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# **The lavage of pulpo-periapical wounds and its clinical outcomes**

**Running title:** Lavage of the pulpoperiapical wound

**Keywords:** wound infection, biofilm, canal anatomy, irrigation, sodium hypochlorite

## **Abstract**

A critical narrative review focusing on studies providing insight about the potential and actual effect of lavage (irrigants and irrigation techniques) on clinical, biological, chemical and mechanical outcomes of root canal treatment, including microbial load reduction, periapical healing, postoperative pain, tooth survival and dentine changes.

## **Introduction**

The specific context of this review is the effect of lavage (therapeutic irrigation of hollow organ) on clinical or sub-clinical manifestations and outcomes of root canal treatment. Root canal treatment aims to manage a spectrum of pulpo-periapical inflammatory conditions; at their two extremes lie, vital pulpectomy at one end and necrotic, infected pulp with apical periodontitis at the other. These extremes represent two biologically distinct entities with significantly different treatment outcomes (1). The inferred reverse remit of this review is also to predict or explain fundamental irrigation challenges or consequences based on outcomes. In order to achieve the aim of this paper, this review evaluates the relevant macro, micro and nanoscale interactions by considering several factors, including the:

1. challenges of lavage (irrigation) of the infected wound as imposed by the physical constraints of root canal anatomy and the further biological constraints imposed by biofilm physiology;
2. efficacy of treatment techniques in addressing both types of treatment constraint;
3. relative impact of root canal lavage on clinical outcomes; and
4. collateral damage of dentine and tooth structure, as expressed in tooth survival and dentine weakening.

## **Concept of endodontic treatment as management of an infected wound**

The pulpo-periapical wound may be defined as a spectrum of pathological states, from exposed dentinal tubules (cervical abrasion, abfraction, attrition, caries, cracks), through exposed pulp, pulpotomy wound, and pulpectomy stump, to periapical tissues.

The initial breach into dentine and associated localised inflammatory response leave the dentine and pulp potentially vulnerable to infection. The outcome of the dynamic encounter between infection and inflammation determines whether the pulp succumbs, or resolves, with the formation of a barrier (2, 3). Failure to wall off the source of inflammation leads to persistent,

chronic inflammation; the greater the number of dentinal tubules involved (effective surface area and remaining dentine thickness), the greater the injury and loss of potential recovery (4). Variations in the picture of pulp inflammation progress result in a wide spectrum of presentations (5, 6), most, amenable to management by root canal treatment.

Intraradicular infection begins with initial microbial contamination of dentinal surfaces (7), successful colonisation, propagation into the pulp cavity and growth and consolidation of a biofilm with exopolymeric substances, succession of bacterial species within the maturing community with establishment of anaerobic properties, leading to critical colonisation. This progression, evident both temporally and spatially (8-10), is predicated upon ecological factors in the root canal system (9). Microbial biofilm maturity and its influence on host defence mechanisms create a bioburden that is critical enough for a state of infection to exist. The “slow-burning” nature of a biofilm infection, with starvation and dormancy states, plays a significant part in sustaining the chronicity of periapical disease (11, 12).

The picture of intra-radicular infection spread is based on synthesis of a variety of data sources (13-18). The distribution and diversity of the microbiota within the root canal system and dentine is completely unique to the individual tooth and notably variable (16, 17), even within each niche. The patchy root canal biofilm has a variable distribution and also penetrates into canal wall dentine but remains confined to the area close to the canal lumen (19). The biofilm thickness varies from a few cells to hundreds. The relative amount and composition of extracellular polysaccharide matrix (ECPM) in biofilms within root canal systems is not precisely known but its importance lies in its influence on biofilm eradication (20). The overall ecological picture is one of a complex polymicrobial community that may function as one in response to its environment and probably to treatment as well. Variations in the nature of intra- and extra-radicular infections coupled with variations in host responses (21-23), define the diverse clinical presentations of apical periodontitis. The latter may therefore predict root canal treatment outcome (24).

Wound infections in other parts of the body follow a similar series of stages, as described for root canal infection (25). The full spectrum of pulpo-periapical diseases may be deemed, in essence, management of wound infection by lavage, consisting of decontamination of the wound and adjacent surfaces, followed by their “dressing” to facilitate healing and closure (12, 26).

### **Principles of wound infection management and challenges of lavage of the root canal space – paradigm shifts in action**

Antimicrobial debridement or lavage (antiseptics) and therapy (antibiotics) form the principal approaches to treatment of infected wounds. Challenges to the debridement and control of endodontic wound infections are imposed by four main factors. Firstly, the *biofilm* nature of the infection (12, 27-30), secondly, the stability of the infecting microbiome (26, 31, 32), thirdly the physical constraints of access to the secluded root canal space (33-35) and fourthly the nature of host response (36). This review will explore how these elements might interact to create a comprehensive challenge.

