods. At 21-, 60-, and 120-day intervals, the Ultrafil gutta-percha was associated with mature granulation tissue with neither oedema nor vascular congestion, in contrast to the responses observed with the other two formulations.

Mcaree & Ellender (130) compared the biocompatibility of SuperEBA and Ketac-silver glass ionomer, an amalgam, a ZOE cement and silver-free AH26 by subcutaneous implantation of these materials in rats. Initially, ZOE, amalgam, and SuperEBA were associated with moderate inflammation. By 100 days, the latter materials and Ketac-silver glass ionomer were encapsulated with fibrous connective tissue. However, AH26 was associated with necrosis and persistent inflammation and granulation tissue formation.

To test the disinfection effect of paraformaldehyde on gutta-percha, Cleary et al. (131), com-

pared the histological response of a 7-day exposure of paraformaldehyde treated gutta-percha with untreated gutta-percha (Obtura) implanted in rat connective tissue. The untreated gutta-percha showed moderate to severe inflammation for 4–7 days, which decreased progressively. At 56 days untreated gutta-percha was associated with mild to no inflammation with fibrous capsule formation. The paraformaldehyde treated gutta-percha showed significantly less inflammation in the early stages (4–7 days) of implantation. The tissue responses to both treated and untreated gutta-percha were very similar at the end of the experiment (56 days). The samples were associated with capsule formation and presence of macrophages and giant cells.

Bhambhani & Bolanos (132) implanted Teflon, IRM, and Prisma VLC Dycal in the mandible of guinea pigs for 4 and 12 weeks. Their histological findings indicated apposition of bone adjacent to the Prisma VLC Dycal; none to mild inflammation and a thin fibrous connective capsule near IRM, and a thick capsule adjacent to well condensed Teflon powder material. Loosely condensed Teflon material caused chronic inflammation and active phagocytosis.

To evaluate the osseous reaction to IRM, Opotow Alumina EBA, and amalgam, Olsen et al. (133) implanted these substances in rat tibias and examined them histologically from 7–100 days. Complete healing occurred around Teflon cups containing IRM or amalgam while EBA specimens had more inflammation at 56 days. At 100 days, healing also progressed to completion adjacent to EBA cement group with only an infrequent presence of leukocytes. Based on their results, the authors stated that both IRM and EBA cement were acceptable biological alternatives to amalgam.

The results of implantation studies show that most potential root-end filling materials initially cause inflammation and they become more biocompatible as they age (122, 130, 131, 133). This is partly as a result of surgical trauma and also release of leachable substances from these materials. In addition these studies show that implanted gutta-percha is usually encapsulated with fibrous connective tissue (122, 129) and there are no histological differences between the tissue responses to zinc-containing and zinc-free amalgams (125, 126). Furthermore, IRM, EBA, and glass ionomer cements are well tolerated by connective tissue as well as bone (127, 128, 130, 132, 133).

Because of possible differences in tissue responses in different animal species and locations, various methods of evaluation and short observation periods, the results of various implantation studies cannot be compared. In subcutaneous implantation, mechanical displacement of implanted material has been recognized and is noted as a disadvantage of this technique (134). This deficiency can cause an inability to achieve proper material-tissue contact.

Despite advantages, an artificial bony cavity and its content (Teflon cup & test material) used in intraosseous implantation are different from a tooth suspended in the periodontal ligament. As stated by Olsson et al. (135), both implantation techniques can provide important information regarding the cellular response to dental materials. However, the authors state that it is difficult to make the assumption these tests are comparable to usage tests. The results of these methods like those obtained in *in vitro* tests should not be used as absolute values and can be only used as indicators of biocompatability of test materials.

IV. Usage tests

As discussed earlier (Section III-B), implantation methods have several shortcomings compared to usage tests. Despite their costs and lack of complete standardization of clinical variables, the usage tests can provide information related to biological properties as well as handling characteristics of test materials under clinical circumstances. The usage tests are performed in experimental animals and clinical trials in man.

