Advances in microbiology and bacteriology

Navid Saberi presents an overview of microbiology and bacteriology in endodontic treatment

CPD Aims and objectives

This clinical article aims to present an overview of the many advances in microbiology and bacteriology, and which bacteria are safe to leave in the root canal after endodontic treatment.

Expected outcomes

Correctly answering the questions on page 34, worth one hour of verifiable CPD, will demonstrate that you understand the many advances in microbiology and bacteriology.

It has been almost 340 years since the birth of microbiology and the discovery of bacteria by Robert Hooke and Antonie Van Leeuwenhoek (Gest, 2004) and around 150 years since the dawn of modern bacteriology by the concurrent and rivalry-driven studies of Robert Koch and Louis Pasteur (Ullmann, 2007).

In the field of endodontology, WD Miller was the first to associate bacteria with pulpal disease in 1894. However, even prior to Miller's experiments, dental practitioners were aware of the important role of disinfection and antiseption in endodontic outcomes. In fact, in 1886, Dental Cosmos published a report titled *Disinfection and antiseption by heat*. According to the author, in this procedure 'a fine wire heated to redness and inserted into the canal to the apical foramen… the application repeated according to circumstances… is all that is necessary' and 'by his [Dr GO Rogers'] method complete disinfection and antiseption are secured' (Pomeroy, 1886).

In addition, devitalisation by means of arsenic trioxide and instrumentation with modified watch springs were commonplace (Grossman, 1982; 1987).

It is now well established that bacteria are essential for the development of pulpal and periradicular diseases (Kakehashi et al, 1965, Möller et al, 1981) and the presence of bacteria in the root canal space or periapical tissue will undermine the success of endodontic treatment (Sjögren et al, 1997; Byström et al, 1987).

Therefore, for a successful treatment, all root canals should be identified, thoroughly disinfected and rendered bacteria free prior to obturation. The aim of modern endodontic therapy is therefore to achieve this goal by mechanical instrumentation and biochemical irrigation of the root canal systems, which can be referred to as biomechanical cleansing of infected root canals.

However, despite being desirable to render the root canals sterile, it is logical and realistic to assume that achieving sterility may not be feasible in every infected root canal. In fact, sterility may not even be required for a successful outcome.

Actor (2012) reported that there are 20 times more bacterial cells in human bodies than eukaryotic cells. These communities live in either a symbiotic or mutualistic manner and benefit from the existence of one another without causing harm. Therefore, at least in theory, it can be assumed that some bacteria in inaccessible areas of the root canal systems may be left behind without compromising the overall outcome of the treatment.

In spite of many advances in microbiology and bacteriology, there still remain many unanswered questions. How can we detect these remaining bacteria? Which bacteria are safe to remain in the root canals? And, more importantly, how many bacteria is it safe to leave in the root canal after endodontic treatment?

The aim of this article is to provide answers to the above questions.

Bacterial identification

In order to determine the safe number of bacteria that may possibly be left in root canals after endodontic treatment, it is imperative for researchers to identify these microorganisms. However, most methods of bacterial detection have been associated with some drawbacks.

Culturing has been advocated as a rather simple means of bacterial identification. However, up until the late 1960s, culturing methods were unable to propagate anaerobic bacteria predictably. The routine use of anaerobic glove-box and sterilised anaerobic culture media has significantly enhanced accurate identification of these bacteria. Furthermore, standardised field decontamination protocols in the late 60s reduced the possibility of false positive results (Moore, 1966, Kantz and Henry, 1974, Ng et al, 2003).

Although cultivability of all bacteria was first reported in

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the 60s, it was not until the 90s that scientists fully recognised that not all bacteria were culturable or identifiable (Socransky et al, 1963; Hugenholtz and Pace, 1996). This meant previous bacteriological studies based on culturing alone probably only discovered half of the true taxa associated with endodontic infections (Paster et al, 2001). These uncultivable bacteria could indeed be the more important entities in the pathogenesis of periapical disease.

