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EDITORIAL

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Endodontic biofilm, technology and pulpal regenerative therapy: where do we go from here?

Introduction

In the past, scientific progress was considered as a series of steady and incremental developments taking place through the accumulation of accepted facts. This concept was then challenged by Kuhn (1962) who described an episodic model in which progress takes place in revolutionary steps when an accepted model undergoes sudden drastic changes or a *paradigm shift*. Microbial diseases, known under various names such as diseases of infection, infectious diseases, transmissible or communicable diseases, were considered to be caused by specific biological agents. Whilst that is true for classical infectious diseases such as tuberculosis and tetanus that are caused by single, specific aetiological agents, there are several chronic diseases that are caused by a consortium of microbial species living in an ecological habitat, known as biofilms. Apical periodontitis belongs to this group of diseases. The purpose of this editorial is to briefly but critically survey the physical and biological challenges that limit our ability to predictably cure apical periodontitis and to explore the emerging opportunities in endodontic microbiology, cell biology and new generative medicine.

A biofilm disease

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by aetiological agents of endodontic origin. Although Antony van Leeuwenhoek (Dobell 1960) described several kinds of microorganisms (animalcules) in the dead pulps of carious human teeth in 1697, and two centuries later, the presence of several kinds of bacteria was rediscovered (Miller 1894) in necrotic pulps within root canals of diseased human teeth, the role of microorganisms in the causation of apical periodontitis remained uncertain until 1965 when Kakehashi and others convincingly demonstrated (in a rat model) the essential role of microbes in apical periodontitis (Kakehashi *et al.* 1965). In the mid-1970s, using advanced anaerobic technology, Sundqvist extended the aetiological role of microbes to apical periodontitis associated with human teeth containing necrotic pulps (Sundqvist 1976). In 1987, using the technique of correlative light and transmission electron microscopy, Nair presented the ultrastructural visualization of intracanal microbial flora and described it as embedded in '. . .extracellular matrix of bacterial origin' and existing as sessile '. . . intricate communities promoting a symbiotic relationship' (Nair 1987), what is now known as endodontic biofilms.

Today, the confocal laser scanning microscopy (CLSM) has become the foremost imaging tool for biofilm research. The CLSM enables optical serial sectioning in different planes of fluorescence labelled living and fixed biofilm specimens. The CLSM contributed much to our understanding of biofilms in general and also of endodontic biofilms – the latter mostly by extrapolation of information from general and medical microbiology or by imaging biofilms extirpated from root canals. To the author's knowledge, there has been only one attempt to apply CLSC to endodontic biofilms *in vivo* (Schaudinn *et al.* 2009), and it has not yet been applied successfully to obtain *in situ* visualization of biofilms in root canals of diseased teeth, with the accuracy, strength of evidence and clarity of information that was achieved repeatedly (Nair 1987, Nair *et al.* 1990a, Nair *et al.* 2005) with the precision of correlative light and transmission electron microscopy (Fig. 1).

To my knowledge, the term 'biofilm' was coined for the first time by Poul Harremoes, a Danish physicist, in a paper dealing with the diffusion kinetics of fluids into slimy biofilters that he called 'biofilms'. Recognizing the crux of the problem he wrote, '. . . little attention has

