On the causes of persistent apical periodontitis: a review

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Abstract


Apical periodontitis is a chronic inflammatory disorder of periradicular tissues caused by etiological agents of endodontic origin. Persistent apical periodontitis occurs when root canal treatment of apical periodontitis has not adequately eliminated intraradicular infection. Problems that lead to persistent apical periodontitis include: inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation, debridement and leaking temporary or permanent restorations. Even when the most stringent procedures are followed, apical periodontitis may still persist as asymptomatic radiolucencies, because of the complexity of the root canal system formed by the main and accessory canals, their ramifications and anastomoses where residual infection can persist. Further, there are extraradicular factors – located within the inflamed periapical tissue – that can interfere with post-treatment healing of apical periodontitis. The causes of apical periodontitis persisting after root canal treatment have not been well characterized. During the 1990s, a series of investigations have shown that there are six biological factors that lead to asymptomatic radiolucencies persisting after root canal treatment. These are: (i) intraradicular infection persisting in the complex apical root canal system; (ii) extraradicular infection, generally in the form of periapical actinomycosis; (iii) extruded root canal filling or other exogenous materials that cause a foreign body reaction; (iv) accumulation of endogenous cholesterol crystals that irritate periapical tissues; (v) true cystic lesions, and (vi) scar tissue healing of the lesion. This article provides a comprehensive overview of the causative factors of non-resolving periapical lesions that are seen as asymptomatic radiolucencies post-treatment.

Keywords: aetiology, endodontic failures, persistent apical radiolucency, non-healing apical periodontitis, refractory periapical lesions, persistent apical periodontitis.

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Introduction

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by persistent microbial infection within the root canal system of the affected tooth (Kakehashi et al. 1965, Sundqvist 1976). The infected and necrotic pulp offers a selective habitat for the organisms (Fabricius et al. 1982b). The microbes grow in sessile biofilms, aggregates, coaggregates, and also as planktonic cells suspended in the fluid phase of the canal (Nair 1987). A biofilm (Costerton et al. 2003) is a community of microorganisms embedded in an exopolysaccharide matrix that adheres onto a moist surface whereas planktonic organisms are free-floating single microbial cells in an aqueous environment.
Microorganisms protected in biofilms are greater than one thousand times more resistant to biocides as the same organisms in planktonic form (Wilson 1996, Costerton & Stewart 2000).

There is consensus that apical periodontitis persisting after root canal treatment presents a more complex aetiological and therapeutic situation than apical periodontitis affecting teeth that have not undergone endodontic treatment. The aetiological spectrum and treatment options of persistent apical periodontitis are broader than those of teeth that have not undergone previous root canal treatment. Further, the process of decision-making regarding the management of persistent apical periodontitis is more complex and less uniform among clinicians than in the management of apical periodontitis affecting non-treated teeth (Friedman 2003). For optimum clinical management of the disease a clear understanding of the aetiology and pathogenesis of the disease is essential. Therefore, the purpose of this communication is to provide a comprehensive overview of the causes and maintenance of persistent apical periodontitis that is radiographically visualized as periapical radioluencies which are often asymptomatic.

Intraradicular microorganisms being the essential aetiological agents of apical periodontitis (Kakehashi et al. 1965, Sundqvist 1976), the treatment of the disease consists of eradicating the root canal microbes or substantially reducing the microbial load and preventing re-infection by root canal filling (Nair et al. 2005). When the treatment is done properly, healing of the periapical lesion usually occurs with hard tissue regeneration, that is characterized by reduction of the radiolucency on follow-up radiographs (Strindberg 1956, Grahné & Hansson 1961, Seltzer et al. 1963, Storms 1969, Molven 1976, Kerekes & Tronstad 1979, Molven & Halse 1988, Sjögren et al. 1990, 1997, Sundqvist et al. 1998). Nevertheless, a complete healing of calcified tissues or reduction of the apical radiolucency does not occur in all root canal-treated teeth. Such cases of non-resolving periapical radioluencies are also referred to as endodontic failures. Periapical radioluencies persist when treatment procedures have not reached a satisfactory standard for the control and elimination of infection. Inadequate aseptic control, poor access cavity design, missed canals, insufficient instrumentation, and leaking temporary or permanent restorations are common problems that may lead to persistent apical periodontitis (Sundqvist & Figdor 1998). Even when the most careful clinical procedures are followed, a proportion of lesions may persist radiographically, because of the anatomical complexity of the root canal system (Hess 1921, Perrini & Castagnola 1998) with regions that cannot be debrided and obturated with existing instruments, materials and techniques (Nair et al. 2005). In addition, there are factors located beyond the root canal system, within the inflamed periapical tissue, that can interfere with post-treatment healing of the lesion (Nair & Schroeder 1984, Sjögren et al. 1988, Figdor et al. 1992, Nair et al. 1999, Nair 2003a,b).

Microbial causes

Intraradicular infection

Microscopical examination of periapical tissues removed by surgery has long been a method to detect potential causative agents of persistent apical periodontitis. Early investigations (Seltzer et al. 1967, Andreasen & Rud 1972, Block et al. 1976, Langeland et al. 1977, Lin et al. 1991) of apical biopsies had several limitations such as the use of unsuitable specimens, inappropriate methodology and criteria of analysis. Therefore, these studies did not yield relevant information about the reasons for apical periodontitis persisting as asymptomatic radioluencies even after proper root canal treatment.

In one histological analysis (Seltzer et al. 1967) of persistent apical periodontitis, there was not even a mention of residual microbial infection of the root canal system as a potential cause of the lesions remaining unhealed. A histobacteriological study (Andreasen & Rud 1972) using step-serial sectioning and special bacterial stains, found bacteria in the root canals of 14% of the 66 specimens examined. Two other studies (Block et al. 1976, Langeland et al. 1977) analysed 230 and 35 periapical surgical specimens, respectively, by routine paraffin histology. Although bacteria were found in 10% and 15% of the respective biopsies, only in a single specimen in each study was intraradicular infection detected. In the remaining biopsies in which bacteria were found, the data also included those specimens in which bacteria were found as ‘contaminants on the surface of the tissue’. In yet another study (Lin et al. 1991) ‘bacteria and or debris’ was found in the root canals of 63% of the 86 endodontic surgical specimens, although it is obvious that ‘bacteria and debris’ cannot be equated as potential causative agents. The low reported incidence of intraradicular infections in these studies is primarily due to a methodological
inadequacy as microorganisms easily go undetected when the investigations are based on random paraffin sections alone. This has been convincingly demonstrated (Nair 1987, Nair et al. 1990a). Consequently, historic studies on post-treatment apical periodontitis did not consider residual intraradicular infection as an aetiological causative factor.