The development of polymerase chain reaction (PCR) in the late 80s and its use in endodontics by the early 90s radically changed the dogma that had been founded on a culture-based understanding of endodontic microbiology (Mullis and Faloona, 1987, Pollard et al, 1989, Spratt, Weightman and Wade, 1999).

PCR, however, is not without pitfalls. This highly sensitive method of nucleic acid amplification requires a strict decontamination protocol if false positive results are to be avoided (Ng et al, 2003). In addition, Hayden and colleagues (1991) found that PCR amplification methods inherently detect bacteria without specifying whether the microorganisms were dead or alive at the time of sampling. This predicament can be overcome by amplifying genomic ribosomal ribonucleic acid (RNA), which can only be detected in active and dividing cells. However, bacteriological results based on ribosomal ribonucleic acid (rRNA) amplification should also be scrutinised for methodology as a very short half-life of rRNA could potentially lead to false negative results if the procedure is not carried out swiftly.

In spite of all major improvement in bacteriological identification methods, one major shortcoming still remains unsolved and that is access to intraradicular microorganisms. Even though the main root canal and associated lateral canals may be accessible for direct or indirect sampling, most of the root canal system including canal walls, dentinal tubules, isthmuses, fins and webs may be untouched, inaccessible or blocked by debris, hence making accurate sampling an impossible task. In addition, the discovery of biofilms transformed our understanding of bacterial ecology, according to Costerton and colleagues (1994; 1999). Planktonic root canal bacteria seldom sustain periapical disease (Siqueira and Rocas, 2009a). A successful endodontic ecosystem may be composed of complex arrangements of multispecies bacteria living in symbiotic biofilms, in which homeostasis is achieved by collaboration between the species that are attached firm and deep into root canal dentinal walls and tubules (Siqueira and Rocas 2009a,b; Avila et al, 2009). These biofilms may be pathogenic to the host or live in symbiosis with the host. Current bacteriological sampling techniques