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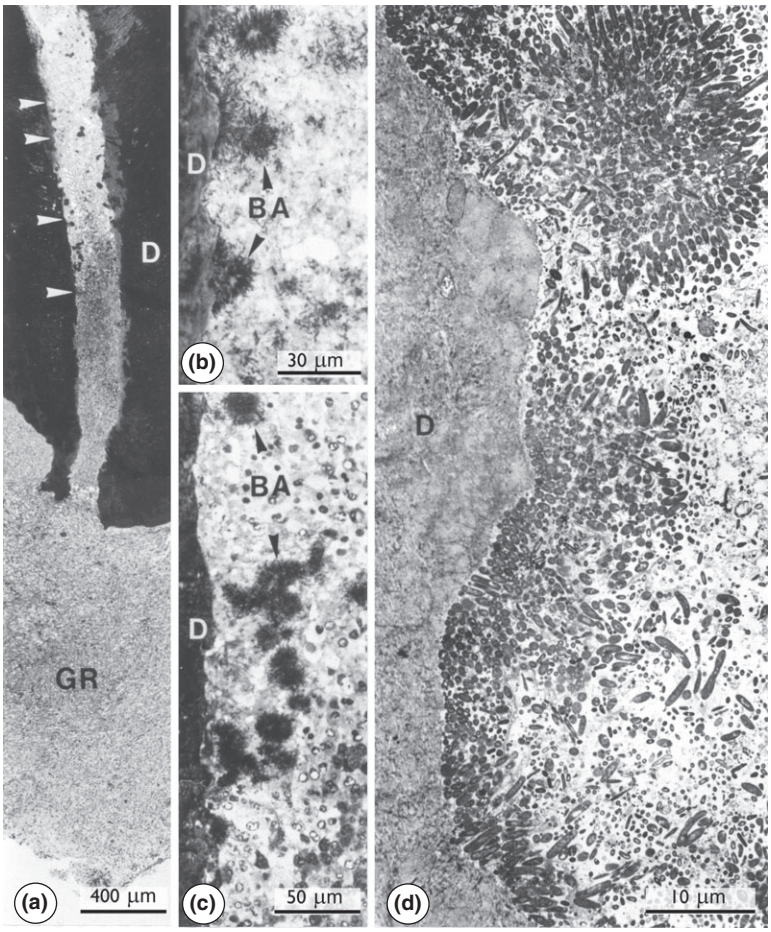

Figure 1 Axial view of endodontic microbial biofilm in a human tooth with apical periodontitis (GR) and previously untreated root canal. The areas within the axial section of the tooth (a) in between the upper two and the lower two arrowheads in are magnified in (b, c), respectively. Note the biofilm as dense bacterial aggregates (BA) sticking (b) to the dentinal (D) wall and also remaining suspended amongst neutrophilic granulocytes in the fluid phase of the root canal (c). A transmission electron microscopic view (d) of the pulp–dentinal interface shows bacterial condensation on the surface of the dentinal wall forming sessile biofilm. Magnifications: a ×46; b ×600; c ×370; d ×2350. (From Nair, *Periodontology 2000* **13,** 121–148, 1997).

been given to . . . the fact that organisms are gathered in folks into which the substrate has to diffuse. This phenomenon is of particular significance for attached biofilms' (Harremoes 1977). A biofilm (Costerton *et al.* 1995) is a polymicrobial community, with a biofilm growth pattern, living embedded in a self-produced matrix of highly hydrated extracellular polymeric substances (EPS). The microbes in a biofilm adhere to each other and/or to a moist surface as against planktonic organisms, which are free-swimming/floating single microbial cells in an aqueous environment. The intracanal microbes living in a biofilm (Fig. 1) can advance or their products can egress into the periapex. Consequently, the body mounts an array of defence consisting of several classes of cells, of intercellular messengers, and of effector molecules (Nair 2004). In spite of this formidable defence, the body cannot remove the microbes, well entrenched as biofilms, in the sanctuary of the necrotic pulp within root canals. In essence, apical periodontitis is not self-healing. The biofilm microbes and the host defence clash, and strike an equilibrium that allows the persistent biofilms in the root canal and limited host response leading to various categories of lesions that are given the overarching name of apical periodontitis (Nair 1997).

Treatment with remarkable success

Because intracanal biofilm is the essential aetiological agent of primary apical periodontitis, the logical

treatment for the disease consists of mechanically disrupting the biofilm, removing or at least reducing substantially the intracanal microbial load with an irrigating solution and then preventing reinfection by root filling. Currently, this is achieved by root canal instrumentation, irrigation with sodium hypochlorite (NaOCl) solution, rinsing with EDTA and, in multiple visit treatments, application of a microbicide dressing. Investigations (Nair *et al.* 2005), however, show that the infectious agents are mostly located in uninstrumented recesses of the main canals, the isthmus communicating between them and in accessory canals. The microbes in these untouched locations exist primarily as biofilms that are not removed predictably by instrumentation and irrigation with NaOCl in one treatment session (Nair *et al.* 2005), nor after two-visit treatment protocols (Vera *et al.* 2012). The application of two-visit protocols did result in microbial reduction in the treated root canals as compared to one-visit protocols, but even so, microbes remained in isthmuses and other inaccessible areas of the canal system.