A. Periradicular tissue responses to root-end filling materials

To examine the periradicular tissue responses to potential root-end filling materials, the root canals of experimental animals are usually cleaned, shaped, obturated, and after root-end resection and preparation of root-end cavities, they are filled with test materials. The animals are then sacrificed and their periradicular tissues are examined histologically to determine the biocompatibility of test materials at different time intervals. A number of histological evaluations of periradicular tissue reponses of monkeys, dogs and ferret to some of the commonly used and potential root-end filling materials have been reported (77, 78, 136–143(.

Marcotte et al. (136) compared the periradicular tissue responses of the permanent anterior teeth of two rhesus monkeys to gutta-percha (following apicoectomy) and zinc-free amalgam. After performing root canal fillings and apicoectomies on 12 anterior teeth, they filled six root-end cavities with amalgam. Their histological evaluation of

two specimens per time interval at 3–15 weeks showed healing of surgical defects regardless of which of the two root-end filling materials had been used. Starting at 5 weeks, collagenous fibrous connective tissue covered gutta-percha and amalgam. Maturation of this tissue and formation of bone were observed at subsequent time intervals. New cementum covered resected root-ends starting at the 7 week interval.

Kimura (77) evaluated the periradicular tissue reaction of four dog teeth retrofilled with zinc- or zinc-free alloys. Histological examination of four specimens at various time intervals (1–22 months) showed presence of some inflammation in the 1- and 7-month specimens and severe inflammation adjacent to both materials after 12 and 22 months. In the second part of his study, Kimura (78) determined the concentration of elements by optical emission spectrographic analysis in eight specimens at each time interval (1–22 months) and found no zinc precipitate in periapical bone adjacent to root-ends filled with zinc-containing or zinc-free alloys.

Callis & Santini (137) compared the periapical tissue response of 10 ferrets to gutta-percha and glass ionomer cement when used as root-end filling materials for 7 and 28 days. Both materials caused only a mild inflammatory response after 7 days. At 28 days the glass ionomer samples showed more bone formation and no inflammation. The gutta-percha specimens had good healing with mild inflammation. The sealer (Tubiseal) used with the gutta-percha might have contributed to the presence of inflammation. In contrast to the presence of a layer of fibrous connective tissue separating gutta-percha from bone, bone formed in direct contact with the glass ionomer cement (Ketacfil) used.

Mangkornkarn & Harrison (138) evaluated amalgam and thermoplasticized gutta-percha as root-end filling materials in six monkeys at three time intervals (4–16 weeks). Their histological findings indicated slower bone healing associated with thermo-plasticized gutta-percha than amalgam at 4 and 8 weeks and no significant difference in the osseous healing at 16 weeks. Both materials appeared to be well tolerated and biocompatible to the periapical tissues.

Zetterqvist et al. (139) compared the periradicular tissue responses to glass ionomer and amalgam in eight monkeys after 2 weeks, 1, 3, and 6 months. At 2 weeks, inflammatory cells were observed close to the root-end filling materials. One month after surgery, the granulation tissue had started to be replaced by new bone. After 3 and 6 months, complete healing was noted adjacent to both root-end filling materials. Based on their results, the authors recommended glass ionomer cement to be considered as an alternative to amalgam for patients suffering from allergic or toxic reactions to mercury present in amalgam.

Pitt Ford & Roberts (140) examined the periradicular tissue response of eight central incisors in four Cynomolgus monkeys to a radiopaque glass ionomer cement with and without root canal filling materials. Contaminated canals with apical glass ionomer as a root-end filling material but without a root canal filling had severe inflammation and abscess formation adjacent to root-end filling material. However, teeth with glass ionomer root-end fillings and gutta-percha root canal fillings were associated with either no inflammation or a mild response. Bacteria were found in the interface of glass ionomer and dentine in every tooth without a root canal filling and had extended apical to the root-end filling material.

Rud et al. (141) examined the periradicular tissue response of two green Vervet monkeys to a composite resin (Retroplast) as a root-end filling material. After filling root canals of two maxillary incisors and canines and placing Retroplast at their resected root-ends, the tissues were examined histologically 1 year following surgery. In some cases epithelium and inflammatory cells were seen in periapical tissues. In other cases, there was an absence of inflammatory cells adjacent to the filling material, but close contact between the Retroplast and fibroblasts with collagen fibres. In one case, cementum and Sharpey's fibres were found in contact with the filling material.