Lavage of the apical canal termini, as defined by predictable, controlled and continually sustained delivery of antibacterial fluids to the site is currently not possible without some degree of canal enlargement by abrasive or rotary shaping of the root canal system. Although the innovative, “non-instrumentation technique”, attempted to meet this elusive goal (37), it has not resulted in a commercial application.

To place into context, mechanical preparation of the “entire” wall of the root canal system was once deemed the most important phase for bacterial infection control (38). A paradigm shift in thinking has seen this phase alternatively regarded as no more than an extension of the coronal access into the root, and dubbed a “radicular access” for the subsequent irrigation (39). This is principally because about half the surface of the root canal system remains uninstrumented (40, 41) due to geometric variations in canal morphology that simply cannot be reached by available modes of instrumentation even with the use of innovative

instruments (42, 43). The consequence is that the uninstrumented wall must be debrided of biofilm by means other than “surface-planing” by metal instruments. In addition, the small volume of the root canal system imposes viscous limitations on the resident fluid dynamics (33, 35) and compromises delivery, flow and mixing of fluids throughout this space. Added to this, its variations in spatial configuration further complicate irrigant delivery and lavage.

Root canal space lavage is aided by the chemical potency of irrigants but this is limited by progressive depletion of their activity by reaction upon contact, at a rate dictated by their concentration, temperature, diffusion and mixing (45). Fresh unreacted fluid thus needs to be constantly circulated into the root canal system and spent, reacted fluid, out of the system without stagnation at the wall boundaries; a feat that is currently technically challenging.

The role of the host response in infection management has recently acquired a more prominent place (36, 46). This may be the second paradigm shift. In periodontology, improving but incomplete insight of host biology is modifying our thinking on the relative importance of the different players in the dynamic interaction between microbiota and immune response. From a different perspective, meta-analyses have questioned the efficacy of antimicrobial debridement of surface wounds because the resident microbiome forms part of its defence against pathogens. Although this concept cannot be strictly translated to the root canal system, which is not normally infected and does not harbour a resident microbiome, its principles are worthy of consideration. The approach deploys micropore particle technology (MPPT) as a passive form of immunotherapy to disrupt the weaponry used by microbiota to inhibit the immune system, allowing it to recover and challenge the infection (46). MPPT removes wound infections 60% more rapidly than antibiotics and antiseptics and promotes the healing of unresponsive chronic wounds, demonstrating the impact of the host response.

The morphological complexity of the root canal system combined with the nature of microbial infection and host response, pitted against the limitations of contemporary instrumentation and irrigation techniques serve to characterise the severity of the clinical challenge.

## **Practical aspects of chemo-mechanical debridement of root canal systems**

The cumulative chemo-mechanical effect in the root canal system is a function of the combined interaction of canal shaping, irrigant (type, concentration), lavage (irrigant delivery method, frequency, rate, agitation method), and post-irrigation medication (type, potency, activity and substantivity). Laboratory studies allow exploration of the relative impact of these variables under controlled conditions (33) but during clinical application, even standardised chemo-mechanical protocols are rendered diverse by virtue of intra-operator or inter-operator variation and influence (47, 48). The outcomes of clinical/microbiological studies on root canal treatment reflect this complexity and variation (49). Intuitive synthesis of laboratory and clinical data allow detection of underlying patterns and trends to yield new hypotheses, as well as possible pathways for treatment optimisation.

Conventional root canal treatment requires canals to be reshaped and partially enlarged by (conical) instruments to produce a conical cavity disposed longitudinally along the central axis of the canal or root; with minimum diameter at the canal terminus, and maximum at the coronal origin, the shape defined by its taper. The central axis should remain un-deviated regardless of curvature, whilst the taper should be compatible with: 1) delivery of irrigant and root filling material; and balanced by 2) maintenance of root integrity and strength. The optimal apical diameter and taper of the canal are the subject of debate due to conflicting evidence of superiority (1, 50-54). Widely tapered canals allow better irrigant penetration, debridement and obturation but they compromise root strength and long-term tooth survival (55). The clinician must optimally balance these opposing requirements and avoid unnecessarily wide canal preparation (56).

