

Bacteria Present in the Root Canal after Obturation Are the Result of Primary Endodontic Failure, Regardless of the Radiographic Appearance of the Completed Root Canal Filling

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Abstract

Objective: The technical, radiographic appearance of a completed root canal filling is widely used by clinicians as a proxy for treatment success. This narrative review examines whether the presence of viable bacteria within the root canal system at the time of obturation constitutes the principal determinant of endodontic failure, independent of how the completed root filling appears on subsequent radiographs. **Methodology:** A narrative literature review was conducted using PubMed, Scopus and Google Scholar, combining terms relating to endodontic microbiology, persistent and secondary infection, obturation, radiographic outcome, and histobacteriologic and molecular detection methods. Landmark prospective clinical studies, histobacteriologic investigations, molecular microbiology studies and imaging-accuracy studies published between 1990 and 2026 were synthesised narratively. **Major findings:** Prospective clinical cohorts demonstrate markedly lower healing rates when canals yield positive bacterial cultures immediately before obturation, irrespective of the technical standard of the subsequent filling. Histobacteriologic studies of extracted and surgically retrieved root-filled teeth show viable bacteria and organised biofilm in the apical root canal system in the great majority of teeth with post-treatment apical periodontitis, even when the root filling appears dense and well-condensed radiographically. Culture-independent molecular techniques repeatedly detect bacterial DNA in canals that are bacteriologically "silent" by culture and radiographically unremarkable. Conventional periapical radiography under-detects periapical disease relative to histopathology and cone-beam computed tomography, meaning an apparently successful radiograph does not reliably indicate bacteriological cure. **Conclusion:** The weight of clinical, histobacteriologic and molecular evidence indicates that bacteria persisting in the canal at obturation are the primary determinant of endodontic failure. Radiographic appearance of the completed filling, being a surrogate marker of osseous host response rather than of canal sterility, cannot be relied upon in isolation to confirm elimination of infection.

Keywords: Endodontic failure; Root canal bacteria; Persistent infection; Apical periodontitis; Obturation; Enterococcus faecalis

1. Introduction

The biological objective of root canal treatment is not the placement of a radiographically acceptable filling but the elimination, or substantial reduction, of intraradicular microorganisms and the prevention of their re-entry into the root canal system [1]. Necrotic pulp tissue supports a polymicrobial, predominantly anaerobic flora that is the principal aetiological agent of apical periodontitis, and chemomechanical preparation followed by obturation is intended to remove this flora before the canal is sealed [1]. Clinically, however, success is most often judged retrospectively by periapical radiography: the presence of a

homogeneous, well-condensed root filling extending close to the radiographic apex, together with resolution or absence of a periapical radiolucency, is commonly equated with a successful outcome.

This equation is an oversimplification. A well-obtured, radiographically dense root filling describes only the technical outcome of the filling procedure; it says nothing directly about whether the canal was rendered free of viable microorganisms before it was sealed. Conversely, the presence or absence of a periapical radiolucency reflects the state of osseous remodelling at the periradicular bone-marrow interface, a process that is only indirectly, and often only after a considerable delay, coupled to the underlying

bacteriological status of the root canal space [2,3]. Landmark prospective outcome studies established more than two decades ago that a positive bacterial culture obtained immediately before obturation is one of the strongest independent predictors of subsequent failure, regardless of technical filling quality [4,5]. More recent histobacteriologic and molecular microbiology studies have reinforced and extended this observation, demonstrating that viable bacteria, frequently organised as biofilm, are recoverable from the apical portion of the root canal system in the great majority of root-filled teeth that develop or retain apical periodontitis, even when the root filling itself is technically satisfactory [6,7].

The purpose of this narrative review is to synthesise the clinical, histobacteriologic, microbiological and imaging literature bearing on a single proposition: that bacteria present in the canal at the time of obturation represent a primary endodontic failure in themselves, and that this failure exists independently of, and cannot be excluded by, the radiographic appearance of the completed root canal filling.

2. Materials and Methods

This is a narrative review; no original human or animal data were generated or analysed, and formal ethical approval and informed consent were therefore not applicable. A literature search was performed using PubMed/MEDLINE, Scopus and Google Scholar for articles published between January 1990 and September 2026. Search terms, used alone and in combination, included: "endodontic failure", "root canal bacteria", "persistent infection", "secondary endodontic infection", "apical periodontitis", "root canal obturation", "bacterial culture root filling", "histobacteriologic", "biofilm apical periodontitis", "Enterococcus faecalis", "16S rRNA root canal", "molecular detection endodontic", "cone-beam computed tomography periapical", and "radiographic outcome root canal treatment".