In order to identify the aetiological agents of asymptomatic persistent apical periodontitis by microscopy, the cases must be selected from teeth that have had the best possible root canal treatment and the radiographic lesions remain asymptomatic until surgical intervention. The specimens must be anatomically intact block-biopsies that include the apical portion of the roots and the inflamed soft tissue of the lesions. Such specimens should undergo meticulous investigation by serial or step-serial sections that are analysed using correlative light and transmission electron microscopy. A study that met these criteria and also included microbial monitoring before and during treatment (Nair et al. 1990a) revealed intraradicular microorganisms in six of the nine block biopsies (Fig. 1). The finding showed that the majority of root canal-treated teeth with asymptomatic apical periodontitis harboured persistent infection in the apical portion of the complex root canal system. However, the proportion of cases with persistent apical periodontitis having intraradicular infection is likely to be much higher in routine endodontic practice than the two-thirds of the nine cases reported (Nair et al. 1990a) for several reasons. At the light microscopic level it was possible to detect bacteria in only one of the six cases (Nair et al. 1990a). Microorganisms were found as a biofilm located within the small canals of apical ramifications (Fig. 1) in the root canal or in the space between the root fillings and canal wall. This demonstrates the inadequacy of conventional paraffin techniques to detect infections in apical biopsies.

The microbial status of apical root canal systems immediately after non-surgical root canal treatment was unknown. However, in a recent study (Nair et al. 2005), 14 of the 16 root filled mandibular molars contained residual infection in mesial roots when the treatment was completed in one-visit and included instrumentation, irrigation with NaOCl and filling. The infectious agents were mostly located in the uninstrumented recesses of the main canals, isthmuses communicating them and accessory canals. The microbes in such untouched locations existed primarily as biofilms that were not removed by instrumentation and irrigation with NaOCl. In view of the great anatomical complexity of the root canal system, particularly of molars (Hess 1921, Perrini & Castagnola 1998) and the ecological organization of the flora into protected sessile biofilms (Costerton & Stewart 2000, Costerton et al. 2003) composed of microbial cells embedded in a hydrated exopolysaccharide-complex in micro-colonies (Nair 1987), it is very unlikely that an absolutely microorganism-free canal-system can be achieved by any of the contemporary root canal preparation, cleaning and root filling procedures. Then, the question arises as to why a large number of apical lesions heal after non-surgical root canal treatment. Some periapical lesions heal even when infection persists in the canals at the time of root filling (Sjögren et al. 1997). Although this may imply that the organisms may not survive post-treatment, it is more likely that the microbes may be present in quantities and virulence that may be sub-critical to sustain the inflammation of the periapex (Nair et al. 2005). In some cases such residual microbes can delay or prevent periapical healing as was the case with six of the nine biopsies studied and reported (Nair et al. 1990a).

On the basis of cell wall ultrastructure only Gram-positive bacteria were found (Nair et al. 1990a) (Fig. 2), an observation fully in agreement with the results of purely microbiological investigations of root canals of previously root filled teeth with persisting periapical lesions. Of the six specimens that contained intraradicular infections, four had one or more morphologically distinct types of bacteria and two revealed yeasts (Fig. 3). The presence of intracanal fungi in root-treated teeth with apical periodontitis was also confirmed by microbiological techniques (Waltimo et al. 1995, Love et al. 1997, Love & Jenkinson 2002).

**Microbial flora of root canal-treated teeth**

The endodontic microbiology of treated teeth is less understood than that of untreated infected necrotic dental pulps. This has been suggested to be a consequence of searching for non-microbial causes of a purely technical nature for lesions persistent after root canal treatments (Sundqvist & Figdor 1998). Only a small number of species has been found in the root canals of teeth that have undergone proper endodontic treatment that, on follow-up, revealed persisting.
Figure 1 Light microscopic view of axial semithin sections through the surgically removed apical portion of the root with a persistent apical periodontitis. Note the adhesive biofilm (BF) in the root canal. Consecutive sections (a, b) reveal the emerging widened profile of an accessory canal (AC) that is clogged with the biofilm. The AC and the biofilm are magnified in (c) and (d) respectively. Magnifications: (a) ×75, (b) ×70, (c) ×110, (d) ×300. Adapted from Nair et al. (1990a).
asymptomatic periapical radiolucencies. The bacteria found in these cases are predominantly Gram-positive cocc, rods and filaments. By culture-based techniques, species belonging to the genera Actinomyces, Enterococcus and Propionibacterium (previously Arachnia) are frequently isolated and characterized from such root canals (Möller 1966, Sundqvist & Reuterving 1980, Happonen 1986, Sjögren et al. 1988,}