For irrigants to reach all root canal surfaces, the process of conical preparation must avoid obstructing any lateral anatomy by compaction of residual pulp tissue, dystrophic calcification

and pulp stones into it. This heterogenous mix of organic and inorganic canal content should ideally be flushed out of the canal system during preparation. The use of rotary instruments may enhance compaction of resident material into accessory anatomy (57-61) and therefore irrigants with lubricating and dissolving properties are preferable (57, 60).

Effective lavage (flushing) is restricted by the viscously-dominated environment of the root canal system (33, 35, 62). Strategies for delivering, mixing and replacing the canal irrigant must be effective. Pure flushing action is only effective for loose debris; attached canal contents such as pulp tissue must be loosened by agitation or chemical action. Flushing should occur intermittently during mechanical preparation and then after mechanical preparation is complete.

Root canal system anatomy has a dominant influence on lavage efficacy and may be classified into those that allow easier (simple) irrigant access to all walls against those that do not (complex) (63). Simple canal systems include those with narrow circular cross-sections (Type 1), readily encompassed by a regularly tapered preparation, where biofilm removal may indeed be achieved wholly by mechanical means; the irrigant then mostly acting as a flushing agent and bactericide (Fig 1). Simple canals also include oval or flattened canal configurations (also Type 1 - mandibular incisors, canines, premolars, distal and palatal roots of molars, "C"-shaped canals), where regularly tapered preparations may still leave the "poles" (as viewed in transverse section), uninstrumented, and potentially undebrided (Fig 2). However, lavage may not be that difficult in such morphology.

In complex canal systems with narrow fins, isthmi, variable lateral extensions and ramifications (mesial roots of molars, some mandibular incisors, some premolars), a tapered mechanical preparation would mechanically remove biofilms from the instrumented surfaces but not the uninstrumented accessory anatomy (Fig 3). Removal from the uninstrumented anatomy would require chemical dissolution but the agent must reach the biofilm structure in adequately potent concentration. There is potential for compaction of canal contents into such blind-ending peripheral anatomy, which may restrict access for adequate lavage. Furthermore, having

reached the biofilm, the chemical agent must penetrate in potent concentration, to the deepest layers of the multi-layered microbial biofilm with its polysaccharide matrix protection. A combination of mechanical and chemical action would synergistically enhance biofilm removal. Once the mechanical shaping is completed, a final effort should be made to reach all uninstrumented surfaces through chemical “scrubbing”. The strategies for agitating, mixing and delivering the irrigant into the complex anatomy have increased multi-fold as the need became clearer. Agitation methods include manual (hand-files, gutta-percha) (64-66), sonic (SonicAir®, EndoActivator®, Vibringe®, EDDY®) (67-69), ultrasonic (Irrisafe®, ProUltra® syringe, GentleWave®) (68, 70), pressure variation (EndoVac®, RinsEndo®, NIT system) (37, 71, 72) and laser (73-76).

Apart from mixing through agitation, the exchange of irrigant relies on diffusion along a concentration gradient from the main radicular access preparation, where the bolus is deposited towards peripheral anatomy. The extent of diffusion will be dictated by the concentration and temperature of the irrigant, frequency of replacement and the dwell time of a resident bolus of irrigant.

### **Effect of chemo-mechanical debridement on the resident microbiota**

The efficacy of chemo-mechanical debridement has been evaluated using *in vitro*, *ex vivo* and *in vivo* study models and a range of outcome measures. The effect of different stages of root canal treatment on the intraradicular microbiota has been evaluated both qualitatively and quantitatively through bacterial sampling and detection or microscopy. Studies either report positive cultures or identify and quantify intraradicular taxa detected before and after various stages of treatment using culture-dependent (14, 77-85) or culture-independent (86-96) approaches.

In the majority of cases, chemo-mechanical debridement is better able to decontaminate the middle and coronal parts of the root canal system than the apical, where bacterial biofilm may

remain virtually intact (97). Additional antibacterial measures effective in the inter-appointment period help control the residual apical infection (98, 99).

'Mechanical preparation' of canals coupled with lavage using water or saline (14, 100) may yield negative cultures on average in 25% of the cases (range 4.6–53%). When coupled with lavage using sodium hypochlorite (concentration range 0.5–5.0%), the frequency of negative cultures may increase to an average of 75% (range 25–98%) (77, 78, 101). The antibacterial action reduces the number of bacteria from an initial range of  $10^2$ – $10^8$  cells per unit sample to  $10^2$ – $10^3$  cells after debridement, further reducing down to no recoverable cells (from the sampled part of the root canal system) after inter-appointment dressing with calcium hydroxide (102, 103). The bacterial load reduction is directly but negatively influenced by the diversity of the initial infection (14, 90, 93).