	Phylum	Genus	Morphology	Species	Pathogenicity
1	Firmicutes	Dialister	Gram negative anaerobic rod	D. invisus D. pneumosintes Gram negative uncultivated species	.++ .++ .++
		Filifactor	Gram positive anaerobic rod	F. alcois	.+
		Peptostreptococcus	Gram positive anaerobic coccus	P. micros P. anaerobius Gram positive uncultivated species	 .++
		Pseudoramibacter	Gram positive anaerobic rod	P. alactolyticus	.+
		Enterococcus	Gram positive facultative coccus	E. faecalis	.+
		Eubacterium	Gram positive anaerobic rod	E. infirmum E. saphenum E. nodatum E. brachy E. minitum	.+/++ .+/++ .+/++ .+/++ .+/++
		Mogibacterium	Gram positive anaerobic rod	M. timidum M. pumilum M. neglectum M. vescum	.+ .+ .+ .+
		Streptococcus	Gram positive facultative coccus	S. mitis S. sanguinis S. gordonii S. oralis	.+/++ .+/++ .+/++ .+/++
			Gram positive anaerobic coccus	S. anginosus S. constellatus S. intermedius	.++ .++ .++
		Veilonella	Gram negative anaerobic coccus	V. parvula Gram negative uncultivated species	.+ .+
		Lactobacillus	Gram positive anaerobic rod Gram positive facultative rod	L. catenaformis L. salivarius L. acidophilus L. paracasei	.+ .+ .+ .+
		Cantonella	Gram negative anaerobic rod	C. morbi	.+
		Granulicatella	Gram negative facultative coccus	G. adiacens	.+
		Selemomonas	Gram negative anaerobic rod	S. sputigena S. Noxia Gram negative uncultivated species	.++ .++ .++
		Finegoldia	Gram positive anaerobic coccus	F. magna	.++
		Peptoniphilus	Gram positive anaerobic coccus	P. asaccharolyticus P. lacrimalis	.+ .+
		Anaerococcus	Gram positive anaerobic coccus	A. prevotii	.+
		Gemella	Gram positive anaerobic coccus	G. morbillorum	.++
2	Bacteroidetes	Prevotella	Gram negative anaerobic rod	P. intermedia P. nigrescens P. tannerae P. multissachariovorax P. baroniae P. denticola Gram negative uncultivated species	.++/+++ .++/+++ .++/+++ .++/+++ .++/+++ .++/+++ .++/+++
		Porphyromonas	Gram negative anaerobic rod	P. endodontalis P. gingivalis	.+++ .+++
		Tannerella	Gram negative anaerobic rod	T. forsythia	.+++
		Capnocytophaga	Gram negative facultative rod	C. gingivalis C. ochracea	.+ .+
3	Spirochaetes	Treponema	Gram negative anaerobic spirilla	T. denticola T. parvum T. socranskii T. maltophilum T. lecithinolyticum	.++/+++ .++/+++ .++/+++ .++/+++ .++/+++
4	Proteobacteria	Campylobacter	Gram negative anaerobic rod	C. rectus C. gracilis C. curvus C. showae	.++ .++ .++ .++
		Eikenella	Gram negative facultative rod	E. corrodens	.++
		Neisseria	Gram negative facultative cocci	N. mucosa N. sicca	.+ .+
		Aggregatibacter	Gram negative anaerobic rod	A. aphrophilus	.+
5	Fusobacteria	Fusobacterium	Gram negative anaerobic rod	F. nucleatum F. periodonticum Gram negative uncultivated species	.++ .++ .++
6	Actinobacteria	Actinomyces	Gram positive anaerobic rod Gram positive facultative rod	A. israelli A. gerencseriae A. meyeri A. odontolyticus A. naeslundii	.+/++ .+/++ .+/++ .+/++ .+
		Corynebacterium	Gram positive facultative rod	C. matruchotti	.+
		Propionibacterium	Gram positive anaerobic rod	P. acnes P. propionicum	.++ .++
		Olsenella	Gram positive anaerobic rod	O. uli O. profusa	.+ .+
		Slackia	Gram positive anaerobic rod	S. exigua	.+
		Eggerthella	Gram positive anaerobic rod	E. lenta	.+
		Bifidobacterium	Gram positive anaerobic rod	B. dentium	.+
		Atopobium	Gram positive anaerobic rod	A. parvulum A. minutum A. rimae	.+ .+ .+
7	Synergistes	Synergistes	Gram negative anaerobic rod	Gram negative uncultivated species	
8	TM7			Clone I025	
9	SR1			Clone X112	

Table 1: Common bacterial species identified in association with most primary endodontic infections

	Phylum	Genus	Morphology	Species	
1	**Firmicutes**	Dialister	Gram negative anaerobic rod	D. pneumosintes D. invisus Gram negative uncultivated species	Clone BS095
		Filifactor Peptostreptococcus	Gram positive anaerobic rod Gram positive anaerobic coccus	F. alcois P. micros P. stomatis	
		Pseudoramibacter Enterococcus	Gram positive anaerobic rod Gram positive facultative coccus	P. alactolyticus E. faecalis	
		Eubacterium	Gram positive anaerobic rod	E. minitum E. infirmum E. saburreum E. sulci E. yurii	
		Mogibacterium	Gram positive anaerobic rod	M. diversum M. neglectum M. timidum	
		Streptococcus	Gram positive facultative coccus Gram positive anaerobic coccu s	S. gordonii S. cristatus S. oralis S. sanguinis S. mitis S. mutans S. sp S. sp S. australis S. infantis S. anginosus S. constellatus	Oral taxon 071 C8
		Staphylococcus	Gram positive facultative coccus	S. capare S. warneri	
		Veilonella	Gram negative anaerobic coccus	V. dispar	
		Lactobacillus	Gram positive facultative rod		
		Cantonella	Gram negative anaerobic rod		
		Granulicatella	Gram negative facultative coccus		
		Selemomonas	Gram negative anaerobic rod		
		Finegoldia	Gram positive anaerobic coccus		
		Gemella	Gram positive anaerobic coccus	G. haemolysans	
2	**Bacteroidetes**	Prevotella	Gram negative anaerobic rod	P. intermedia	
		Porphyromonas	Gram negative anaerobic rod	P. gingivalis P. endodontalis	
		Tannerella	Gram negative anaerobic rod	T. forsythia	
		Capnocytophaga	Gram negative facultative rod		
3	**Spirochaetes**	Treponema	Gram negative anaerobic spirilla	T. denticola	
4	**Proteobacteria**	Campylobacter	Gram negative anaerobic rod	C. rectus C. gracilis	
5	**Fusobacteria**	Fusobacterium	Gram negative anaerobic rod	F. nucleatum	
6	**Actinobacteria**	Actinomyces	Gram positive anaerobic rod	A. radicidentis	
		Propionibacterium	Gram positive anaerobic rod	P. acnes P. propionicum	
		Olsenella	Gram positive anaerobic rod	O. uli O. profusa	
		Slackia	Gram positive anaerobic rod	S. exigua	
7	**Synergistes**	Synergistes	Gram negative anaerobic rod	Gram negative uncultivated species	Clone BA121