**Figure 2** Transmission electron microscopic view of the biofilm (BA upper inset) illustrated in Fig. 1. Morphologically the bacterial population appears to be composed of only Gram-positive, filamentous organisms (arrowhead in lower inset). Note the distinctive Gram-positive cell wall. The upper inset is a light microscopic view of the biofilm (BA). Magnifications: ×3400; insets: upper ×135, lower ×21 300. From Nair et al. (1990a). Printed with permission from Lippincott Williams & Wilkins®.
Figure 3 Fungi as a potential cause of non-healed apical periodontitis. (a) Low-power view of an axial section of a root-filled (RF) tooth with a persistent apical periodontitis (GR). The rectangular demarcated areas in (a) and (d) are magnified in (d) and (b), respectively. Note the two microbial clusters (arrowheads in b) further magnified in (c). The oval inset in (d) is a transmission electron microscopic view of the organisms. Note the electron-lucent cell wall (CW), nuclei (N) and budding forms (BU). Original magnifications: (a) ×35, (b) ×130, (c) ×330, (d) ×60, oval inset ×3400. Adapted from Nair et al. (1990a). Printed with permission from Lippincott Williams & Wilkins®.
Fukushima et al. 1990, Molander et al. 1998, Sundqvist et al. 1998, Hancock et al. 2001, Pinheiro et al. 2003). The presence of Enterococcus faecalis in cases of persistent apical periodontitis is of particular interest because it is rarely found in infected but untreated root canals (Sundqvist & Figdor 1998). Enterococcus faecalis is the most consistently reported organism from such former cases, with a prevalence ranging from 22% to 77% of cases analysed (Möller 1966, Molander et al. 1998, Sundqvist et al. 1998, Peciuliene et al. 2000, Hancock et al. 2001, Pinheiro et al. 2003, Siqueira & Rôças 2004, Fouad et al. 2005). The organism is resistant to most of the intracanal medicaments, and can tolerate (Byström et al. 1985) a pH up to 11.5, which may be one reason why this organism survives antimicrobial treatment with calcium hydroxide dressings. This resistance occurs probably by virtue of its ability to regulate internal pH with an efficient proton pump (Evans et al. 2002). Enterococcus faecalis can survive prolonged starvation (Figdor et al. 2003). It can grow as monoinfection in treated canals in the absence of synergistic support from other bacteria (Fabricius et al. 1982a). Therefore, E. faecalis is regarded as being a very recalcitrant microbe among the potential aetiological agents of persistent apical periodontitis. However, the presence of E. faecalis in cases of persistent apical periodontitis is not a universal observation. This is because one microbial culture (Cheung & Ho 2001) and a molecular based (Rolph et al. 2001) study, in which the presence of E. faecalis in such cases was investigated, failed to detect the organism. Further, the prevalence of E. faecalis was found to be 22% and 77%, respectively, of cases analysed by two molecular techniques (Siqueira & Rôças 2004, Fouad et al. 2005). In this context the long reported correlation between the prevalence of enterococci in root canals of primary and retreatment cases and that in other oral sites, such as gingival sulcus and tonsils, of the same patients, is worth noting (Engström 1964). The enterococci may be opportunistic organisms that populate exposed root filled canals from elsewhere in the mouth (Fouad et al. 2005). Therefore, in spite of the current focus of attention, it still remains to be shown, in controlled studies, that E. faecalis is the pathogen of significance in most cases of non-healing apical lesions after endodontic treatment (Nair 2004). Microbiological (Möller 1966, Waltimo et al. 1997) and correlative electron microscopic (Nair et al. 1990a) studies have shown the presence of yeasts (Fig. 3) in canals of root filled teeth with unresolved apical periodontitis. Candida albicans is the most frequently isolated fungus from root filled teeth with apical periodontitis (Molander et al. 1998, Sundqvist et al. 1998).

Extraradicular infection

Actinomycosis

Actinomycosis is a chronic, granulomatous, infectious disease in humans and animals caused by the genera Actinomyces and Propionibacterium (McGhee et al. 1982). The aetiological agent of bovine actinomycosis, Actinomyces bovis, was the first species to be identified (Harz 1879). The disease in cattle, known as ‘lumpy jaw’ or ‘big head disease’, is characterized by extensive bone rarefaction, swelling of the jaw, suppuration and fistulation. The causative agents were described as non-acid fast, non-motile, Gram-positive organisms revealing characteristic branching filaments that end in clubs or hyphae. Because of the morphological appearance these organisms were considered fungi and the taxonomy of Actinomyces remained controversial for more than a century. The intertwining filamentous colonies are often called ‘sulphur granules’ because of their appearance as yellow specks in exudates. On careful crushing, the tiny clumps of branching microorganisms with radiating filaments in pus, give a ‘starburst appearance’ which prompted Harz (1879) to coin the name Actinomyces or ‘ray fungus’. Four years later Actinomyces israelii was isolated from humans in pure culture, characterized and its pathogenicity in animals demonstrated (Wolff & Israel 1891). Many researchers, nevertheless, considered the human and bovine isolates as identical. However, A. bovis and A. israelii are now classified as two distinct bacterial species and in natural infections the former is restricted to animals and the latter to humans.

Human actinomycosis is clinically divided into cervicofacial, thoracic and abdominal forms. About 60% of the cases occur in the cervicofacial region, 20% in the abdomen and 15% in the thorax (Kapsimalis & Garrington 1968, Oppenheimer et al. 1978). The most common species isolated from humans is A. israelii (Wolff & Israel 1891), which is followed by Propionibacterium propionicum (Buchanan & Pine 1962), Actinomyces naeslundii (Thompson & Lovestedt 1951), Actinomyces viscosus (Howell et al. 1965) and Actinomyces odontolyticus (Batty 1958) in descending order.

Periapical actinomycosis (Fig. 4) is a cervicofacial form of actinomycosis. The endodontic infections are generally a sequel to caries. Actinomyces israelii is a commensal of the oral cavity and can be isolated from tonsils, dental plaque, periodontal pockets and carious
lesions (Sundqvist & Reutervng 1980). Most of the publications on periapical actinomycosis are case reports and have been reviewed (Browne & O'Riordan 1966, Samanta et al. 1975, Weir & Buck 1982, Martin & Harrison 1984, Nair & Schroeder 1984, Sakellariou 1996). Although periapical actinomycosis is considered

Figure 4 An actinomyces-infected periapical pocket cyst affecting a human maxillary first premolar (radiographic inset). The cyst is lined with ciliated columnar (CEP) and stratified squamous (SEP) epithelia. The rectangular block in (a) is magnified in (c). The typical 'ray-fungus' type of actinomycotic colony (AC in b) is a magnification of the one demarcated in (c). Note the two black arrow-headed, distinct actinomycotic colonies within the lumen (LU). Original magnifications: (a) x20, (b) x60, (c) x210. From P.N.R. Nair et al. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 94: 485–93, 2002.
to be rare (Nair & Schroeder 1984), it may not be so infrequent (Monteleone 1963, Hylton et al. 1970, Sakellariou 1996). The data on the frequency of periapical actinomycosis among apical periodontitis lesions are scarce. A microbiological control study revealed actinomycotic involvement in two of the 79 endodontically treated cases (Byström et al. 1987). A histological analysis showed the presence of characteristic actinomycotic colonies (Fig. 5) in two of the 45 investigated lesions (Nair & Schroeder 1984). An identification and aetiological association of the species involved can be established only through laboratory culturing (Sundqvist & Reuterving 1980) of the organisms, molecular techniques and by experimental induction of the lesion in susceptible animals (Fígodo et al. 1992). However, the strict growth requirements of \textit{A. israelii} make isolation in pure culture difficult. A histopathological diagnosis has generally been reached on the basis of demonstration of typical colonies (Nair & Schroeder 1984) and by specific immunohistochemical staining of such colonies (Sundqvist & Reuterving 1980, Happonen et al. 1985). Today, an unequivocal identification of the organism can be achieved by molecular methods. The characteristic light microscopic feature of an actinomycotic colony is the presence of an intensely dark staining, Gram and PAS positive, core with radiating peripheral filaments (Fig. 5) that gives the typical ‘star burst’ or ‘ray fungus’ appearance. Ultrastructurally (Nair & Schroeder 1984, Fígodo et al. 1992), the centre of the colony consists of a very dense aggregation of branching filamentous organisms held together by an extracellular matrix (Fig. 5). Several layers of PMN usually surround an actinomycotic colony.