Prospective and retrospective clinical outcome studies correlating pre-obturation bacterial culture status with healing outcome, histobacteriologic studies of extracted or surgically retrieved root-filled teeth, culture-independent molecular microbiology studies of treated canals, and diagnostic-accuracy studies comparing periapical radiography, panoramic radiography and cone-beam computed tomography (CBCT) against histopathological or higher-resolution imaging reference standards were considered eligible. Landmark historical studies were included where they remain the primary evidential basis for widely cited clinical principles, together with more recent studies extending or refining those principles. Case reports, non-peer-reviewed sources, and articles not available in English were excluded. Because of the heterogeneity of study designs, sampling methods (culture versus molecular), and outcome definitions across the identified literature, a narrative rather than a quantitative meta-analytic synthesis was undertaken.

3. Results and Discussion

3.1 Bacterial status at obturation as a predictor of clinical outcome

The clearest clinical demonstration that residual bacteria at obturation drive failure comes from prospective cohort studies in which canals were sampled microbiologically immediately before filling and outcome was assessed radiographically at long-term follow-up. In the frequently cited study by Sjögren and colleagues, 55 single-rooted teeth with apical periodontitis were instrumented and irrigated with sodium hypochlorite, sampled bacteriologically, and obturated in a single visit; canals that were culture-negative immediately before obturation healed in approximately 94% of cases, whereas canals that remained culture-positive healed in only around two-thirds of cases at follow-up [4]. This relationship was independent of the technical standard of the subsequent obturation: teeth were filled to a comparable technical standard across both groups, yet outcome diverged sharply according to bacteriological status at the moment of filling. An earlier cohort of 356 patients followed for 8 to 10 years by the same research group similarly found that the pre-operative pulpal and periapical status was a dominant determinant of long-term healing, although the level and technical quality of the root filling also contributed [5].

Subsequent studies have reaffirmed this relationship using varied disinfection protocols and sampling strategies. Waltimo and colleagues confirmed that achievement of a negative intracanal culture before filling was strongly associated with periapical healing at one-year follow-up [8], and more recent reviews of endodontic microbiology continue to identify detectable bacteria in the canal at the time of filling as a consistent, reproducible risk factor for the subsequent development of post-treatment apical periodontitis [9]. Collectively, these prospective data establish the central premise of this review: it is the bacteriological status of the canal immediately before obturation, not the technical or radiographic appearance of the filling placed afterwards, that predicts whether the tooth will go on to fail.

3.2 Histobacteriologic evidence of bacterial persistence in root-filled teeth

Prospective culture studies necessarily sample only what conventional culture techniques can grow, and only immediately before filling. Histobacteriologic examination of teeth extracted or surgically retrieved after root canal treatment allows direct visualisation of what remains within the canal system after the filling has been placed, sometimes years earlier. In a retrospective histologic study of 24 root-filled teeth with persistent apical periodontitis, Ricucci and colleagues found bacteria within the apical portion of the root canal system in virtually every specimen examined, strongly implicating this residual intraradicular infection, rather than extraradicular or foreign-body phenomena, in the maintenance of post-treatment disease [3]. A larger histobacteriologic survey of 106 biopsy specimens from untreated and root-filled teeth with apical periodontitis, again by Ricucci and Siqueira, found bacteria in all but one specimen, with intraradicular biofilm arrangements present in the apical segment of 77% of

canals overall, and in 74% of previously treated canals specifically [6]. These biofilms were frequently observed not only in the main canal lumen but also covering the walls of lateral ramifications and isthmuses, anatomical regions that instrumentation and irrigation reach poorly and that are not resolvable on a two-dimensional radiograph regardless of how well the main canal appears to be filled [6,10].

Foundational work by Nair and colleagues using light and electron microscopy of root-filled, asymptomatic teeth with therapy-resistant periapical lesions similarly demonstrated intraradicular bacteria and fungi persisting within the canal system despite a technically completed root filling, implicating this persistent intraradicular flora, rather than any feature visible on the radiograph itself, as the driver of non-healing [11]. In his later review of the causes of persistent apical periodontitis, Nair emphasised that while extraradicular factors (true cysts, foreign-body reactions, extruded material, cholesterol crystals) can contribute to non-resolving lesions in a minority of cases, the dominant and most common cause of persistent post-treatment disease remains intraradicular infection that has survived treatment [2]. Because these bacteria are almost always confined within the canal space or its ramifications, ahead of and independent of the radiolucency visible on a two-dimensional image, their presence cannot be inferred, confirmed, or excluded from the radiographic appearance of the completed filling.