Table 2: Common bacterial species identified in association with failed root canal treated teeth (secondary, persistent and refractory)

may not be able to identify all the constituents of these biofilms, especially if they are situated in inaccessible parts of root canal systems.

In order to envisage and analyse different possible combinations of bacteria in endodontic ecosystem and its effect on pathogenicity, host response and eventual endodontic outcome, it is prudent to review the characteristics of root canal microbiota.

Endodontic microbiota

It is believed that over 300 bacterial species are capable of colonising root canals containing necrotic pulp, according to Sundqvist (1994). However, only a fraction of these bacteria could establish an infection (Sundqvist, 1994; Paster et al, 2006). These bacteria live in symbiosis with the host within the oral cavity (Avila, Ojcius and Yilmaz, 2009). Nonetheless, they are opportunistic microorganisms and may cause disease, particularly where the host loses the ability to maintain the homeostasis in the ecosystem (Actor, 2012).

As explained earlier, almost half of disease-producing endodontic bacteria are uncultivable (Hugenholtz and Pace, 1996). Although with the aid of PCR many uncultivable bacteria have been cloned, they are yet to be named and meticulously analysed (Rolph et al, 2001; Munson et al, 2002, Siqueira and Rocas, 2005). Therefore, a thorough knowledge of the type, characteristics, metabolism, pathogenicity and particularly interactions of these microorganisms is of utmost importance. This is especially essential if an association between required numbers of bacteria, host response and pathogenesis is to be drawn.

Siqueira and Rocas found that most bacteria associated with primary endodontic infections have been classified under nine phyla with the aid of culture and molecular analysis (2009b). These phyla are:

- Firmicutes
- Bacteroidetes
- Spirochaetes
- Proteobacteria
- Fusobacteria
- Actinobacteria
- Synergistes
- TM7
- SR1.

The phyla with the highest species richness are firmicutes, bacteroidetes, actinobacteria and proteobacteria. These phyla contain around 460 taxa belonging to 100 genera.

However, these bacterial profiles associated with apical periodontitis are not the same in all individuals, which implies heterogeneity of the disease.

Therefore, a certain combination of bacteria, a favourable environment, nutrient availability and the host response must be the main determining factors in the pathogenesis of apical periodontitis. (Siqueira and Rocas 2009a,b; Paster et al, 2006)

The main inhabitants of primary endodontic infection are gram negative anaerobic rods with different pathogenicities. The most common bacterial species involved in primary endodontic infections with their genera, phyla and degree of pathogenicity are shown in Table 1 (Siqueira and Rocas 2009b,c; Siqueira et al, 2009, Ribeiro, 2011).

It is important to mention, however, that individual virulence of single species does not directly translate to an overall degree of pathogenicity of a bacterial colony (Siqueira and Rocas, 2009a,c). Virulence is a direct result of bacterial colonisation of the root canal as multispecies biofilms, in which the entire community as a whole is responsible for the degree of pathogenecity of the disease. This concept will be discussed later.