Because of the ability of the actinomycotic organisms to establish extraradicularly, they can perpetuate the inflammation at the periapex even after proper root canal treatment. Therefore, periapical actinomycosis is important in endodontics (Sundqvist & Reuterving 1980, Nair & Schroeder 1984, Happonen et al. 1985, Happonen 1986, Sjögren et al. 1988, Nair et al. 1999). \textit{Actinomyces israelii} and \textit{P. propionicum} are consistently isolated and characterized from the periapical tissue of teeth, which did not respond to proper non-surgical endodontic treatment (Happonen 1986, Sjögren et al. 1988). A strain of \textit{A. israelii}, isolated from a case of failed endodontic treatment and grown in pure culture, was inoculated into subcutaneously implanted tissue cages in experimental animals. Typical actinomycotic colonies were formed within the experimental host tissue. This would implicate \textit{A. israelii} as a potential aetiological factor of persistent apical periodontitis following root canal treatment. \textit{Actinomyces} have been shown to posses a hydrophobic cell surface property. Gram-positive cell wall surrounded by a fuzzy outer coat through which fimbiae-like structures protrude (Fígodo & Davies 1997). These may help the cells to aggregate into cohesive colonies (Fígodo et al. 1992). The properties that enable these bacteria to establish in the periapical tissues are not fully understood, but appear to involve the ability to build cohesive colonies that enables them to escape host defence systems (Fígodo et al. 1992). \textit{Propionibacterium propionicum} is known to be pathogenic and associated with actinomycotic infections. But the mechanism of pathogenicity of the organism has not yet been explained.

Other extraradicular microbes
Apical periodontitis has long been considered to be a dynamic defence enclosure against unrestrained invasion of microorganisms into periradicular tissues (Kronfeld 1939, Nair 1997). It is, therefore, conceivable that microorganisms generally invade extraradicular tissues during expanding and exacerbating phases of the disease process. Based on classical histology (Harndt 1926) there has been a consensus of opinion that ‘solid granuloma’ may not harbour infectious agents within the inflamed periapical tissue, but microorganisms are consistently present in the periapical tissue of cases with clinical signs of exacerbation, abscesses and draining sinus. This has been substantiated by more modern correlative light and transmission electron microscopic investigations (Nair 1987).

However, in the late 1980s, there was a resurgence of the concept of extraradicular microbes in apical periodontitis (Tronstad et al. 1987, 1990, Iwu et al. 1990, Wayman et al. 1992) with the controversial suggestion that extraradicular infections are the cause of many failed endodontic treatments; such cases would not be amenable to a non-surgical approach but would require apical surgery and/or systemic medications. Several species of bacteria have been reported to be present at extraradicular locations of lesions described as ‘asymptomatic periapical inflammatory lesions… refractory to endodontic treatment’ (Tronstad et al. 1987). However, five of the eight patients had ‘long-standing fistulae to the vestibule...’ (Tronstad et al. 1987), a clear sign of abscessed apical periodontitis draining by fistulation. Obviously the microbial samples were obtained from periapical abscesses that always contain microbes and not from asymptomatic periapical lesions persisting after proper
Figure 5 Periapical actinomycosis. Note the presence of an actinomycotic colony (AC) in the body of a human apical periodontitis lesion (GR) revealing typical ‘starburst’ appearance (inset in a). The transmission electron microscopic montage (b) shows the peripheral area of the colony with filamentous organisms surrounded by few layers of neutrophilic granulocytes (NG). D, dentine; ER, erythrocyes. Original magnifications: (a) ×70; inset ×250; (b) ×2200. Adapted from Nair & Schroeder (1984). Printed with permission from Lippincott Williams & Wilkins©.
endodontic treatment. Other publications also show serious deficiencies. In one (Iwu et al. 1990), the 16 periapical specimens studied were collected ‘during normal periapical curettage, apicectomy or [during the procedure of] retrograde filling’. Of the 58 specimens that were investigated in another (Wayman et al. 1992), ‘29 communicated with the oral cavity through vertical root fractures or fistulas’. Further, the specimens were obtained during routine surgery and were ‘submitted by seven practitioners’. An appropriate methodology is essential and in these studies (Tronstad et al. 1987, Iwu et al. 1990, Wayman et al. 1992) unsuitable cases were selected for investigation or the sampling was not performed with the utmost stringency needed to avoid bacterial contamination (Møller 1966).

Microbial contamination of periapical samples is generally believed to occur from the oral cavity and other extraneous sources. Even if such ‘extraneous contaminations’ are avoided, contamination of periapical tissue samples with microbes from the infected root canal remains a problem. This is because microorganisms generally live at the apical foramen (Fig. 6) of teeth with persistent apical periodontitis (Nair et al. 1990a, 1999) and also of those that have not undergone root canal treatment (Nair 1987). Here microbes can be easily dislodged during surgery and the sampling procedures. Tissue samples contaminated with intraradicular microbes may be reported positive for the presence of an extraradicular infection. This is probably the reason behind the repeated reporting of bacteria in the periapical tissue of asymptomatic persistent apical lesions by microbial culture (Abou-Rass & Bogen 1997, Sunde et al. 2002) and molecular techniques (Gatti et al. 2000, Sunde et al. 2000) in spite of using strict aseptic sampling procedures.

Although there is an understandable enthusiasm with molecular techniques, they seem less suitable to solve the problem of extraradicular infection. Apart from the unavoidable contamination of the samples with intraradicular microbes, the DNA-based molecular genetic analysis: (1) does not differentiate between viable and non-viable organisms, (2) does not distinguish between microbes and their structural elements in phagocytes from extracellular microorganisms in periapical tissues and (3) exaggerates the findings by PCR amplification.

In summary, extraradicular infections do occur in: (i) exacerbating apical periodontitis lesions (Nair 1987), (ii) periapical actinomycosis (Sundqvist & Reuterving 1980, Nair & Schroeder 1984, Happonen et al. 1985, Happonen 1986, Sjögren et al. 1988), (iii) association with pieces of infected root dentine that may be displaced into the periapex during root canal instrumentation (Holland et al. 1980, Yusuf 1982) or having been cut off from the rest of the root by massive apical resorption (Valderhaug 1974, Laux et al. 2000) and (iv) infected periapical cysts (Fig. 4), particularly in periapical pocket cysts with cavities open to the root canal (Nair 1987, Nair et al. 1996, 1999). These situations are quite compatible (Nair 1997, Bengenholtz & Spångberg 2004) with the long-standing and still valid concept that solid granuloma generally do not harbour microorganisms. Therefore, the main target of treatment of persistent apical periodontitis should be the microorganisms located within the complex apical root canal system.