Pitt Ford et al. (142), examined the effect of IRM and amalgam as root-end filling materials prior to replantation in 42 roots of mandibular molars of monkeys. Following extraction and contamination of canals with the monkey's oral flora and placement of IRM or amalgam as root-end filling materials, the teeth were replanted and the tissues examined after 8 weeks. The tissue response to IRM was less severe than that to amalgam. Sixty percent of roots filled with IRM were associated with inflammation. In contrast, 92% of roots filled with amalgam had periapical inflammation. No root with IRM as the root-end filling material had inflammation in the bone marrow space. In contrast, inflammation was present in the alveolar bone marrow space with every root-end filled with amalgam. Using a similar surgical technique, Pitt Ford et al. (143) investigated the periradicular tissue response to root-end fillings of SuperEBA in eight roots of monkeys and reported a mild inflammatory response.

Except for one study Pitt Ford et al. (143), the

sample size in usage tests in experimental animals for each time interval seems very small (2–4 samples). The observation periods in most of these studies are relatively short and may or may not represent clinical circumstances. Except for four studies (77, 78, 140, 142), which contaminated the root canals of teeth purposely, the rest of these studies filled the canals with root-end filling materials under ideal circumstances. Most of these studies used subjective criteria for their histologic assessment. Because of limited sample numbers no statistical tests were used to analyze their findings.

B. Clinical usage

Sealing ability, marginal adaptation, *in vitro* cytotoxicity tests, implantation and usage tests in experimental animals are screening means to eliminate materials with unacceptable degrees of leakage and high levels of toxicity. These tests are prerequisite exercises and are not substitutes for clinical studies.

Clinical comparison of potential root-end filling materials under similar operative and postoperative conditions is the ultimate and the most reliable method for evaluation of their clinical usefulness and their long-term efficiency. Clinical investigations are performed in retrospective or prospective manners. A number of clinical studies have been reported on various root-end filling materials (144–162).

Harty et al. (144) in a retrospective study examined the success rate of apicoectomy in 1,016 cases at 6 and 12 months and yearly thereafter at least for 2 years and reported no significant difference between using amalgam as a root-end filling material and nonsurgical root canal therapy using gutta-percha or silver points in conjunction with a ZOE sealer. They reported teeth with preoperative radiolucencies had more failures than those without them.

Rud et al. (145) examined the course of healing of 1000 teeth treated by surgical endodontics and reported that most completely healed or unsuccessfully healed cases had no significant changes (were stable) irrespective of the observation period. Because significant changes were noted in incompletely healed cases within the first few years following periapical surgery, they recommended a final 4-year follow-up observation period in cases showing uncertain healing. Rud et al. (146) performed a multivariate analysis of the same surgical cases which had been followed from 1 to 15 years after operation. Their results showed that cases with gutta-percha root fillings had significantly less inflammation than those retrofilled; retreated cases had more periapical inflammation than teeth without previous root canal filling; medium sized bone cavities had more periapical inflammation; maxillary lateral incisors were mor frequently associated with scar formation than maxillary canines and premolars; perforation of lingual cortical bone was associated with a later occurrence of scar tissue; scar tissue was found more often after operation on cysts; and the age group from 20–40 years often showed scar tissue at final follow-up times.

In a radiographic and clinical examination of 93 apicoectomized roots over a period of 1–6 years, Altonen & Mattila (147) found over 80% with complete healing, 6% uncertain healing, and 13% complete failure. No difference in healing was noted between cases with cysts compared with those with granulomas. Their findings also indicated that placement of a root-end filling material improved the success rate of existing orthograde filling materials. In addition, they found that the presence of multiple periapical lesions adversely affected the prognosis of teeth requiring surgical endodontic therapy and removal of one half of the root led to more complete healing (89%) than removal of apical $^{1}/_{3}$ of the roots.

Finne et al. (148) conducted a 3-year postoperative clinical evaluation of patients who had Cavit (102 cases) and amalgam (116 cases) as root-end filling materials, and concluded that amalgam was significantly better than Cavit. They made the statement that retreatment of incomplete root canal fillings is not necessary when amalgam is used as a retrofilling because of its superior seal. Twenty-five teeth root-end filled with Cavit demonstrated a considerable amount of dissolution of this material.