Longitudinal sampling has not revealed persistence of any particular species (14, 77, 78) during debridement, excluding specific bacteria as a cause of persistent infections. However, samples from previously root-filled canals show certain species to be more prevalent pre-operatively, suggesting that resistant types possibly do exist. These include *Enterococcus faecalis*, *Streptococcus* species, *Staphylococcus* species, *Lactobacillus* species, *Propionibacterium* species, *Actinomyces* species, yeasts and other Gram-positive bacteria (104-108).

A monkey-model study (109) shed some light on this difference, in finding that facultative bacteria were more resistant to chemo-mechanical treatment than anaerobic species in a 4-strain infection (*Streptococcus milleri*, *Peptostreptococcus anaerobius*, *Prevotella oralis*, *Fusobacterium nucleatum*) compared to a 5-strain infection that included *Enterococcus faecalis*. The addition of *E. faecalis* made the consortium more difficult to eradicate.

Persistent root canal bacteria decrease the probability of periapical healing by an average of 12% (range 0–26%); the healed rate for teeth with no detectable bacteria was 80% in one study compared to 33% for those with pre-obturation bacteria (104). Another monkey-model study (110) corroborated these findings in showing that when bacteria remained, 79% of the root-filled

root canals were associated with non-healed periapical lesions, compared with 28%, when no bacteria were found. A more diverse residual microbiota more frequently leads to non-healed lesions.

### **Modes of chemo-mechanical effect on microbiota**

Typical and favoured root canal irrigants display multiple modes of effect on microbial biofilms (28), through disruption by dissolution or killing of microbial cells by penetration of the extra-cellular polysaccharide matrix (ECPM). Different agents exhibit different combinations of properties in these respects, which may be characterised visually by polygonal graphs (Fig 4).

Microbial biofilms contain an aggregate of microbial cells and ECPM in variable proportions depending on diversity of the microbiota and nutrient availability. Disruption involves organic tissue dissolution by sodium hypochlorite or break-up by chelating agents such as EDTA (28, 111, 112). EDTA chelates and sequesters heavy metal ions that act as bridges to bond bacteria together in the biofilm (113).

The most significant action of the irrigant is its ability to reach and kill microbial cells. In this respect, sodium hypochlorite is the most potent, followed by iodine and then chlorhexidine but only sodium hypochlorite displays the dual benefits of both disruption and killing (28, 114).

Biofilm communities are inter-dependent amongst its constituent members (115); interference with the ecology of the niche, even in a nonspecific way, may kill the fastidious members, triggering a chain reaction akin to a “domino effect” by deprivation of nutrients or stimulants (quorum sensing) to their neighbouring partner species (116). Root canal irrigation therefore probably works by a combination of direct and indirect killing effects. The importance of indirect killing is underestimated in modern root canal treatment and is why, knowledge of biofilm physiology and dysbiosis (32) may be therapeutically, highly advantageous.

Multiple resistance or survival mechanisms in biofilms have encouraged multiple avenues of attack to optimise their eradication, known as “multiple concurrent therapy” (29, 117). Whilst multiple chemical agents may potentiate each other (NaOCl alternated with EDTA), they may

also neutralize each other, or have a negative effect (NaOCl and chlorhexidine) (118-120). A long-established method for potentiating chemical effect is to agitate the irrigant to cause mixing and interference with chemical equilibria (45, 121).

The inability to predictably replenish irrigants in the apical anatomy, allows the resident microbial population to survive in the majority of cases (97, 99). Nevertheless, the paradoxically high probability of periapical healing, coupled with slow healing dynamics, suggest that the continued survival of apical microbiota is dependent upon their interaction with host defences (98).

The surviving organisms may also seed recontamination and reinfection of the root canal system, so insufficient attempts at chemo-mechanical debridement may result in a more recalcitrant infection through partial killing and regrowth (14, 77). It is therefore biologically most sensible to launch the most comprehensive lavage effort at the first attempt.

### **Effect of chemo-mechanical debridement on periapical healing**

The principles of root canal treatment were established long ago (122) based on the notional aetio-pathogenesis of apical periodontitis and the intuitive premise that controlling intra-radicular infection should calm or switch-off the pro-inflammatory stimulus. Thus, chemo-mechanical preparation alone should be sufficient to achieve periapical healing (123), the further step of obturation serving to control any residual infection and recontamination (110). Technological advances have made root canal preparation, irrigation and root-filling procedures more efficient but these have not improved their efficacy over an entire century (47). Nevertheless, clinical outcome studies have helped forge a clearer understanding of the factors influencing positive outcomes.