**Extraradicular viruses**

A series of publications appeared recently (Sabeti et al. 2003a,b,c, Sabeti & Slots 2004) that report the presence of certain viruses in inflamed periapical tissues with the suggestion of an ‘etio-pathogenic relationship’ to apical periodontitis. The findings were reviewed in another publication even before some of the original works appeared in print (Slots et al. 2003). It is almost impossible to provide controls for such claims because the reported viruses are present in almost all humans in latent form from previous primary infections. The possibility that the periapical inflammatory process activates the viruses, existing in latent form, cannot be excluded.

**Non-microbial causes**

**Cystic apical periodontitis**

The question as to whether or not periapical cysts heal after non-surgical root canal treatment has been longstanding. Oral surgeons are of opinion that cysts do not heal and should be removed by surgery. Many endodontists, on the other hand, hold the view that majority of cysts heal after endodontic treatment. This conflict of opinion is probably an outcome of the reported high incidence of cysts among apical periodontitis and the reported high ‘success rate’ of root canal treatments. There have been several studies on the prevalence of radicular cysts among human apical periodontitis (Table 1). The recorded incidence of cysts among apical periodontitis lesions varies from 6% to 55%. Apical periodontitis cannot be differentially diagnosed into cystic and non-cystic lesions based on radiographs alone (Priebe et al. 1954, Baumann & Rossman 1956, ...
A correct histopathological diagnosis of periapical cysts is possible only through serial sectioning or step-serial sectioning of the lesions removed in toto. The vast discrepancy in the reported incidence of periapical cysts is probably due to the difference in the interpretation of the sections. Histopathological diagnosis based on random or limited number of serial sections, usually leads to the incorrect categorization of epithelialized lesions as radicular cysts. This was clearly shown in a study using meticulous serial sectioning (Nair et al. 1996) in which an overall 52% of the lesions (n = 256) were found to be epithelialized but only 15% were actually periapical cysts. In routine histopathological diagnosis, the structure of a radicular cyst in relation to the root canal of the affected tooth has not been taken into account. As apical biopsies

**Figure 6** Well-entrenched biofilm at the apical foramen of a tooth affected with apical periodontitis (GR). The apical delta in (a) is magnified in (b). The canal ramifications on the left and right in (b) are magnified in (c) and (d), respectively. Note the strategic location of the bacterial clusters (BA) at the apical foramina. The bacterial mass appears to be held back by a wall of neutrophilic granulocytes (NG). Obviously, any surgical and/or microbial sampling procedures of the periapical tissue would contaminate the sample with the intraradicular flora. EP, epithelium. Original magnifications: (a) ×20, (b) ×65, (c, d) ×350. (From P.N.R. Nair, Pathology of the periapex. In: Cohen S, Burns RC, eds. Pathways of the Pulp. St Louis, MO, USA, 2002; Reprinted with permission from Mosby®.)
Table 1 The incidence of radicular cysts among apical periodontitis lesions

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<th>Others (%)</th>
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The therapeutic significance of the structural difference between apical true cysts and pocket cysts should also be considered. The aim of root canal treatment is the elimination of infection from the root canal and the prevention of reinfection by root filling. Periapical pocket cysts, particularly the smaller ones, may heal after root canal therapy (Simon 1980). A true cyst is self-sustaining as the lesion is no longer dependent on the presence or absence of root canal infection (Simon 1980, Nair et al. 1996). Therefore, the true cysts, particularly the large ones, are less likely to be resolved by non-surgical root canal treatment. This has been reported in a long-term radiographic follow-up (Fig. 9) of a case and subsequent histological analysis of the surgical block-biopsy (Nair et al. 1993). It can be argued that the prevalence of cysts in persistent apical periodontitis should be substantially higher than that in primary apical periodontitis. However, this remains to be clarified by research based on a statistically reliable number of specimens. Limited investigations (Nair et al. 1990a, 1993, 1999) on 16 histologically reliable block biopsies of persistent apical periodontitis revealed two cystic specimens (13%), which is higher than the 9% of true cysts observed in a large study (Nair et al. 1996) on mostly primary apical periodontitis lesions. The two distinct histological categories of periapical cysts and the low prevalence of cystic lesions among apical periodontitis would question the rationale of disproportionate application of apical surgery based on unfounded radiographic diagnosis of apical lesions as cysts, and the widely held belief that majority of cysts heal after non-surgical root canal treatment. Nevertheless, clinicians must recognize the fact that the cysts can sustain apical periodontitis post-treatment, and consider the option of apical surgery, particularly when previous attempts at non-surgical retreatment have not resulted in healing (Nair 2003b).

Cholesterol crystals

Although the presence of cholesterol crystals in apical periodontitis lesions has long been observed to be a common histopathological feature, its aetiological significance to failed root canal treatments has not yet been fully appreciated (Nair 1999). Cholesterol (Taylor 1988) is a steroid lipid that is present in abundance in all ‘membrane-rich’ animal cells. Excess blood level of cholesterol is suspected to play a role in atherosclerosis as a result of its deposition in the vascular walls (Yeagle 1988, 1991). Deposition of cholesterol crystals in tissues and organs can cause ailments such as otitis...
such deposits of cholesterol appear as narrow elongated clefts because the crystals dissolve in fat solvents used for the tissue processing and leave behind the spaces they occupied as clefts (Fig. 10). The incidence of cholesterol clefts in apical periodontitis varies from 18% to 44% of such lesions (Shear 1963, Browne 1971, ...
Figure 9 Longitudinal radiographs (a–d) of a periapically affected central maxillary incisor of a 37-year-old woman for a period of 4 years and 9 months. Note the large radiolucent asymptomatic lesion before (a), 44 months after root-filling (b), and immediately after periapical surgery (c). The periapical area shows distinct bone healing (d) after 1 year postoperatively. Histopathological examination of the surgical specimen by modern tissue processing and step-serial sectioning technique confirmed that the lesion was a true radicular cyst that also contained cholesterol clefts. Selected radiographs from Nair et al. (1993).
Figure 10 Cholesterol crystals and cystic condition of apical periodontitis as potential causes persistent apical periodontitis. Overview of a histological section (upper inset) of an asymptomatic apical radiolucent (Fig. 9) lesion that persisted after nonsurgical root canal treatment. Note the vast number of cholesterol clefts (CC) surrounded by giant cells (GC) of which a selected one with several nuclei (arrowheads) is magnified in the lower inset. D = dentine, CT = connective tissue, NT = necrotic tissue. Original magnifications: ×68; upper inset ×11; lower inset ×412. From Nair (1999). Printed with permission from Australian Endodontic Journal.
The crystals are believed to be formed from cholesterol released by: (i) disintegrating erythrocytes of stagnant blood vessels within the lesion (Browne 1971), (ii) lymphocytes, plasma cells and macrophages which die in great numbers and disintegrate in chronic periapical lesions, and (iii) the circulating plasma lipids (Shear 1963). All these sources may contribute to the concentration and crystallization of cholesterol in periapical area. Nevertheless, locally dying inflammatory cells may be the major source of cholesterol as a result of its release from disintegrating membranes of such cells in long-standing lesions (Seltzer 1988, Nair et al. 1993).