Tay et al. (149) examined the relationship between the size of periradicular lesions and the success or failure of apicoectomy in 86 cases. Their findings indicated that large lesions (>12 mm in diameter) healed as completely as the small ones, and removal of cysts increased the chances for complete healijg.

Hirsch et al. (150) examined the influence of clinical factors in the healing of 572 cases following periapical surgery up to 3 years. Factors found to be important for prognosis included: extent of bone destruction, quality of root canal filling, age of patient, and presence of marginal buccal bone. Cases with smaller lesions, better root canal filling, and intact buccal cortical plates in older patients healed better than their counterparts.

In a clinial examination, Goel et al. (151) replanted 40 mandibular molars and used ZOE-

based cements, amalgam, Durelon, or gold foil as root-end filling materials. Their clinical assessment (biting force) up to 6 months showed gold foil to be the best material, followed by amalgam, Durelon, and ZOE-based cements. They attributed the low success rate in teeth with ZOE/based cements as root-end filling material to the presence of eugenol in these compounds.

Mikkonen et al. (152) studied clinically and radiographically the success of apicoectomy in 174 teeth root filled with chloropercha and gutta-percha with and without amalgam. Their results showed location of teeth in the jaws, sex, age, and orthograde or retrograde methods had no significant influence on success rate. Lack of periradicular lesions, root resorption, and presence of good-quality root canal fillings in teeth requiring periradicular surgery significantly increased the success rate.

Reit and Hirsch (153) performed retrograde root canal treatment using Hedstrom files and sodium hypochlorite to clean the canals and chloroform-softened gutta-percha technique to obturate 35 teeth whose root canals contained posts. Their clinical and radiographic examination performed "every other year" showed evidence of 71% successful healing of these cases.

In a clinical retrospective study, Dorn & Gartner (154) examined the success rate of 488 cases whose root-end cavities had been filled with SuperEBA, IRM, and zinc-free high-copper amalgam for a minimum of 6 months to 10 yeras. Their clinical and radiographic examinations showed that both SuperEBA and IRM had significantly higher success rates than amalgam. The success rates were 75% for amalgam, 91% for IRM, and 95% for SuperEBA.

Grung et al. (155) assessed radiographic healing of 477 teeth treated by endodontic surgery for a period of 1–8 years and reported no correlation between placement of root-end filling materials and healing. They found that 28% of the cases treated with retrograde fillings failed, compared with 4% in cases with orthograde root canal fillings. In their cases, apical curettage was performed if the root canal could be completely prepared and adequately obturated; otherwise apicoectomy was performed. This might have caused the difference in the result. Furthermore, they found that there was a marked correlation between the presence of a larger periapical rarefaction or perforation of lingual and buccal cortical plates and the occurrence of incomplete healing in the maxillary lateral incisors.

Lustmann et al. (156) examined the relation of various operative factors to the treatment results of apical surgery in 103 premolar or molar teeth. They reported a significantly higher success rate in roots obturated ≤2 mm of the apex than in roots obturated to or beyond the apex. In addition they found roots with posts had significantly more failures than those without posts. They attributed the failures in these roots to the presence of fracture before periapical surgery, or to that of a retrofilling material contacting the post and formation of corrosin products. Using the data from the above study, Friedman et al. (157) investigated the long-term prognosis of surgical cases in premolars and molars for a period of 6 months to 8 years. Their clinical and radiographic findings showed that only 44.1% of the roots were successful, and the rest were either unsuccessful (33.1%) or doubtful (22.8%). Furthermore, their results show that well-obturated canals have significantly higher success rates than poorly obturated canals.

Rapp et al. (158) studied the effects of various factors on 428 surgically treated endodontic cases and found that patients over 60 years of age had the highest percentage of complete healing. Their explanation was that canals in these patients are smaller and are easier to seal during surgery. Presence or absence of retrofilling had no significant effect on healing. In addition, they reported no significant difference between healing when different root-end filling materials were used. They also reported that significantly better healing was seen in teeth that were permanently restored following surgery.

Waikakul & Punwutikorn (159) compared healing in 66 teeth of patients who had either gold leaf or amalgam as root-end filling materials. A follow/up of 6 to 24 months showed no significant difference between the two groups. The authors recommended gold foil as an alternative to amalgam because of the following characteristics: its ease of sterilization, fewer or no residual particles, no setting time, cohesiveness, and its antibacterial effect.