An important point regarding the effectiveness of root canal treatment is whether or not residual microorganisms really matter? The long-term impact of residual biofilm depends on several factors such as its location within the root canal system, the size and composition of the microbial population in the biofilm, and the availability of nutrients for the microbes. Root canals containing microbes in 'subcritical' numbers or situated in locations where they or their products are inaccessible to periapical tissues can remain 'harmless'. Depending on the factors outlined above and those that have been dealt with at length (Sundqvist & Figdor 2003, Nair *et al.* 2005); remaining microbes may or may not pose an immediate obstacle for healing. It will be a challenging task to find out which residual infections in what composition, quantity and location will persist and thus impair apical healing. However, the occurrence of post-treatment apical disease some years after initial root canal treatment that appeared to heal initially (radiographically) may be an indication that residual biofilms (or possibly renewed infection through coronal microleakage) are of concern for apical healing. Therefore, the problem of microorganisms surviving in the inaccessible, remote areas of the canal system beyond the reach of contemporary treatment is a potential risk that there may result over time an unfavourable apical healing response (Sjögren *et al.* 1997). When the treatment is performed to a sufficient standard, healing of the periapical lesion usually occurs with osseous regeneration, which is characterized by gradual reduction and resolution of the radiolucency on subsequent follow-up radiographs.

Post-treatment apical periodontitis

For various reasons, complete bone healing of a lesion or a reduction in the volume of a lesion may not occur in all root filled teeth. Such nonhealing post-treatment periapical radiolucencies are also known as persistent apical periodontitis or simply as endodontic failures. Taken together, there are six biologic factors that contribute to persistent post-treatment apical radiolucencies (Nair 2006). They are as follows: (i) residual intraradicular biofilm in the complex apical root canal system (Nair *et al.* 1990a) (Figs 2 and 3), (ii) extraradicular infection, generally in the form of periapical actinomycosis (Nair & Schroeder 1984), (iii) extruded root canal filling or other exogenous materials that cause a foreign body reaction (Nair *et al.* 1990b), (iv) accumulation of endogenous cholesterol crystals that irritate periapical tissues (Nair *et al.* 1993, 1998), (v) true cystic lesions (Nair *et al.* 1993) and (vi) scar tissue healing of the lesion (Nair *et al.* 1999).

Microbe-free canals?

There has been considerable interest as to whether the apical root canal system contains microbes immediately after completing root canal treatment. It has been shown that 14 of 16 instrumented and root filled canals in mandibular molars with apical radiographic lesions had residual biofilm in mesial roots when the treatment was completed in one-visit (Nair *et al.* 2005). Further, microcomputer tomography (μCT) has detected inadequacies in root canal instrumentation techniques. For example, approximately 40% of the walls of root canals remained untouched by instruments (Peters *et al.* 2001, 2003, Huebscher *et al.* 2003). The great anatomical complexity of the root canal system (Hess 1921, Perrini & Castagnola 1998) and the ecological organization of the flora into biofilms (Costerton *et al.* 1995, 2003, Costerton & Stewart 2000) make the goal of achieving a microbe-free canal by root canal treatment currently unattainable. Thus, biology sets limits to technology in endodontics.

Then, the question arises as to why a large number of lesions of apical periodontitis heal after conventional