Root canal treatment outcome, based on absence of signs and symptoms, coupled with radiographic evidence of periapical lesion resolution, show the mean success rate to be 83% after vital pulpectomy but reduced to 72% when aimed at controlling established infection associated with a periapical lesion (1). This suggests the treatment rationale for teeth with or

without apical periodontitis is biologically different. In the absence of a periapical lesion, the main treatment goal will be to prevent introduction of microbial infection into the apical anatomy. The treatment focus is therefore on prevention of microbial contamination and infection spread and requires meticulous maintenance of a sterile field and a crown-down approach to avoid contaminating apical pulpal and periapical tissues from any coronal infection. The technical and biological challenges posed are therefore less severe, reflected in the fact that a wide variety of chemo-mechanical protocols suffice and yield equally high success rates by adhering to the main principles. The emphasis in irrigation in such cases will be on dissolution and flushing out of residual or necrotic pulp tissue, for which the irrigant of first choice is sodium hypochlorite (124, 125) .

In the case of established periapical disease, the treatment challenge, in addition to prevention of contamination, is to eradicate bacterial biofilm from the apical root canal anatomy to help switch off the host immune response and stimulate wound healing. Larger periapical lesions signify a greater host response, often associated with more diverse microbiota, both of which pose a more serious challenge. Management of established periapical lesions, therefore, demands more exacting chemo-mechanical protocols, as these have a greater impact on outcome. Efforts to effectively deliver and debride the apical anatomy through irrigation are therefore crucial; and by intuitive inference, the need to maintain patency of all exits to reach their resident microbiota (24).

Treatment factors having a major impact on root canal treatment outcome are apical extent of instrumentation (and thus irrigation) and root filling to the canal terminus, microbial culture test result prior to obturation, quality of root filling (as surrogate measure of quality of entire procedure), and quality of the final coronal restoration (24).

Treatment factors not having a strong impact include the specific nature of the treatment protocol (method of canal preparation, irrigation regimen, root-filling material and technique); the sole treatment factor having a powerful effect is apical proximity of treatment to the canal

terminus, without extrusion. The intuitive inference is that *all* root canal treatment protocols encounter the *same* powerful apical buffer, neutralising and equalising their apical impact (24). Laboratory studies show these apical buffers to be the phenomenon of “apical stagnation zone” and “apical vapour lock”, which obfuscate attempts to control the apical microbiota (33). The latest multi-sonic device (GentleWave®) gave promising outcomes of 92% healed, one year post-operatively (126, 127), although the majority of these were vital pulpectomies, which would not have had established apical infections.

### **Effect of chemo-mechanical debridement on post-operative pain**

Patients undergoing root canal treatment should anticipate some post-operative discomfort or pain that gradually resolves over 3-7 days. Numerous factors influence the occurrence of such pain, which varies widely in prevalence; pain experienced within one day post-treatment has a pooled mean prevalence of 40%±24%, reducing to 11%±14% by day 7 (128). These outcomes though may include persistent preoperative pain (128), as well as the effect of both chemo-mechanical debridement and root filling.

Pain experienced specifically after root canal debridement ranges even more widely from 2% to 90% (129-142) and reduces to 10% by day 7 (141). The factors influencing such pain have not been systematically evaluated and a literature search only yields comparisons on isolated and discrete questions.

Root canal debridement of teeth associated with apical periodontitis up to the canal terminus or 1 mm short of it, did not have a significant effect on post-operative pain (142). Debridement using reciprocation of the WaveOne® system coupled with 2% chlorhexidine caused significantly more severe post-operative pain of longer duration, compared with rotational use of the ProTaper® system using the same irrigant (143); the difference was attributed to the shorter duration of canal preparation, resulting in shorter irrigation periods with the former system (143). Irrigation regimens applied to teeth with apical periodontitis also reveal that an antiseptic irrigant (Solvidont®) may result in a significantly lower incidence of post-operative pain compared to

saline (144). Other studies, however, found no significant difference in postoperative pain, when comparing NaOCl (5.25%) with chlorhexidine (2%) (145); or the supplementary use of EDTA (17%) or MTAD with NaOCl (146).