Frank et al. (160) evaluated the long-term (10-years) success of 104 teeth with amalgam as a root-end filling material. They listed a case as a failure if there was a root-end radiolucency, even when a previous radiograph indicated that healing had occurred. These investigators reported that only 57% of cases retrofilled with amalgam were successful after a 10-year observation period and the rest failed in the same time period.

In a clinical and radiographic examination of 103 teeth whose root-end cavities had been filled with amalgam of EBA, Pantschev et al. (161) found 52% and 57% success rates for amalgam and EBA samples respectively 3 years after surgery. The percentage of teeth classified as uncertain was

23% for EBA and 19% for amalgam. No significant differences were noted between the success rates of the two treatment groups.

Jesslén et al. (162) evaluated healing of root-end filled teeth with amalgam or glass ionomer cement clinically and radiographically and reported success rates of 90% at 1 year and 85% at 5 years in both groups. The authors concluded that presence of moisture during periapical surgery did not affect healing adversely and recommended the glass ionomer as an alternative to amalgam as a root-end filling material.

Examination of clinical studies using various root-end filling materials shows that there are many variables in these investigations. The main variables include: the number of cases, follow-up observation periods, materials tested, different procedures and techniques used during nonsurgical and surgical endodontic treatments, and lack of standardization of evaluation criteria for qualitative results obtained in these studies. Because of the presence of these variables in these studies, they are difficult to compare with one another. Except for studies by Finn et al. (148) and Grung et al. (155), the rest are retrospective studies.

Standardization of clinical parameters such as cleaning and shaping of the root canal system, obturation of the root canals, root-end preparations, taking radiographs, and post operative time intervals are easier to achieve in prospective studies compared with the retrospective ones. In addition, the information obtained in retrospective studies is restricted to available information. More well-controlled multi-centre prospective studies with standardized clinical procedures along with large sample sizes and appropriate statistical analysis are needed to investigate and compare the effectivenss of various root-end filling materials.

Based on the review of the literature it appears to date that exising root-end filling materials do not possess "ideal" characteristics. Recently an experimental material, Mineral Trioxide Aggregate (MTA), has been investigated in a series of tests: *in vitro* dye leakage without and with blood contamination, *in vitro* bacterial leakage, SEM examination of replicas for marginal adaptation, setting time, compressive strength, solubility, antibacterial properties, cytotoxicity, implantation in bone, and an usage test in root-ends in dogs. Existing materials were used for comparison (163–173).

The sealing ability of MTA was superior to that of amalgam or SuperEBA in both dye and bacterial leakage methods, and was not adversely affected by blood contamination (163–165). The marginal adaptation of MTA was better than that of amalgam, IRM or SuperEBA (166). The setting time of MTA was found to be <3 hours, which is much longer than amalgam or IRM. Compressive strength and solubility of MTA were similar to IRM and SuperEBA respectively (167).

The antibacterial effects of MTA and three existing materials were investigated on facultative and strictly anaerobic bacteria; none were found to be completely antibacterial (168). The cytotoxicity of MTA was investigated by two methods, agar overlay and radiochromium release. The MTA was ranked less cytotoxic than IRM or SuperEBA, but more cytotoxic than amalgam in the agar overlay method. It was found to be less cytotoxic than amalgam, IRM or SuperEBA when the radiochromium release method was used (169). When the Ames test was used to determine the mutageniticty of root-end filling materials, MTA and commonly used root-end filling material proved to be non-mutagenic (170). With implantation of materials in guinea pig mandibles, there was no observable difference between MTA and SuperEBA (171).

When root-end fillings of MTA or amalgam were placed in the premolar teeth of dogs and examined histologically at various postoperative periods up to 18 weeks, there was less inflammation around the root-ends filled with MTA, and there was evidence of healing of the surrounding tissues. In addition, with the longer-term teeth filled with MTA, new cementum was found on the surface of the material; this was not the case with amalgam (172). Similar periradicular tissue responses were noted when MTA was used as root-end filling material in the maxillary incisors of monkeys (173). These studies supported the further development of MTA for use as a root-end filling material in man.

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