Apart from bacteria, other microorganisms such as fungi, archaea and viruses may also be found within an infected root canal ecosystem (Peciuliene et al, 2001; Jiang et al, 2009; Vianna et al, 2009, Sabeti et al, 2003). However, in order to focus on the main aim of this article, the role of bacteria will only be discussed here.

Although the bacterial profiles of most primary endodontic infections illustrate vast diversity of microorganisms comprising a few hundred species, the majority of these microorganisms get destroyed and removed by biomechanical cleansing action of root canal treatment procedure or become inactivated and die after

root canal obturation due to lack of nutrients and an unfavourable ecosystem (Siqueira and Rocas, 2004; 2005; 2009a,b,c; Niazi et al, 2010). These bacteria do not appear in samples taken from failed root canal treated cases. Hence, the attention and focus should be drawn towards the bacteria involved in secondary and persistent intracanal infections. In addition, their mode of action, characteristics, especially with regard to withstanding biomechanical cleaning of endodontic procedure, inter- and intraspecies associations and their symbiotic affiliations must be analysed.

Root canal treated teeth have been shown to harbour only a mean number of one to six bacterial species per tooth (Siqueria and Rocas, 2009b,c).

In contrast to primary endodontic infections, culture-dependent studies demonstrated that persistent and secondary endodontic infections were more likely to contain more gram positive bacteria (Chávez de Paz, 2004). These included streptococci, lactobacilli, staphylococci, enterococcus faecalis, propionibacterium spp, poliana micra, and pseudoramibacter alactolyticus, to name just a few (Byström and Sundqvist, 1985; Sjögren et al, 1997; Gomes et al, 1996; Peters et al, 2002; Chávez de Paz et al, 2003; 2004; 2005; Chu et al, 2006). However, molecular analysis of persistent endodontic infections has revealed that almost 42% of the samples contained uncultivated bacteria, which were the dominant taxa (Sakamoto et al, 2007). The main bacteria associated with secondary and persistent endodontic infections are presented in Table 2 (Siqueira and Rocas, 2005, Niazi et al, 2010).

Unfortunately, most outcome, medication and irrigation studies that evaluated the association between bacteria and success of root canal treatment have only concentrated on the presence or total absence of cultivable bacteria. Most of these studies have demonstrated that only a negative culture at the time of obturation will lead to a successful outcome (Engstrom et al, 1964; Heling and Shapira, 1978, Byström and Sundqvist, 1985; Sjogren et al, 1997; Shuping et al, 2000; McGurkin-Smith et al, 2005; Fabricius et al, 2006). However, there are two major flaws with these studies and the conclusions and theories drawn from them.

The first issue is the fact that, as discussed above, not all bacteria are cultivable (Hugenholtz and Pace, 1996; Paster et al, 2001; Sakamoto et al, 2007). In fact, the majority of bacterial taxa that remain inside the root canals and withstand biomechanical cleaning procedures were only recently discovered by means of PCR and are yet to be cultivable (Sakamoto et al, 2007). Therefore, negative cultures in the previous studies that illustrated endodontic success may have, in actual fact, contained bacteria.

The second issue is the access to the bacteria. Not all bacteria are accessible to direct or indirect sampling. Isthmuses, dentinal tubules, lateral canals, webs and fins may all harbour bacteria in biofilm structures. Negative cultures in previous outcome studies might have failed to demonstrate these bacteria. Therefore, previous successful cases and acceptable outcomes may have been achieved in the presence of bacteria. As a result, it may be concluded that although total sterility is indeed ideal and desirable for a successful outcome, rendering root canals bacteria-free is neither practical nor achievable in every case.

As most bacteria can be eliminated by adequate biomechanical cleaning of the endodontic procedures (Byström and Sundqvist 1985; Shuping et al, 2000; Spratt et al, 2001; McGurkin-Smith et al, 2005) and a successful outcome may be achieved even in the presence of residual bacteria, other factors must contribute towards the sequelae of apical periodontitis. These may be the host, the number of bacteria, the correct combination of bacteria, and/or an interaction between these elements.