The employment of higher (5.25%), rather than lower concentration (2.5%) of NaOCl in teeth with irreversible pulpitis, results in a significantly lower frequency of post-operative pain (147). Agitation of NaOCl in the canal did not have any significant influence on post-operative pain, regardless of the agitation method (gutta-percha pumping, sonic or ultrasonic) in teeth with apical periodontitis (148, 149). In contrast, another study found that agitation of NaOCl (3%) with a sonic device (EndoActivator®) in symptomatic irreversible pulpitis with moderate to severe pain preoperatively, resulted in significantly less postoperative pain and analgesic intake than when agitation was not employed (150). The use of a negative pressure device (EndoVac system®) for NaOCl delivery in teeth with vital pulps resulted in significantly less immediate post-operative pain compared to when it was delivered through a conventional syringe (151, 152). These apparently random observations do coalesce into some form of a logical pattern when analysed in the context of irrigation dynamics coupled with biological interplay.

### **Effect of chemo-mechanical debridement on tooth survival**

The 5-year survival rates for root-filled teeth are above 90% (55, 153), whilst the 10-year survival rates are above 80% (55). The only irrigation factor influencing tooth survival positively was *achieving patency at the canal terminus* during chemo-mechanical debridement (153). Although, “tooth or root fracture” was the most common reason for extraction of root-filled teeth in one study (153), there was no evidence that root canal debridement regimens caused this. This may mean that irrigation has no significant effect or that the relevant parameters have not been investigated adequately in clinical studies. It is therefore prudent to explore the effect of irrigants and root canal treatment procedures at tooth and dentine level in laboratory studies, to gauge the impact of any sub-clinical changes.

## **Effect of chemo-mechanical debridement on chemical and mechanical properties of dentine**

Canal dentine suffers collateral damage during root canal treatment due to mechanical (instrumentation), chemical (NaOCl & EDTA) and thermal (heated materials and instruments) stresses. The procedure leads to profound changes in the physical (154), mechanical (155-157), and chemical (158-160) properties of root dentine.

NaOCl acts predominantly on the organic component of dentine, with no (158-161) or some (162-164) effect on the mineral content. NaOCl denatures or dissolves collagen in dentine (158, 160, 162, 165, 166), which reduces its elastic modulus, microhardness, flexural strength (157, 167) and visco-elasticity (168). It also increases strain upon loading of dentine or whole teeth (156, 169).

The effect of NaOCl on dentine mineral content varies with study. A number (162, 170-172) found a decrease in the carbonate content of dentine following treatment with NaOCl, whereas others (158, 165) reported the carbonate and phosphate FTIR peaks to remain constant. Demineralising agents do not affect collagen, *per se* (158-160) but may disrupt the matrix and alter its fibrillar structure, leaving it exposed to further damage from NaOCl (160, 165, 166, 173). All the described mechanical and physical changes resulting from exposing dentine to NaOCl can be ascribed to a loss or denaturation of collagen in dentine. NaOCl and EDTA independently influence dentine visco-elasticity in different and self-limiting ways over the duration of exposure but cause accumulative damage if they are used alternately (158, 168, 174).

Root dentine may become heated during root canal treatment (175) for a variety of reasons, including irrigation with heated NaOCl (176), thermoplasticisation of gutta-percha, and use of ultrasonic or rotary tools. Heat may cause loss of unbound dentine water through evaporation, as well as loss of bound water at temperatures above 200°C (177). The contribution of water to the viscoelastic properties of dentine (178) means its loss may alter the mechanical properties

of teeth significantly (179-181). Heat may also affect dentine by altering collagen properties. Collagen, consisting of 3 helical polypeptide chains bound together by hydrogen and covalent bonds into a super-helix (164), undergoes various temperature- and time-dependent changes (182). Collagen structure is reportedly altered to different degrees at different temperatures (20-200°C) and levels of hydration and physical confinement (182-188). At temperatures above 60°C, vibrations between molecules have sufficient energy to break hydrogen bonds and covalent cross-links. Consequently, the tertiary tri-helical structure of collagen is denatured into a random coil (184) with a reduction in its tensile strength and a loss of its physical properties (184, 189, 190). This is reflected in the fact that NaOCl reduces the flexural strength and elastic modulus of dentine bars (155, 157), as well as their visco-elasticity (168).

Exposure of dentine bars to direct heat and NaOCl, independently and accumulatively, produces moderate changes in quasi-static mechanical properties but more obvious and marked changes in viscoelastic properties measured by dynamic mechanical analysis (191). Kafantari (2011) (192) found that heated NaOCl (60°C or 80°C) significantly increased the visco-elastic behaviour of dentine bars tested by dynamic mechanical analysis (DMA), compared to NaOCl at room temperature.