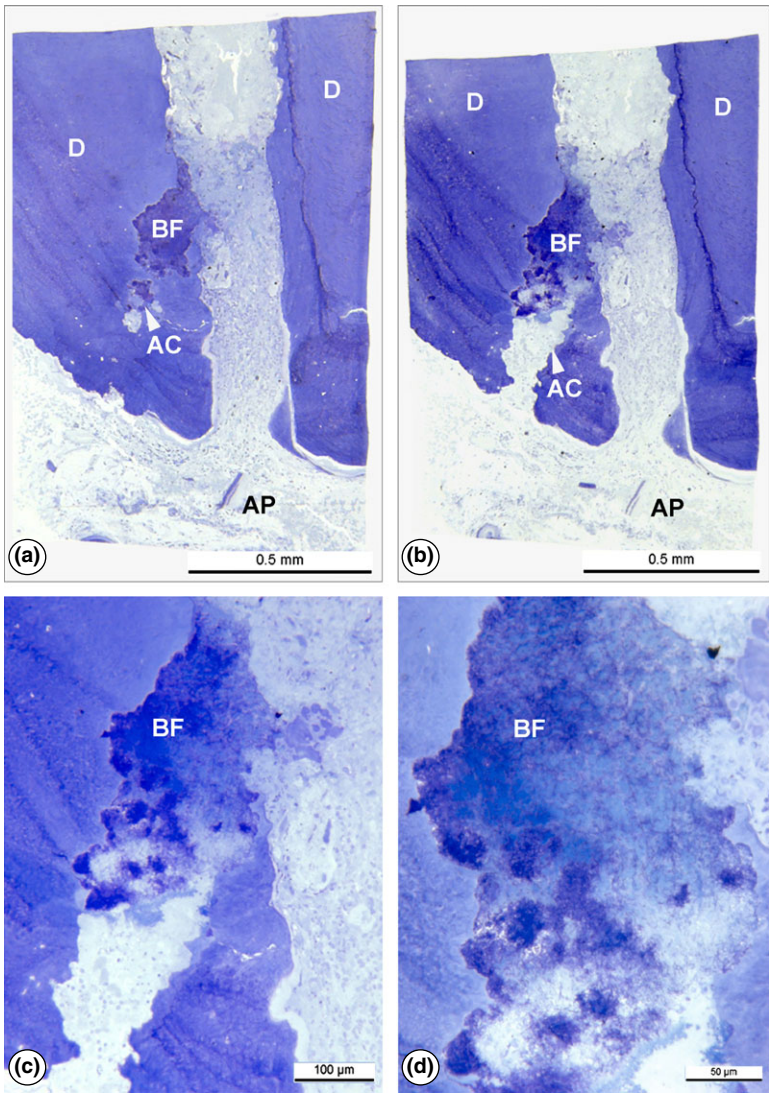

Figure 2 Photomicrographs of axial semi-thin sections through the surgically removed apical portion of the root with a persistent apical periodontitis. Note the adhesive biofilm (BF) in the root canal. Consecutive sections (a and b) reveal the emerging widened profile of an accessory canal (AC) that is clogged with the biofilm (BF). The biofilm in (a and b) is magnified in (c and d), respectively. Magnifications: a ×75, b ×70, c × 110 d ×300. (Reprinted from Nair, Int Endod J, 2006, 39, 241–81).

root canal treatment. This is because the root canal treatment results in the following: (i) a substantial reduction in the intracanal microbial load to a subcritical level to sustain the inflammation of the periapex (Nair *et al.* 2005), (ii) the drastic disturbance to the delicate microbial ecosystem, (iii) destruction of the microbial habitat, (iv) the robust host defence contributes to the healing of apical radiolucent lesions after root canal treatment and further, (v) it also depends on access of the biofilm microbes to the periapical tissues (Sundqvist & Figdor 2003).

Future: is it bright or bleak?

As there are physical and biological limits to technological innovations in endodontics, further progress in Endodontology lies with advances in endodontic microbiology and cell biology.

Endodontic microbiology

The new frontiers of the chemo-mechanical landscape of endodontic treatment will be drawn by

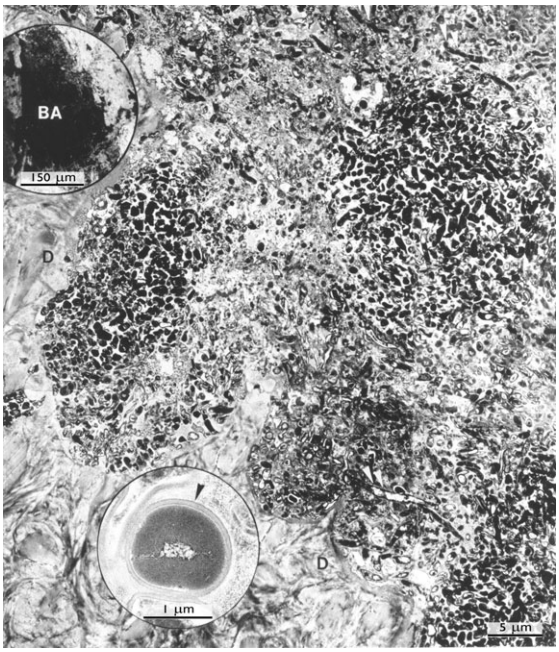

Figure 3 Composite transmission electron micrographs of the biofilm (BA, upper inset) illustrated in Fig. 2. The biofilm is composed of only Gram-positive, filamentous organisms (arrowhead in lower inset). Note the distinctive Gram-positive cell wall. The upper inset is a light microscopic view of the biofilm (BA). Magnifications: x3400; insets: upper ×135, lower ×21, 300. (Reprinted from Nair, Int Endod J, 2006, 39, 241–81).