Siqueira and Rocas (2009c) found that this interaction may lead to a successful outcome if all aforementioned elements are in a 'homeostatic symbiotic environment'. Any breach in this equilibrium may consequently lead to disease formation. This breach could arise as a result of host defence depression, an increase in the numbers or a development of a more favourable environment for the 'essential bacteria' within the community, such as in an event of a rise in nutrient availability.

The host

As explained above, some of the major factors that contribute towards the sequelae of apical periodontitis are the host and host-bacteria interactions within a homeostatic symbiotic environment (Actor, 2012).

It is generally acknowledged that in an event of host defence depression, opportunistic bacteria that possess essential virulence factors may give rise to disease, according to Actor (2012). Disease initiation is carried out by means of exotoxin secretion via viable bacterial cells, by endotoxins arising from by-products of cell lysis or from certain components of bacterial cell wall such as lipopolysaccharides, proteins, lipoproteins, glycoproteins, carbohydrates and lipids, to name just a few (Casadevall and Pirofski, 2001; Lawrence, 2005; Actor, 2012).

Moreover, bacterial toxins include enterotoxins, neurotoxins, cytotoxins and lysins. These toxins are virulence factors and are recognised by the host as pathogens and trigger cytokine release, such as interleukins, which leads to the activation of the inflammation system (Actor, 2012).

Therefore, it may be assumed that if endodontic bacteria lack the ability to produce virulence factors, or more importantly, the host defence mechanism efficiently depresses the formation of such factors within the homeostatic symbiotic environment, at least in theory, apical periodontitis may not ensue.

The other aspect of the host that is as important as the host defence inflammatory reaction is the dentine and intracanal host ecosystem, as bacteria require a suitable surface to be able to colonise on. The dentine is capable of influencing the ecosystem by regulating the pH, modifying the anaerobic metabolism of bacteria, providing nutrients and suitable attachment surface and accommodating root filling materials and sealers.

Unfortunately, studies with regard to the influence of the dentine on intracanal homeostatic symbiotic environment and bacterial interaction are scarce.

Bacteria can also directly invade and damage the host cells by releasing enzymes such as collagenases and

hyaluronidases and metabolites such as short chain fatty acids. These elements can sometimes exert their mode of action without being detected by the host defence mechanism (Hashioka, 1994; Lawrence, 2005).

Interactions between bacteria

Bacteria are intelligent microorganisms. As explained previously, almost all bacteria that inhabit root canals are oral cavity commensals. Although many of these commensal bacteria are opportunistic pathogens waiting for an 'opportune' moment to initiate disease, several bacteria do not inherently possess high virulence but can still cause pathogenicity if accompanied by certain other bacterial species within a biofilm (Casadevall and Pirofski, 2001; Moine and Abraham, 2004; Drenkard, 2003).

Bacterial virulence acquisition can be performed by means of gene switching and horizontal gene transferring in which valuable genetic material can be transferred between species through extrachromosomal plasmids (Lawrence, 2005; Kunin et al, 2005). This is a very beneficial mechanism for propagating vital genes. Gene transferring can take place between species; therefore, it will benefit all bacteria within a community (Lorenz and Wackernagel, 1994; Wang et al, 2002; Chen et al, 2004; Nallapareddy et al, 2005). In addition, it is faster than chromosomal gene propagation by means of cell division.

Furthermore, it enables all species within a bacterial community to rapidly adapt themselves to an adverse change in the ecosystem.

Adaptation to changes in the pH, nutrition availability, lack of oxygen and developing resistance to medicaments are a few examples (Hayes, 2003; Martinez and Baquero, 2002; Socransky and Haffajee, 2000).

For this precise interaction to occur, bacteria need to exist in a cohesive, suitable and protective environment. This setting facilitates the communication and transfer of information and materials between different bacteria and improves their chance of survival. This cohesive, suitable and protective setting is referred to as the biofilm.

Biofilm, community and colony-forming units

According to Costerton and colleagues (1994), a biofilm is 'a mode of microbial growth where dynamic communities of interacting sessile cells are irreversibly attached to a solid substratum, as well as each other, and are embedded in a self-made matrix of extracellular polymeric substances'.

