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Microbial analysis of root canal and periradicular lesion associated to teeth with endodontic failure

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ABSTRACT

The quantification of ten microorganisms at the root ends and in the surrounding periradicular lesions was performed. Thirty 3 mm samples root ends and 30 samples of the surrounding chronic periapical infection were collected during apical microsurgery. Samples were triturated, and the bacterial DNA was obtained. The bacterial quantification was performed by using the SYBR Green system. At least one microorganism was detected in all patients. In both the root end and periapical samples, *Fusobacterium nucleatum* (71.6%), *Dialister pneumosintes* (58.3%) and *Tannerella forsythia* (48.3%) were the most prevalent species. *Dialister pneumosintes* showed statistically significant values in the root end, and *F. nucleatum* was also significant in the apical periodontitis samples. A statistically significant association between *T. forsythia* and *Porphyromonas gingivalis* in the root ends was observed. Bacterial associations from 2 to 7 species were observed in most samples. Extra-radicular and/or intra-radicular infections were present in all teeth with failed endodontic treatment, and showed polymicrobial infection in most cases, with a predominance of *F. nucleatum*, *D. pneumosintes* and *T. forsythia*. When present, *Enterococcus faecalis* was never found to be the most prevalent species. The presence of a microbial diversity in post-treatment apical periodontitis confirms the polymicrobial and synergistic characteristic of this process. Our results show that the bacterial array associated with the 3 mm root ends and periradicular lesions in post-treatment apical periodontitis are complex and with a high inter-individual variability. These results might be useful to delineate treatment strategies for microbial elimination in apical periodontitis. Further studies are necessary to elucidate the role of these microorganisms in endodontic treatment failures.

1. Introduction

Endodontic therapy aims to eliminate infection from the inner root canal system and prevent re-infection by obturation [1]. However, several authors have recognized that one of the main causes of root canal treatment failure leading to post-treatment apical periodontitis is the presence of residual microorganisms after endodontic therapy (persistent infection). The reinfection of a previously disinfected root canal environment (secondary infection) can also lead to endodontic failure [2,3].

Over the years, the majority of authors have stated that the major cause of endodontic treatment failure is the presence of microorganisms within the root canal system [4,5]. Studies using molecular methods have shown contamination on the external root surface of treated teeth [6,7] and within soft-tissue lesions in the periapical region [8–10].

The endodontic therapy should treat the infected root canal as a complex system. The main canal includes a system of lateral canals, apical ramifications and an isthmus, all of which can be challenging to reach with endodontic therapy, as bacteria can spread and remain unaffected by treatment procedures in these areas [11]. The dental community is in agreement that the elimination of microorganisms from the root canal system is critical in preventing and treating apical periodontitis [12].

Traditionally, bacterial identification has been accomplished through biochemical methods, but these can be laborious, expensive, and time-consuming and have limitations in terms of the

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Table 1
Target microorganism and species-specific primers used in the bacterial quantification.