Despite the insight about the potential for such damaging effects, the precise depth of effect of irrigants on dentine had been unclear. Ramírez-Bommer *et al.* (2018) (160) estimated the depth of effect in pulverised dentine, alternately exposed to NaOCl and EDTA, using FTIR; NaOCl reduced the collagen content within the first four minutes of reaction, leading to a plateauing effect, thereafter. Conversely, EDTA continuously reduced the phosphate content of dentine over twenty-four hours and exposed the collagen content in the process. The depth of hypochlorite reaction was  $16 \pm 13 \mu\text{m}$  after 10 minutes exposure, whilst the depth of EDTA reaction increased with duration of exposure ( $19 \pm 12 \mu\text{m}$  by 10 minutes,  $27 \pm 13 \mu\text{m}$  by 60 minutes, and  $89 \pm 43 \mu\text{m}$  by 24 hours) and also by pre-treatment with NaOCl ( $62 \pm 28 \mu\text{m}$  by 10 minutes). NaOCl/EDTA/NaOCl alternated treatment resulted in an estimated further  $62 \pm 28 \mu\text{m}$

plus  $7\pm 4$   $\mu\text{m}$  thick collagen-depleted surface compared to the  $16\pm 13$   $\mu\text{m}$  depletion by initial NaOCl treatment, alone.

Morgan *et al.* (2018)(193) used an FTIR protocol for judging the depth of effect of NaOCl irrigation in an *ex-vivo* tooth model, *in situ*; the effect on dentinal collagen extended to at least 0.5 mm from the canal wall. This supports Zou *et al.* (2010)(194), who assessed the depth of penetration of NaOCl to be 0.3 mm using its bleaching effect on crystal violet dye as a tracer. The depth of effect of irrigants on dentine collagen is likely to be a function primarily of penetration along dentinal tubules, but secondarily and over longer time periods, as a function of inter-tubular matrix degradation. The extent of any tooth weakening is likely to be dictated by the breadth and depth of chemical changes in dentine, *relative to the remaining bulk of unaffected dentine* (156, 160, 193).

### **Concluding remarks**

Residual infection in the complex apical anatomy of teeth with apical periodontitis is the norm after root canal treatment (97, 99), yet long-term follow-up shows progressive periapical healing, even though it may take 12 months or more (47), sometimes even 20 years (195). The implication is an ongoing interaction between residual apical infection and host response beyond the active treatment (98). It is predicted that both residual infection and host defences play a definitive role in determining the final biological outcome. This explains why, despite variations and changes in the technical aspects of the chemo-mechanical protocol, the success rates of root canal treatment have not improved over the last century (47). It also explains why the success rates are so sensitive to the apical extent of root canal debridement and further why, the periapical lesion can take so long to heal beyond the end of the treatment procedure. Future challenges to improving success rates or make them more predictable depend upon effective research in biofilm physiology and dysbiosis, host response (including genetics), treatment effects on microbiota and host, and the physics of apical fluid dynamics. Viewing the

management of pulp and periapical disease as an infected wound that requires adequate lavage should yield important benefits in treatment success.

The most effective root canal debridement protocols unfortunately have a collateral damaging effect on dentine; but fortunately, the depth of this effect seems to be limited to the inner 0.5 mm of dentine adjacent to the canal wall. Treatment protocols should facilitate periapical healing without damaging tooth structure.