It is important to state that while bacteria create the biofilms to improve their interaction and survival, they inherently require a host and a surface – in this case the root canal dentine – to colonise.

This is yet another example of the importance of the host in the sequelae of apical periodontitis.

Furthermore, Costerton and colleagues (1994; 1999) reported that the bacteria within biofilms must be able to self-organise, achieve homeostasis, perform better in a community as opposed to in isolation, and be able to respond to changes as a unit.

Apart from proficient communication through quorum sensing and genetic transfers within and in between species, biofilms improve the survival of the bacteria by nutrient trapping, establishing metabolic cooperativity, and reducing the risk of desiccation by means of extracellular polysaccharide exertions and efficient internal water channels (Costerton et al, 1994; 1997; 1999).

Not all bacteria are capable of forming biofilms. However, once a biofilm is formed by the interaction of one or several species, other species can join the community as long as they possess the specific characteristics mentioned above (Costerton et al, 1999; Cowan, Taylor and Doyle, 1987; Al-Hashimi and Levine, 1989; Handley, Carter and Fielding, 1984). In root canals, E. faecalis can form different biofilms based on the changes in the ecosystem and survive in difficult conditions (Gentry-Weeks et al, 1999; Capiaux et al, 2000; Fidgor, Davies and Sundqvist, 2003).

Siren and colleagues (1997) reported that fusobacterium nucleatum is capable of coexisting with enterococcus faecalis within the same community by means of coaggregation. Fusobacterium nucleatum may in fact be an important link between different species in endodontic microbiota. Fusobacterium nucleatum and many streptococci have been revealed to coaggregate by bridging (Kolenbrander, Andersen and Moore, 1989; Lancy et al, 1983). Furthermore, positive associations have been identified between fusobacterium nucleatum and poliana micra, porphyromonas endodontalis, selenomonas sputigena, and campylobacter rectus in teeth with apical periodontitis (Sundqvist, 1992).

Other community-forming species appear to be streptococcus anginosus, peptostreptococcus anaerobius and prevotella oralis and prevotella, streptococcus and fusobacterium in acute endodontic infections (Johnson, Flannagan and Sedgley, 2006; Khameleelakul, Baumgartner and Pruksakom, 2006). In contrast, species of streptococci, probionibacterium propionicum, capnocytophaga ochracea, and veillonella parvula have been demonstrated to exhibit no or negative associations with one another (Sundqvist, 1992). Moreover, porphyromonas gingivalis and streptococcus gordonii have been shown to coexist within a community but not in the presence of S. mutans (Love and Jenkinson, 2002; Love et al, 2000).

In the field of endodontic microbiology many studies have been conducted in order to identify the bacteria responsible for the pathogenicity of apical periodontitis.

However, not even one high quality research could be found on the issue of the relation of bacterial load, with regard to the numbers, to pathogenicity.

It is logical to assume that total sterility of root canals, despite being desirable, is seldom achievable. Furthermore, sterility may not even be essential for the treatment outcome.

Hence, identifying bacterial load threshold for the development of apical periodontitis could be of utmost importance.

The number of colony-forming units (CFU) in infected root canals has been estimated to be between 103 and 108 (Siqueira and Rocas, 2005, Vianna et al, 2006) and a direct association has been established between the radiographic dimensions of periapical lesions and the number of CFUs. This number is probably somewhere between 102 and 103 in secondary and persistent infections with lower number of species involved per root canals. However, there is no

evidence on the bacterial load (CFU) required for the induction of periapical disease. This lack of evidence may be a result of three main problems facing research in this field.

The first major problem associated with analysing the required CFU is the sensitivity of the tests. The sensitivity of culture-dependent investigation is at best around 103 cells per millilitre, according to Zambon and Haraszthy (1995). Siqueria and Rocas showed that PCR can potentially reduce this number to one cell per millilitre (2003). However, at least for the time being, no study has managed to achieve this number within the field of endodontics. Realistic and achievable sensitivity for the PCR is estimated to be 10 cells per millilitre. This lack of sensitivity hampers the accurate estimation of bacterial numbers required to establish apical periodontitis (Siqueira and Rocas, 2003).