Cholesterol crystals are intensely sclerogenic (Abdulla et al. 1967, Bayliss 1976). They induce granulomatous lesions in dogs (Christianson 1939), mice (Spain et al. 1959, Adams et al. 1963, Abdulla et al. 1967, Adams & Morgan 1967, Bayliss 1976) and rabbits (Hirsch 1938, Spain et al. 1959, Spain & Aristizabal 1962). In an experimental study that specifically investigated the potential association of cholesterol crystals and non-resolving apical periodontitis lesions (Nair et al. 1998), pure cholesterol crystals were placed in Teflon cages that were implanted subcutaneous in guinea-pigs. The cage contents were retrieved after 2, 4 and 32 weeks of implantation and processed for light and electron microscopy. The cages revealed (Fig. 11) delicate soft connective tissue that grew in through perforations on the cage wall. The crystals were densely surrounded by numerous macrophages and multinucleate giant cells forming a well circumscribed area of tissue reaction. The cells, however, were unable to eliminate the crystals during an observation period of 8 months. The accumulation of macrophages and giant cells around cholesterol crystals suggests that the crystals induced a typical foreign-body reaction (Coleman et al. 1974, Nair et al. 1990b, Sjögren et al. 1995).

The macrophages and giant cells that surround cholesterol crystals are not only unable to degrade the crystalline cholesterol but are major sources of apical inflammatory and bone resorptive mediators. Bone resorbing activity of cholesterol-exposed macrophages due to enhanced expression of IL-1 has been experimentally shown (Sjögren et al. 2002). Accumulation of cholesterol crystals in apical periodontitis lesions (Fig. 10) can adversely affect post-treatment healing of the periapical tissues as has been shown in a long-term longitudinal follow-up of a case in which it was concluded that ‘the presence of vast numbers of cholesterol crystals … would be sufficient to sustain the lesion indefinitely’ (Nair et al. 1993). The evidence from the general literature reviewed (Nair 1999) is clearly in support of that assumption. Therefore, accumulation of cholesterol crystals in apical periodontitis lesions can prevent healing of periapical tissues after non-surgical root canal treatment, as such retreatment cannot remove the tissue irritating cholesterol crystals that exist outside the root canal system.

**Foreign bodies**

Foreign materials trapped in periapical tissue during and after endodontic treatment (Nair et al. 1990b, Koppang et al. 1992) can perpetuate apical periodontitis persisting after root canal treatment. Materials used in non-surgical root canal treatment (Nair et al. 1990b, Koppang et al. 1992) and certain food particles (Simon et al. 1982) can reach the periapex, induce a foreign body reaction that appears radiolucent and remain asymptomatic for several years (Nair et al. 1990b).

**Gutta-percha**

The most frequently used root canal filling material is gutta-percha in the form of cones. The widely held view that it is biocompatible and well tolerated by human tissues is inconsistent with the clinical observation that extruded gutta-percha is associated with delayed healing of the periapex (Strindberg 1956, Seltzer et al. 1963, Kerekes & Tronstad 1979, Nair et al. 1990b, Sjögren et al. 1990). Large pieces of gutta-percha are well encapsulated in collagenous capsules (Fig. 12), but fine particles of gutta-percha induce an intense, localized tissue response (Fig. 13), characterized by the presence of macrophages and giant cells (Sjögren et al. 1995). The congregation of macrophages around the fine particles of gutta-percha is important for the clinically observed impairment in the healing of apical periodontitis when teeth are root filled with excess material. Gutta-percha cones contaminated with tissue irritating materials can induce a foreign body reaction at the periapex. In an investigation on nine asymptomatic apical periodontitis lesions that were removed as surgical block biopsies and analysed by correlative light and electron microscopy, one biopsy revealed the involvement of contaminated gutta-percha (Nair et al. 1990b). The radiolucency grew in size but remained asymptomatic for a decade of post-treatment follow-up (Fig. 14). The lesion was characterized by the presence of vast numbers of multinucleate giant cells with...
Figure 11 Photomicrograph (a) of guinea-pig tissue reaction to aggregates of cholesterol crystals after an observation period of 32 weeks. The rectangular demarcated areas in (a), (b) and (c) are magnified in (b), (c) and (d), respectively. Note that rhomboid clefts left by cholesterol crystals (CC) surrounded by giant cells (GC) and numerous mononuclear cells (arrowheads in d). AT = adipose tissue, CT = connective tissue. Original magnifications: (a) ×10, (b) ×21, (c) ×82 and (d) ×220. From Nair (1999). Printed with permission from Australian Endodontic Journal.
birefringent inclusion bodies (Fig. 15). In transmission electron microscope the birefringent bodies were highly electron dense (Fig. 16). An X-ray microanalysis of the inclusion bodies using scanning transmission electron microscope (STEM) revealed the presence of magnesium and silicon (Fig. 17). These elements are presumably the remnants of a talc-contaminated gutta-percha that protruded into the periapex and had been resorbed during the follow-up period.