control of the biofilm. In the short term, it should be possible to develop quick acting, low viscous, deep diffusing and body compatible microbicides. Microbes in biofilms are more than a thousand times resistant to antimicrobial agents as against the same organisms in free-floating (planktonic) form (Costerton *et al.* 1995, Wilson 1996). Therefore, the reported positive effects of microbicides of root canal disinfectants based on short-term *in vitro* experiments (Sirén *et al.* 2004) may not be clinically valid! Further, the biofilm is a massive genetic lending library for microbes. This is because the EPS matrix is flooded with the components of lysed microbial cells, which include DNA fragments for lateral or horizontal gene transfer. Therefore, caution must be exercised in the use of intracanal antibiotics so as to avoid horizontal gene transfer, allowing pathogenic bacteria, to share gene resistance to drugs.

Understanding the complexities of biofilms is the key for further progress in root canal treatment. It should be possible to develop biofilm inhibitors and agents that weaken the biofilm structure, particularly the EPS. Microbes in the biofilm communicate with each other through a biochemical signalling language known as the *quorum sensing*, which literally means to judge the population size or density of the microbes in the biofilm. If the population size is small or subcritical for them to advance into the host tissue, they may be able to 'lie low' by 'switching off' offensive genes by the mechanism known as gene silencing (Fire *et al.* 1998). As in human wars, an army with compromised lines of communication is vulnerable to isolation and defeat, disrupting the microbial communication lines should help to control microbes in root canal biofilms.

Gene silencing

Also known as RNA interference (RNAi) (Fire *et al.* 1998), gene silencing was discovered in 1998, a discovery that won the Nobel Prize for Andrew Fire and Craig Mello in 2006. In gene silencing, certain molecules trigger the inactivation of the messenger RNA from a particular gene, so that no related protein is produced. Thus, the gene is silenced, inactivated, blocked or turned off by a cellular mechanism that is distinctly different from genetic modification by chromosomal changes and gene mutation.

Gene editing

Gene editing is another fast emerging cell-based gene therapy to restore or modify gene function by correcting the disease-causing gene by removing it/or replacing it with a normal or modified version of the gene. It is 'cut and paste' (Komiyama 2013) molecular technology or genetic 'surgery' using molecular 'scissors'! Could gene silencing and gene editing lead to new treatments? It has been shown in monkeys that blood cholesterol levels can be lowered by suppressing a gene (Soutschek *et al.* 2004). Thus, attaining the capability to correct or block disease-promoting microbial genes should lead to new treatment lines against microbes in the biofilm.

Thus, there is considerable interest to develop antibiofilm strategies that can be clinically applied to treat biofilm diseases. These include (i) improving methods for physical debridement of biofilm, ii) application of chemical microbicides, (iii) developing biological strategies that change the genotype, phenotype and/or behaviour of microbes so as to make them poor

biofilm builders and (iv) that weaken the EPS. In a nutshell, our contemporary chemo-mechanical treatment of root canals needs to evolve into broader mechano-chemo-biological procedures.

The 'elephant in the room'

The problem of the currently intractable residual biofilm in root filled teeth has important implications in other areas of endodontics, for instance, on pulpal regenerative treatments designed to replace 'damaged root structures as well as the pulp–dentine complex' (Murray *et al.* 2007). The complexity of the issues involved in endodontic regenerative therapy is being debated (Spångberg 2009, Hargreaves & Law 2010, Andreasen & Bakland 2012, Fouad & Nosrat 2013, Lin *et al.* 2013). On the other hand, there is a consensus that many teeth with infected and necrotic pulps cannot be made completely microbe-free using contemporary treatment procedures (Nair *et al.* 2005, Vera *et al.* 2012). Further, biofilms may be present even in root filled teeth with radiographically healed apical lesions. These pockets of residual biofilm pose some risk of apical healing around a root filled canal, but have significant potential to perturb regeneration of a healthy pulp. Nevertheless, many clinicians and researchers seem to believe that therapeutic replacement of damaged dental body parts is within practical reach in spite of the unpredictable and variable outcomes of pulp regenerative procedures (Kahler *et al.* 2014). Even if an immature tooth with pulp disease but with a vital apical growth region may undergo 'revascularization' and may become to some extent stronger, longer and be preserved in a better state than if untreated (Cotti *et al.* 2008), there remains the threat of subsequent breakdown under microbial activity (Lin *et al.* 2014).