3.3 Discordance between radiographic appearance and true bacteriological/histological status

A central plank of the argument that radiographic appearance is an unreliable surrogate for bacteriological cure comes from studies directly comparing what a periapical radiograph shows against what histopathology or higher-resolution three-dimensional imaging reveals. Using extracted human jaw specimens with histopathologically confirmed apical periodontitis as the reference standard, Kanagasalingam and colleagues found that both conventional periapical radiography and CBCT under-detected disease relative to histology, with periapical radiography performing considerably worse [12]. Comparable diagnostic-accuracy work by Estrela and colleagues found that apical periodontitis was identified in only 17.6% of endodontically treated teeth using panoramic radiographs and 35.3% using periapical radiographs, compared with 63.3% of the same teeth using CBCT [13]. A recent meta-analysis summarised by Cotti and Schirru reported pooled sensitivity of only around 50% for periapical radiography in detecting apical periodontitis, compared with approximately 95% for CBCT, and estimated that roughly one-third of periapical lesions present are missed altogether on conventional periapical films [14]. Longitudinal follow-up work by Patel and colleagues, tracking root-filled teeth with both digital periapical radiography and CBCT over one year, likewise demonstrated that periapical radiography substantially under-represents the true extent and persistence of periapical pathosis compared with three-dimensional imaging [15].

These findings have a direct bearing on the present thesis. If a two-dimensional radiograph systematically fails to detect a

substantial proportion of the periradicular bone changes that histopathology confirms are present, then the absence of a visible radiolucency, or the presence of an apparently dense, well-condensed root filling with no obvious radiolucency, cannot be taken as reliable evidence that the canal was rendered free of bacteria. The radiograph is, at best, an indirect and delayed reflection of the periradicular host response to whatever irritants, bacterial or otherwise, remain within or have escaped the canal system; it was never designed to, and does not, directly image bacteria.

A further, related line of evidence comes from studies of the relative contribution of root-filling quality versus coronal restoration quality to periapical health. In their frequently cited study of 1,010 endodontically treated teeth, Ray and Trope found that teeth with a good coronal restoration had significantly less periapical inflammation than teeth with a poor coronal restoration, regardless of the technical quality of the root filling itself, and that the coronal restoration exerted a greater influence on periapical status than the technical quality of the root canal filling [16]. This finding has been replicated in more recent retrospective cohorts [17]. Its relevance here is that it further separates the technical, radiographic appearance of the obturation from the biological outcome: a radiographically "good" root filling beneath a leaking coronal restoration, or containing bacteria sealed in at the time of filling, can still be associated with periapical disease, demonstrating that radiographic filling quality alone is an incomplete and sometimes misleading proxy for the biological, bacteriological state of the tooth.

3.4 Microbiological mechanisms permitting bacterial survival despite an adequate obturation

Several microbiological features of the infected root canal system explain how bacteria can survive chemomechanical preparation and remain viable beneath a technically well-placed, radiographically satisfactory root filling. Bacteria within the root canal system are typically organised as structured, matrix-embedded biofilm communities rather than as free-floating planktonic cells, a configuration that confers substantially greater resistance to antimicrobial irrigants and intracanal medicaments than is seen with the same species grown in planktonic culture [6,10]. Anatomical complexity compounds this problem: instrumentation and irrigation cannot reliably reach isthmuses, fins, lateral and accessory canals, or the apical ramifications of the canal system, all of which can harbour biofilm that is left largely undisturbed by preparation and is subsequently entombed, rather than eliminated, by the obturation material [6,10].

Dentinal tubules represent a further reservoir. Peters and colleagues demonstrated experimentally that bacteria can penetrate into dentinal tubules and survive there for extended periods, sheltered from the direct antibacterial effect of irrigants and medicaments used in the canal lumen [18]. *Enterococcus faecalis*, the species most consistently associated with persistent and secondary endodontic infection, is particularly well adapted to this niche: its surface adhesins and lytic enzymes assist penetration and colonisation of dentine, and it can tolerate prolonged nutrient deprivation and

the highly alkaline environment created by calcium hydroxide dressings, surviving the buffering capacity of dentine as small numbers of dormant or slow-growing cells that evade both culture-based detection and conventional disinfection protocols [19,20]. A systematic review by Alghamdi and Shakir concluded that *E. faecalis* is disproportionately represented among cases of confirmed endodontic failure precisely because of this constellation of survival properties, rather than because it is a particularly common component of primary, untreated infections [7]. Because these surviving organisms are, by definition, walled off from the canal lumen at the moment the master cone is seated, their presence is not merely undetected by the completed radiograph; it is fundamentally outside what a radiograph is capable of representing.