Other plant materials

Vegetable food particles, particularly leguminous seeds (pulses), and materials of plant origin that are used in endodontics can get lodged in the periapical tissue before and/or during the treatment procedures and prevent healing of the lesion. Oral pulse granuloma is a distinct histopathological entity (King 1978). The lesions are also referred to as the giant cell hyaline angiopathy (Dunlap & Barker 1977, King 1978), vegetable granuloma (Harrison & Martin 1986) and food-induced granuloma (Brown & Theaker 1987). Pulse granuloma has been reported in lungs (Head 1956), stomach walls and peritoneal cavities (Sherman & Moran 1954). Experimental lesions have been induced in animals by intratracheal, intraperitoneal and submucous introduction of leguminous seeds (Knoblich 1969, Talacko & Radden 1988b). Periapical pulse granuloma are associated with teeth damaged by caries and with the antecedence of endodontic treatment (Simon et al. 1982, Talacko & Radden 1988a). Pulse granuloma are characterized by the presence of intensely iodine and PAS positive hyaline rings or bodies surrounded by giant cells and inflammatory cells (Mincer et al. 1979, Simon et al. 1982, Talacko & Radden 1988a,b). Leguminous seeds are the most frequently involved vegetable food material in such granulomatous lesions. This indicates that certain components in pulses such as antigenic proteins and mitogenic phytohaemagglutinins may be involved in the pathological tissue response (Knoblich 1969). The pulse granuloma are clinically significant because particles of vegetable food materials can reach the periapical tissue via root canals of teeth exposed to the oral cavity by trauma, carious damage or by endodontic procedures (Simon et al. 1982).

Apical periodontitis developing against particles of predominantly cellulose-containing materials that are used in endodontic practice (White 1968, Koppang et al. 1987, 1989, Sedgley & Messer 1993) has been denoted as cellulose granuloma. The cellulose in plant materials is a granuloma-inducing agent (Knoblich...
Figure 13 Disintegrated gutta-percha as potential cause of persistent apical periodontitis. As clusters of fine particles (a) they induce intense circumscribed tissue reaction (TR) around. Note that the fine particles of gutta-percha (*in c, GP in d) are surrounded by numerous mononuclear cells (MNC). Original magnifications: (a) ×20, (b) ×80, (c) ×200, (d) ×750. From Nair (2003b).
Endodontic paper points (Fig. 18) are utilized for microbial sampling and drying of root canals. Sterile and medicated cotton wool has been used as an apical seal. Particles of these materials can dislodge or get pushed into the periapical tissue (White 1968) so as to induce a foreign body reaction at the periapex. The resultant clinical situation may be a ‘prolonged, extremely troublesome and disconcerted course of events’ (White 1968). Presence of cellulose fibres in periapical biopsies with a history of previous endodontic treatment has been reported (Koppang et al. 1987, 1989, Sedgley & Messer 1993). The endodontic paper points and cotton wool consists of cellulose that cannot be degraded by human body cells. They remain in tissues for long periods of time (Sedgley & Messer 1993) and induce a foreign body reaction around them. The particles, in polarized light, are birefringent due to the regular structural arrangement of the molecules within cellulose (Koppang et al. 1989). Infected paper points can protrude through the apical foramen (Fig. 18) and allow a biofilm to grow around it. This will sustain and even intensify the apical periodontitis after root canal treatment eventually leading to a failure of treatment.

Other foreign materials

They include amalgam, endodontic sealants and calcium salts derived from periapically extruded Ca(OH)₂.

In a histological and X-ray microanalytical investigation of 29 apical biopsies 31% of the specimens were found to contain materials compatible with amalgam and endodontic sealer components (Koppang et al. 1992).

Scar tissue healing

There is evidence (Penick 1961, Bhaskar 1966, Seltzer et al. 1967, Nair et al. 1999) that unresolved periapical radiolucencies may occasionally be due to healing of
Figure 15 Talc-contaminated gutta-percha as a potential cause of non-healing apical periodontitis. Note the apical periodontitis (AP) characterized by foreign-body giant cell reaction to gutta-percha cones contaminated with talc (a). The same field when viewed in polarized lights (b). Note the birefringent bodies distributed throughout the lesion (b). The apical foramen is magnified in (c) and the dark arrow-headed cells in (c) are further enlarged in (d). Note the birefringence (BB) emerging from slit-like inclusion bodies in multinucleated (N) giant cells. B, bone; D, dentine. Magnifications: (a, b) ×25; (c) ×66; (d) ×370. From P.N.R. Nair, Pathology of apical periodontitis. In: Ørstavik D, Pitt Ford TR, eds. Essential Endodontology. Oxford, 1998.
the lesion by scar tissue (Fig. 19) that may be misdiagnosed as a radiographic sign of failed endodontic treatment. Little is known about the tissue dynamics of periapical healing after non-surgical root canal treatment and periapical surgery. However, certain deductions can be made from the data available on normal healing and guided regeneration of the marginal periodontium. Various tissue cells participate in the healing process. The pattern of healing depends on several factors, two of which are decisive. They are the regeneration potential and the speed with which the tissue cells bordering the defect react (Karring et al. 1980, 1993, Nyman et al. 1982, Schroeder 1986). A periapical scar probably develops because precursors of soft connective tissue colonize both the root tip and periapical tissue; this may occur before the appropriate cells, which have the potential to restore various structural components of the apical periodontium are able to do so (Nair et al. 1999).

**Figure 16** Low magnification transmission electron micrograph showing the profiles of several giant cells within the apical periodontitis shown in Figs. 14 & 15. Note the presence of many slit-like inclusion bodies (BB1 to BB6), which contain a highly electron-dense material. This material may remain intact within the inclusion body, may be pushed away from its original site (BB2) or may appear disintegrated (BB3 and BB4) by the tissue processing. Note the lines of artefacts AL, which are created by portions of the electron dense material having been carried away by the knife-edge, leaving tracts behind. Original magnification ×1880. From Nair et al. (1990b). Printed with permission from Lippincott Williams & Wilkins®.