It has been claimed that application of the principles of tissue engineering can result in the 'regeneration of dental pulp with newly deposited continuous layer of dentine' (Huang *et al.* 2010) in human root segments implanted into immunocompromised mice. Leaving aside the question as to which type of new hard tissue and under what experimental condition was formed inside the hollowed root segments (Huang *et al.* 2010), it will suffice here to say that the experimental model used was sterile. It remains to be shown that regenerative cells establish themselves in an infected root canal and recreate the pulp–odontoblast–dentine complex. The enthusiasts of pulpal regenerative therapy do not see the 'elephant in the room'. There is no evidence yet for the much celebrated claim that a 'paradigm shift is taking place' (Huang 2008) in the clinical management of neither immature nor mature teeth *with infected and totally necrotic pulps* (Nair 2014). This situation is reminiscent of what happened in dental research during the 1970s. A huge sum of money was then spent on developing vaccines against caries and periodontal diseases. In retrospect, with the benefit of hindsight, we know that those investments in money, materials and efforts did not lead to the desired results. The infatuation with pulp regenerating therapy is understandable but may turn out to be 'barking up the wrong tree'. This is because the breakthrough in research is more likely to come elsewhere in dental medicine, namely from the new generative medicine (Nair 2014).

New generative medicine

The cloning of Dolly in 1997 (Wilmut *et al.* 1997) demonstrated that a fully differentiated somatic cell could recreate a whole individual. The experimental basis for the creation of Dolly was worked out long before, in the early 1960s, on tadpoles (Gurdon 1962). Broadly speaking, any organ or tissue could be generated from a fully differentiated adult somatic cell. Less than a decade after the birth of Dolly, researchers (Takahashi & Yamanaka 2006) showed that any adult somatic cell can be reprogrammed to become induced pluripotent stem cells (IPS). The Nobel Prize in Physiology and Medicine for 2012 was jointly awarded to John Gurdon and Shinya Yamanaka for their respective discoveries. In May 2013, human adult somatic cells were converted to embryonic stem cells using the technique of somatic cell nuclear transfer (Tachibana *et al.* 2013).

There is an intense focus to develop quicker, simpler and less ethically fraught methods to reprogramme somatic cells to stem cells. It was reported in January 2014 that exposing blood cells from newborn mice to a weak acid solution for 30 min resulted in the cells becoming pluripotent and were able to form different types of cells (Obokata *et al.* 2014). The experimental protocol, called stimulus-triggered acquisition of pluripotency (STAP), is yet to be successfully replicated by other scientists for validation. It is appropriate that advances in stem cell research undergo stringent and broad scrutiny by other scientists; so too should studies in pulp regeneration to ensure that experimental and clinical protocols are reproducible by other scientists and clinicians in the

endodontic community. In the context of growing new replacement teeth, the findings of a recent study (Volponi *et al.* 2013) deserve particular attention. The researchers isolated epithelial cells from adult human gingiva. The cells were expanded *in vitro* and mixed with mouse embryonic mesenchymal cells. A mixture of the cells was implanted beneath a murine renal capsule and teeth developed with growing roots.

Taken together, the rapid developments in stem cell and new generative technology signify that the problems regarding the availability of ethically and legally applicable stem cells are almost resolved. It is the author's contention that there is greater potential for creating a new tooth in a healthy recipient site, compared with attempting to repair one that may contain pockets of infection. This is because of the limits of current treatment to predictably remove all residual biofilms in teeth with necrotic pulps. In addition to inaccessibility, microbes living in a biofilm have significantly different properties from planktonic organisms of the same species. The environment in the EPS enables them to cooperate and interact in various ways. The dense EPS and the outer layer of cells protect the interior of the biofilm community. In addition to the physical protection, the enhanced lateral gene transfer makes biofilm microbes far more resistant to microbicides and antibiotics (Costerton *et al.* 1995, Wilson 1996). The biological challenges of dealing with residual root canal biofilms have so far compromised our ability to predictably cure apical periodontitis. These circumstances tempted the author to speculate that, 'in future, such severely compromised and missing teeth would be substituted by whole natural replacement teeth growing and erupting *in vivo de novo* using one's own stem cells. In other words ... dentures and dental implants will become obsolete as new whole teeth would be grown in patient's jaw using the stem cell technology' (Nair 2014). This is akin to demolishing of a hopelessly damaged house and building it anew based on the original design, instead of attempting to restore it by soft renovation. A new generative treatment for severely diseased teeth is on the horizon, instead of the much-heralded regenerative endodontics (Nair 2014). The future of Endodontology appears brighter than ever before.