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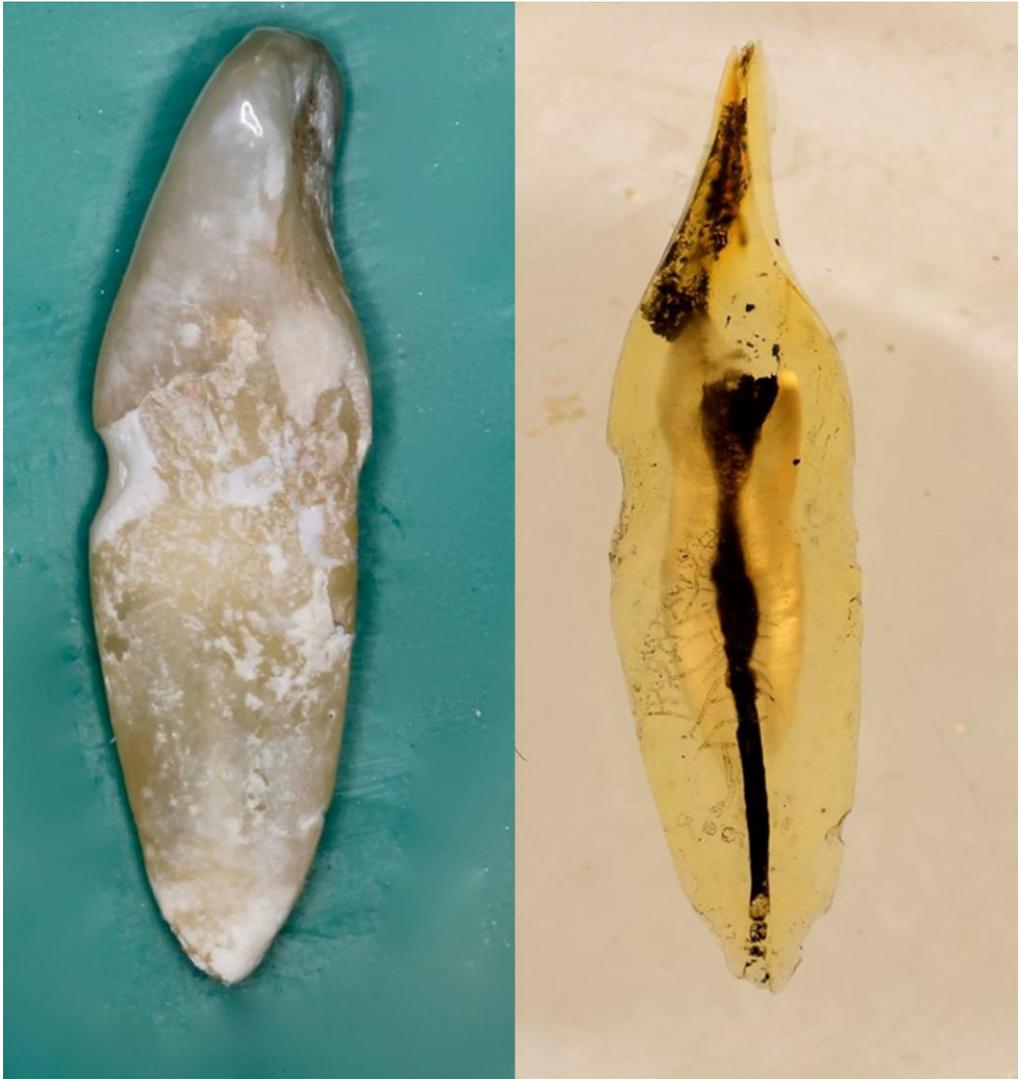


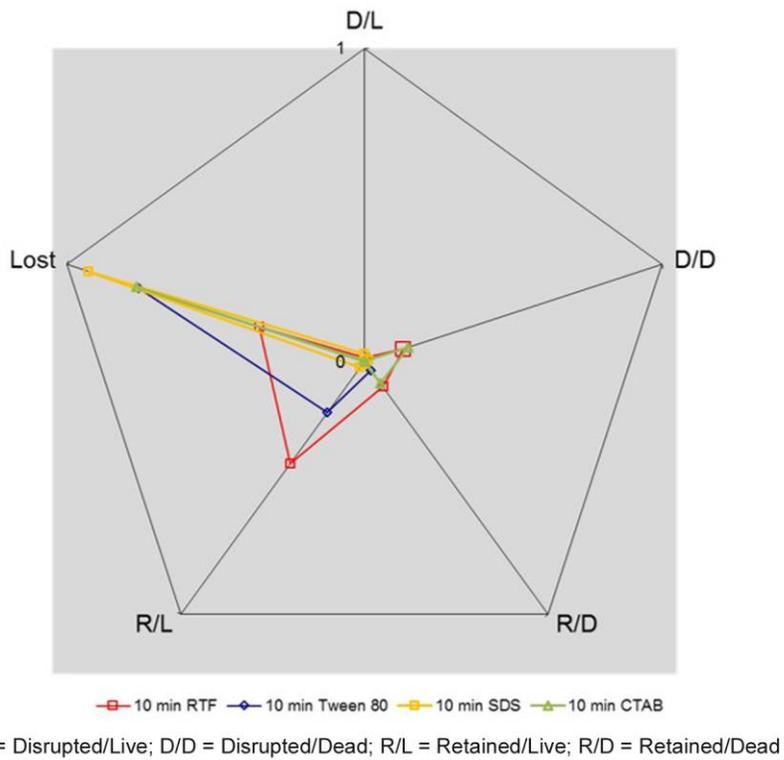
Figure 1. Representative images of a cleared tooth with Type 1 root canal system.



**Figure 2. Representative images of a cleared tooth with Type 2 root canal system.**



Figure 3. Representative images of a cleared tooth with Type 3 root canal system.



**Figure 4 – Five-point polygraph to depict relative disruption and killing effects of irrigants.**