3.5 Molecular evidence of bacteria undetected by culture in radiographically unremarkable canals

Conventional culture, the method used in most of the classical prospective outcome studies, itself under-detects bacterial presence because a substantial proportion of endodontic taxa are fastidious, slow-growing, or presently unculturable by routine laboratory methods [9]. Culture-independent molecular techniques, including polymerase chain reaction (PCR), denaturing gradient gel electrophoresis, checkerboard hybridisation and gene sequencing, have repeatedly identified bacterial DNA, and by extension bacterial presence, in root canals that had already been judged clinically and radiographically satisfactory. Rôças and colleagues used denaturing gradient gel electrophoresis to characterise bacterial communities associated with failed endodontic treatment and confirmed a diverse persisting flora in canals that had previously received what was, at the time, considered adequate treatment [21]. Fouad and colleagues used species-specific molecular detection to identify *Enterococcus* species in the canals of therapy-resistant cases at a substantially higher frequency than culture alone had suggested [22]. Sakamoto and colleagues, using molecular analysis of canals associated with confirmed treatment failure, likewise recovered a broader and more diverse bacterial community than culture-based sampling of the same specimens would have revealed [23], and Tennert and colleagues, comparing the bacterial composition of primary and persistent or secondary endodontic infections against clinical and radiographic findings, found that molecular methods detected organisms in cases that clinical and radiographic assessment alone had not flagged as unusual [24]. Reverse-capture checkerboard analysis of cryogenically ground apical root samples has similarly revealed an extensive and diverse apical microbiota in teeth with apical periodontitis that would not have been apparent from culture or from the radiographic picture alone [25].

The clinical significance of this body of work is that it closes a potential gap in the argument. It is not simply that radiographs fail to show bacteria because bacteria are microscopic; it is that even microbiological sampling by culture, the technique underpinning the landmark outcome studies described in section 3.1, itself misses a substantial

fraction of the organisms actually present. Molecular methods make clear that canals which appear entirely unremarkable, both radiographically and by conventional culture, can nonetheless harbour a persisting bacterial community. This reinforces, rather than weakens, the central proposition of this review: an apparently successful radiographic outcome offers no positive assurance of bacteriological cure, and the reverse inference, that a well-filled canal implies a sterile one, is not supported by the microbiological literature.

3.6 Clinical implications

Taken together, the clinical, histobacteriologic, imaging and molecular evidence reviewed above supports a reframing of how endodontic failure should be conceptualised. A radiographically dense, well-condensed root filling that extends to within an acceptable distance of the radiographic apex, in the absence of a visible periapical radiolucency, remains a reasonable and clinically useful indicator of technical procedural competence. It should not, however, be treated as direct or sufficient evidence that the canal was rendered free of viable bacteria before it was sealed. The primary failure, when it occurs, has already taken place at the moment infected, biofilm-organised tissue and dentine were sealed into the canal system; the radiograph, months or years later, is at best a delayed and imperfect window onto the downstream periradicular consequences of that earlier event, mediated by host immune and osseous remodelling responses that vary between individuals and can lag well behind, or in some cases never fully resolve, the underlying bacteriological reality [2,3,14].

Practically, this supports continued emphasis on maximising intracanal disinfection before obturation, through thorough chemomechanical preparation, the use of an appropriately concentrated sodium hypochlorite irrigation protocol with adequate volume and contact time, supplementary irrigant activation to improve penetration into fins, isthmuses and ramifications, and, where indicated, an interappointment antimicrobial dressing, rather than relying on the technical or radiographic standard of the completed filling as a proxy for adequate disinfection [1,4,8]. It also supports close attention to the quality and timeliness of the coronal restoration, given the demonstrated capacity of a leaking coronal seal to compromise periapical outcome independently of root filling quality [16,17]. Finally, it supports a degree of clinical humility regarding long-term radiographic "success": given the demonstrated tendency of two-dimensional radiography to under-detect periapical disease relative to histopathology and CBCT, an asymptomatic tooth with an unremarkable periapical radiograph should not be regarded as proof of a bacteriologically sterile canal, and where three-dimensional imaging is clinically indicated it may reveal a materially different picture of periradicular health than periapical radiography alone [13,14,15].

4. Conclusion

The literature reviewed here converges on a consistent conclusion: bacteria remaining viable within the root canal system at the time of obturation constitute a primary

endodontic failure in their own right, and this failure exists independently of the radiographic appearance of the completed root canal filling. Prospective clinical cohorts show that pre-obturation bacterial status, not filling technique, predicts long-term outcome. Histobacteriologic studies confirm that bacteria and organised biofilm are recoverable from the apical root canal system in the great majority of teeth with post-treatment apical periodontitis, frequently despite a technically satisfactory root filling. Molecular microbiology demonstrates that a substantial bacterial community can persist even where both culture and radiography appear unremarkable. Diagnostic-accuracy studies confirm that conventional periapical radiography under-detects periradicular disease relative to histopathology and CBCT, undermining its use as a stand-alone indicator of bacteriological cure. Clinicians should therefore treat thorough intracanal disinfection before obturation, rather than the subsequent radiographic appearance of the filling, as the primary determinant of, and the appropriate clinical focus for achieving, a successful endodontic outcome.

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Conflict of Interest

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