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References

Andreasen JO, Bakland LK (2012) Pulp regeneration after non-infected and infected necrosis, what type of tissue do we want? A review. *Dental Traumatology* **28**, 13–8.

Costerton JW, Stewart PS (2000) Biofilms and device-related infections. In: Nataro PJ, Balser MJ, Cunningham-Rundels S, eds. *Persistent Bacterial Infections*. Washington, D.C.: ASM Press, pp. 423–39.

Costerton JW, Lewandowski DE, Caldwell DE, Krober DR, Lappin-Scott H (1995) Microbial biofilms. *Annual Reviews of Microbiology* **49**, 711–45.

Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C (2003) The application of biofilm science to the study and control of chronic bacterial infections. *Journal of Clinical Investigations* **112**, 1466–77.

Cotti E, Mereu M, Lusso D (2008) Regenerative treatment of an immature, traumatized tooth with apical periodontitis: report of a case. *Journal of Endodontics* **34**, 611–6.

Dobell C (1960) *Antony van Leeuwenhoek and his Little Animals*. New York: Dover Publications.

Fire A, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegan*. *Nature* **391**, 806–11.

Fouad AF, Nosrat A (2013) Pulp regeneration in previously infected root canal space. *Endodontic Topics* **28**, 24–37.

Gurdon JB (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *Journal of Embryology and Experimental Morphology* **10**, 622–40.

Hargreaves KM, Law A (2010) The wrong Emporer. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **109**, 327–8.

Harremoes P (1977) Half-order reactions in biofilm and filter kinetics. *Vatten* **33**, 122–43.

Hess W (1921) Formation of root canal in human teeth. *The Journal of the National Dental Association* **3**, 704–34.

Huang GT-J (2008) A paradigm shift in endodontic management of immature teeth: conservation of stem cells for regeneration. *Journal of Dentistry* **36**, 379–86.

Huang G-J, Yamaza T, Shea L *et al.* (2010) Stem/Progenitor cell–mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an *in vivo* model. *Tissue Engineering Part A* **16**, 605–15.

Huebscher W, Barbakow F, Peters OA (2003) Root canal preparation with FlexMaster: canal shapes analysed by micro-computed tomography. *International Endodontic Journal* **36**, 740–7.

Kahler B, Mistry S, Moule A *et al.* (2014) Revascularization outcomes: a prospective analysis of 16 consecutive cases. *Journal of Endodontics* **40**, 333–8.

Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery Oral Medicine and Oral Pathology* **20**, 340–9.

Komiyama M (2013) Cut and paste of DNA using an artificial restriction DNA cutter. *International Journal of Molecular Sciences* **14**, 3343–57.

Kuhn TS (1962) *The Structure of Scientific Revolutions*, 1st edn. Chicago: University of Chicago Press.

Lin LM, Ricucci D, Huang GTJ (2013) Regeneration of the dentine-pulp complex with revitalization/revascularization therapy: challenges and hopes. *International Endodontic Journal*. Accepted article.. doi:10.1111/iej.12210.

Lin LM, Schimizu E, Gibbs JL, Logbin S, Riccuci D (2014) Histologic and histobacteriologic observation of failed revascularization/revitalization therapy: a case report. *Journal of Endodontics* **40**, 291–5.

Miller WD (1894) An introduction to the study of bacteriopathology of the dental pulp. *Dental Cosmos* **36**, 505–27.

Murray PE, Gracia-Godoy F, Hargreaves KM (2007) Regenerative endodonticas: a review of current status and a call for action. *Journal of Endodontics* **33**, 377–90.

Nair PNR (1987) Light and electron microscopic studies of root canal flora and periapical lesions. *Journal of Endodontics* **13**, 29–39.

Nair PNR (1997) Apical periodontitis: a dynamic encounter between root canal infection and host response. *Periodontology* **2000 13**, 121–48.