**Conclusions**

This review of the literature leads to the conclusion that there are six biological factors that contribute to the
Figure 17 High magnification transmission electron micrograph (c) of the intact birefringent body labelled BB1 in Fig. 3. Note the distinct delimiting membrane around the birefringent body (BB). Energy-dispersive X-ray microanalysis of the electron dense material done in scanning-transmission electron microscope (STEM: done at the point where the two hairlines perpendicular to each other cross in the left inset) revealed the presence of silicon (Si), magnesium (Mg) and lead (Pb) in (a) whereas another site in the neighbouring cytoplasm of the same giant cell (right inset) does not show the presence of Si and Mg (b). Lead and uranium (U) are used for section contrasting and emission in copper (Cu) is from the section-supporting grid made of copper. Original magnification ×11 000; insets ×3300. From Nair et al. (1990b). Printed with permission from Lippincott Williams & Wilkins®.
**Figure 18** A massive paper-point granuloma affecting a root-canal-treated human tooth (a). The demarcated area in (b) is magnified in (c) and that in the same is further magnified in (d). Note the tip of the paper point (FB) projecting into the apical periodontitis lesion and the bacterial plaque (BP) adhering to the surface of the paper point. RT, root tip; EP, epithelium; PC, plant cell. Original magnifications: (a) ×20, (b) ×40, (c) ×60, (d) ×150. From P.N.R. Nair, Pathology of apical periodontitis. In: Ørstavik D, Pitt Ford TR, eds: Essential Endodontology. Oxford, 1998.
persistence of periapical radiolucency after root canal treatment. These are: (i) intraradicular infection persisting in the complex apical root canal system; (ii) extraradicular infection, generally in the form of periapical actinomycosis; (iii) extruded root canal filling or other exogenous materials that cause a foreign body reaction; (iv) accumulation of endogenous cholesterol crystals that irritate periapical tissues; (v) true cystic lesions, and (vi) scar tissue healing of the periapex. It must be emphasized that of all these factors, residual microbes in the apical portion of the root canal system is the major cause of apical periodontitis persisting post-treatment in both poorly and properly treated cases. Extraradicular actinomycosis, true cysts, foreign-body reaction and scar tissue healing are of rare occurrence. However, the presence of a suspected causative agent

Figure 19 Periapical scar (SC) of a root canal (RC)-treated tooth after 5-year follow-up and surgery. The rectangular demarcated areas in (b–d) are magnified in (c–e), respectively. The scar tissue reveals bundles of collagen fibres (CO), blood vessels (BV) and erythrocytes due to haemorrhage. Infiltrating inflammatory cells are notably absent. Original magnifications: (a) ×14, (b) ×35, (c) ×90, (d) ×340, (e) ×560. Adapted from Nair et al. (1999). Reprinted with permission from Elsevier.
does not imply an aetiological relationship of the agent to the development and/or maintenance of the disease. It is also necessary to differentiate between a mere presence and the ability of the agent to induce the disease or similar pathological changes in susceptible experimental animals. This is particularly important in infectious diseases in which the microbes have to be present within the body milieu. In apical periodontitis and periodontal diseases, the microbes are stationed in the necrotic pulp or periodontal pocket, which are outside the body milieu. Viable and metabolically active microbes present at those locations would release antigenic molecules that irritate periodontal tissues both at the apical and marginal sites to cause inflammation, irrespective of them living there with or without virulence and tissue invasiveness. Nevertheless, among the viruses (Sabeti et al. 2003a,b,c; Sabeti & Slots 2004) and various species of other microorganisms that have been reported to be associated with persistent apical periodontitis (Molander et al. 1998, Sundqvist et al. 1998, Peciuliene et al. 2000, Hancock et al. 2001, Pinheiro et al. 2003, Siqueira & Roças 2004, Fouad et al. 2005) a positive experimental follow-up has been completed only with Actinomyces israelii (Figdor et al. 1992). The periapical disease-producing ability of other reported infectious agents, either singly or in combination, has yet to be demonstrated. Among the probable non-microbial agents that have been identified in association with persisting apical periodontitis, a positive tissue irritating ability has been experimentally demonstrated for fine particulate gutta-percha (Sjögren et al. 1995) and cholesterol crystals (Nair et al. 1998).

While intraradicular infection is the essential cause of apical periodontitis affecting teeth that have not undergone root canal treatment and probably the major cause of persistent apical periodontitis, the cherished goal of endodontic treatment has been to eliminate infectious agents or to substantially reduce the microbial load from the root canal and to prevent re-infection by root filling (Nair 2004, Nair et al. 2005). Periapical healing of some teeth occurs even when microbes are present in the canals at the time of filling (Sjögren et al. 1997). Microbes may be present in quantities and virulence that may be sub-critical to sustain the inflammation of the periapex, or that they remain in a location where they cannot communicate with the periapical tissues (Nair et al. 2005). The great anatomical complexity of the root canal system (Hess 1921, Perrini & Castagnola 1998) and the organization of the microbes into protected adhesive biofilms (Costerton & Stewart 2000, Costerton et al. 2003) composed of microbial cells embedded in a hydrated exopolysaccharide-complex in micro-colonies (Nair 1987, Nair et al. 2005) make it unlikely that a sterile canal-system can be achieved by contemporary technology in endodontics (Nair et al. 2005). As the primacy of residual intracanal infection in persistent apical periodontitis has been recognized (Nair et al. 1990a), the main target of treatment should be the microorganisms residing within the complex root canal system.

However, the tissue dynamics of apical periodontitis persisting from foreign body reaction and cystic condition are not dependent on the presence or absence of infectious agents/irritants in the root canal. The host defence cells that accumulate in sites of foreign body reaction and reside in cystic lesions are not only unable to resolve the pathology, but are also major sources of inflammatory and bone resorptive cytokines and other mediators. There is clinical and histological evidence that the presence of tissue-irritating foreign materials at the periapex, such as extruded root-filling materials, endodontic paper-points, particles of foods and accumulation of endogenous cholesterol crystals, adversely affect post-treatment healing of the periapical tissues. The overall prevalence of foreign body reaction at the periapex and cystic lesions among persistent apical periodontitis is currently unknown, but the occurrence of such cases may be very rare. Nevertheless, initiation of a foreign body reaction in periapical tissues by exogenous materials, endogenous cholesterol and cystic transformation of the lesion delay or prevent post-treatment healing. In well-treated teeth with adequate root fillings, a non-surgical retreatment is unlikely to resolve the problem, as it does not remove the offending objects, substances and pathology that exist beyond the root canal (Koppang et al. 1989, 1992, Nair et al. 1990a,b, 1993, 1999). Currently, a clinical differential diagnosis for the existence of these extraradicular causative agents of persistent apical periodontitis is not possible. Further, the great majority of persistent apical periodontitis are caused by residual infection in the complex apical root canal system (Hess 1921, Perrini & Castagnola 1998). It is not guaranteed that an orthograde root canal retreatment of an otherwise well-treated tooth can eradicate the residual intraradicular infection. Therefore, with cases of asymptomatic, persistent, periapical radiolucencies, clinicians should consider the necessity of removing the extraradicular factors through apical surgery (Kim 2002), in order to improve the long-term outcome of treatment. Apical
surgery provides an opportunity to remove the extra-radicular agents that sustain the apical radiolucency post-treatment and simultaneously allows a retrograde access to any potential infection in the apical portion of the root canal system that can also be removed or sealed within the canal by a retrograde filling of the apical root canal system (Nair 2003a).

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References
root canal after varied times of closure. Scandinavian Journal of Dental Research 90, 134–44.