Microorganisms	Oligonucleotides (5′- 3′)	PCR conditions	Amplicon (bp)	References
Aggregatibacter actinomycetemcomitans	CCC ATC GCT GGT TGG TTA GGC ACG TAG GCG GAC C	1 Hold: 95 °C, 2 min 40 cycles: 95 °C, 45 s 60 °C, 2 min	696	Kuboniwa et al. [32]
Fusobacterium nucleatum	CTT AGG AAT GAG ACA GAG ATG TGA TGG TAA CAT ACG AAA GG	1 Hold: 95 °C, 2 min 40 cycles: 95 °C, 45 s 60 °C,1 min	140	Periasamy & Kolenbrander [34]
Porphyromonas gingivalis	ACC TTA CCC GGG ATT GAA ATG CAA CCA TGC AGC ACC TAC ATA GAA	1 Hold: 95 °C, 2 min 40 cycles: 95 °C, 45 s 60 °C,1 min	83	Kuboniwa et al. [32]
Porphyromonas endodontalis	GCT GCA GCT CAA CTG TAG TCT TG TCA GTG TCA GAC GGA GCC TAG TAC	1 Hold: 95 °C, 10 min 40 cycles: 95 °C, 15 s 60 °C, 1 min	110	Nonnenmacher et al. [33]
Prevotella intermedia	TCCACCGATGAATCTTTGGTC ATCCAACCTTCCCTCCACTC	1 Hold: 95 °C, 2 min 40 cycles: 95 °C, 45 s 60 °C,1 min	98	Kuboniwa et al. [32]
Prevotella nigrescens	CCG TTG AAA GAC GGC CTAA CCC ATC CCT TAC CGG RA	1 Hold: 95 °C, 10 min 40 cycles: 95 °C, 15 s 57 °C,1 min	82	Kuboniwa et al. [32]
Dialister pneumosintes	GAG GGG TTT GCG ACT GAT TA CCG TCA GAC TTT CGT CCA TT	1 Hold: 95 °C, 10 min 40 cycles: 95 °C, 15 s 55 °C,1 min	166	Nonnenmacher et al. [33]
Tannerella forsythia	AGC GAT GGT AGC AAT ACC TGT C TTC GCC GGG TTA TCC CTC	1 Hold: 95 °C, 10 min 40 cycles: 95 °C, 15 s 57 °C,1 min	88	Kuboniwa et al. [32]
Treponema denticola	CCGAATGTGCTCATTTACATAAAGGT GATACCCATCGTTGCCTTGGT	1 Hold: 95 °C, 10 min 40 cycles: 95 °C, 15 s 57 °C,1 min	122	Kuboniwa et al. [32]
Enterococcus faecalis	CGC TTC TTT CCT CCC GAGT GCC ATG CGG CAT AAA CTG	1 Hold: 95 °C, 10 min 40 cycles: 95 °C, 15 s 60 °C, 1 min	143	Williams et al. [35]
Universal primers 16S rDNA	AGA GTT TGA TCC TGG CTC AG GGC TAC CTT GTT ACG ACT T	1 Hold: 95 °C, 10 min 30 cycles: 95 °C, 30 s 58 °C, 30 s		Amano et al. [36]

Table 2
Bacterial prevalence in the root end and periradicular lesion samples.

Microorganisms	Root end		Periradicular lesion		Samples total	
	N°	%	N°	%	N°	%
Aggregatibacter actinomycetemcomitans	7	23.3	8	26.6	15	25
Fusobacterium nucleatum	22	73.3	21	70	43	71.6
Porphyromonas gingivalis	5	16.6	4	13.3	9	15
Porphyromonas endodontalis	3	10	3	10	6	10
Prevotella intermedia	4	13.3	5	16.6	9	15
Prevotella nigrescens	1	3.3	0	0	1	1.6
Dialister pneumosintes	22	73.3	13	43.3	35	58.3
Tannerella forsythia	16	53.3	13	43.3	29	48.3
Treponema denticola	4	13.3	6	20	10	16.6

microbiological diagnosis. Approximately 50% of oral bacteria are not cultivable; therefore, unknown bacteria are always present in such infections. Molecular analysis has revealed a more diverse array of bacteria associated with endodontic infections than culture methods alone [13].

The qualitative and quantitative polymerase chain reaction (PCR) has been used for bacterial detection from endodontic infections due to its great sensitivity [14–16]. The current findings, based on molecular methods, suggest that new candidates for endodontic pathogens may be responsible for post-treatment apical periodontitis and also suggest that it is a complex and polymicrobial disease, with a high level of interspecies variability [17]. Since the elimination of microorganisms from root canal is necessary for preventing the apical periodontitis, the detection of a specific microbiota involved in this process could collaborate with dentists to delineate a better treatment in cases of root canal failure or persistence of apical periodontitis. Thus, the aim of this study was to investigate and compare the presence and quantity of ten microorganisms from root ends and the associated periradicular tissues collected from cases of failed endodontic therapy.

2. Materials and methods

2.1. Patients

Thirty patients (17 female and 13 males) between the ages of 16 and 58 years old (mean 41 years) were selected. All patients had at least one tooth with a performed satisfactory endodontic treatment, between 1 and 15 years prior to enrollment (mean 4 years). The characteristic radiographic evidence of periradicular bone destruction of post-treatment apical periodontitis was observed in all selected asymptomatic first molar (anterior, posterior, inferior or superior). All treated teeth were coronally restored, and no evidence of root canal filling material exposure to the oral cavity was