Nair PNR (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical Reviews in Oral Biology and Medicine* **15**, 348–81.

Nair PNR (2006) On the causes of persistent apical periodontitis: a review. *International Endodontic Journal* **39**, 249–81.

Nair PNR (2014) What is on the horizon? *Journal of Conservative Dentistry* **17**, 1.

Nair PNR, Schroeder HE (1984) Periapical actinomycosis. *Journal of Endodontics* **10**, 567–70.

Nair PNR, Sjögren U, Kahnberg KE, Krey G, Sundqvist G (1990a) Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. *Journal of Endodontics* **16**, 580–8.

Nair PNR, Sjögren U, Krey G, Sundqvist G (1990b) Therapy-resistant foreign-body giant cell granuloma at the periapex of a root-filled human tooth. *Journal of Endodontics* **16**, 589–95.

Nair PNR, Sjögren U, Schumacher E, Sundqvist G (1993) Radicular cyst affecting a root-filled human tooth: a long-term post-treatment follow-up. *International Endodontic Journal* **26**, 225–33.

Nair PNR, Sjögren U, Sundqvist G (1998) Cholesterol crystals as an etiological factor in non-resolving chronic inflammation: an experimental study in guinea pigs. *European Journal of Oral Sciences* **106**, 644–50.

Nair PNR, Sjögren U, Figdor D, Sundqvist G (1999) Persistent periapical radiolucencies of root filled human teeth, failed endodontic treatments and periapical scars. *Oral Surgery Oral Medicine Oral Pathology* **87**, 617–27.

Nair PNR, Henry S, Cano V, Vera J (2005) Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after 'one-visit' endodontic treatment. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **99**, 231–52.

Obokata H, Wakayama T, Sasai Y *et al.* (2014) Stimulus-triggered fate conversion of somatic cells into pluripotency. *Nature* **505**, 641–7.

Perrini N, Castagnola L (1998) *W. Hess & O. Keller's Anatomical Plates: Studies on the Anatomical Structure of Root Canals In Human Dentition by a Method of Making the Tooth Substance Transparent (1928)*. Lainate (MI), Italy: Altini Communicazioni Grafiche.

Peters OA, Schönenberger K, Laib A (2001) Effects of four Ni–Ti preparation techniques on root canal geometry assessed by micro computed tomography. *International Endodontic Journal* **34**, 221–30.

Peters OA, Peters CI, Schönenberger K, Barbakow F (2003) ProTaper rotary root canal preparation: effects of canal anatomy on final shape analysed by micro CT. *International Endodontic Journal* **36**, 86–92.

Schaudinn C, Carr G, Gorur A, Jaramillo D, Costerton JW, Webster P (2009) Imaging of endodontic biofilms by combined microscopy (FISH/cLSM - SEM). *Journal of Microscopy* **235**, 124–7.

Sirén EK, Haapasalo MPP, Waltimo TMT, Ørstavik D (2004) *In vitro* antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on *Enterococcus faecalis*. *European Journal of Oral Sciences* **112**, 326–31.

Sjögren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. (Published erratum appears in Int Endod J 1998, 31:148). *International Endodontic Journal* **30**, 297–306.

Soutschek J, Akinc A, Bramlage B *et al.* (2004) Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* **432**, 173–8.

Spångberg LSW (2009) The emperor's new cloth. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **108**, 643–4.

Sundqvist G (1976) Bacteriological studies of necrotic dental pulps (Dr. Odont. Thesis). Umeå, Sweden: University of Umeå.

Sundqvist G, Figdor D (2003) Life as an endodontic pathogen: ecological differences between the untreated and root-filled root canals. *Endodontic Topics* **6**, 3–28.

Tachibana M, Amato P, Sparman M *et al.* (2013) Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* **153**, 1228–38.

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–76.

Vera J, Siqueira JF Jr, Ricucci D *et al.* (2012) One-versus two visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. *Journal of Endodontics* **38**, 1040–52.

Volponi AA, Kawasaki M, Sharpe PT (2013) Adult gingival epithelial cells as a source for whole-tooth bioengineering. *Journal of Dental Research* **92**, 329–34.

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–3.

Wilson M (1996) Susceptibility of oral bacterial biofilms to antimicrobial agents. *Journal of Medical Microbiology* **44**, 79–87.