The second problem, as explained before, is the anatomy of most root canals, which makes direct, accurate and predictable sampling almost impossible in most cases. As a result, occurrence of false negative samples will inherently lead to incorrect estimation of bacterial numbers.

The third problem is the bacterial interaction within a biofilm. Task allocation and responsibility sharing amongst different bacterial species, together with genetic information transfer between bacteria, makes identification and counting of the real culprits a very difficult task.

Furthermore, heterogeneity of the bacterial taxa in different individuals makes laboratory extrapolation of the numbers invalid. In addition, even correctly identified and counted bacteria may not necessarily be part of the 'essential bacteria' responsible for the pathogenesis of apical periodontitis. In fact, the unidentified bacteria may be as important, if not more important, in the pathogenesis of the disease than the identified taxa.

Despite the importance of bacterial load, the exact estimation of CFU may not be a crucial factor in managing endodontic infections if we consider the presence and activity of the bacteria within a homeostatic symbiotic environment. The host defence, root canal dentine and intracanal microbiota may be able to live in symbiosis.

To be able to address this issue more studies will be required to focus on:

- Interactions between the endodontic microbiota in secondary root canal infections, identification of the essential bacteria, their necessary numbers to initiate apical periodontitis and their effect on endodontic outcomes
- Microbiota and host/dentine symbiosis and endodontic outcomes
- Development of a live CFU measurement device with high sensitivity
- Development of a standard laboratory 'homeostatic symbiotic environment' model.

It is important to point out that it is not being suggested here that leaving bacteria in root canals should be actively practised. Meticulous root canal treatment with thorough biomechanical cleansing and use of medicaments or sampling, where necessary, must be carried out in every case. Our aim must always be the elimination of the bacteria or the reduction of the numbers to as low as biologically and anatomically possible. Hopefully, if this number is below the disease initiation threshold and stays at this level, success will ensue.

Conclusion

Although over a century has passed since what might once have been referred to as the dark ages of endodontics, and new materials, methods and procedures have been developed, the principles of root canal treatment have somehow remained the same. These principles are the removal of the necrotic pulp tissue and infected dentine from the root canal system and filling the space.

It is now well established that bacteria are essential for the development of pulpal and periradicular diseases, and the presence of bacteria in the root canal space or periapical tissue will undermine the success of endodontic treatment.

Therefore, for a successful treatment, the canals should be thoroughly disinfected and rendered bacteria-free prior to obturation.

However, it can be stated that total sterility of root canals, despite being desirable, is seldom achievable. Furthermore, sterility may not even be essential for successful outcome of the treatment. Hence, identifying bacterial load threshold for the development of apical periodontitis could be of utmost importance.

Unfortunately, the identification of necessary CFUs for the initiation of apical periodontitis poses several technological and biological issues.

It is therefore not possible to estimate this number with certainty. However, it can be concluded that due to heterogeneity of the endodontic microbiota and the complexity of the endodontic ecosystem, a synergistic collaboration of several elements are responsible for a successful endodontic outcome.

These elements are all part of a homeostatic symbiotic environment and include the host, the bacteria (numbers and essential species), the ecosystem and the interaction between these elements.

More research is needed to analyse the effect of these elements, both individually and as a unit, on endodontic outcomes. In addition, the role of other microorganisms such as fungi and archaea in the ecosystem should also be further investigated.

In the future, endodontic treatment may move towards active biologic therapeutics with the aid of site-specific and highly selective probiotics that can provide a cascade of desirable biofilms across the root canals, and disturb all pathogenic activity of the microbiota that are capable of initiating apical periodontitis.

In addition, the development of advanced methods of real-time detection of bacteria presence will be extremely valuable. A definitive sealing of the system including its extremities will then prevent reinfection, and is of utmost importance. ■

The full list of references that accompany this article can be found overleaf.

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