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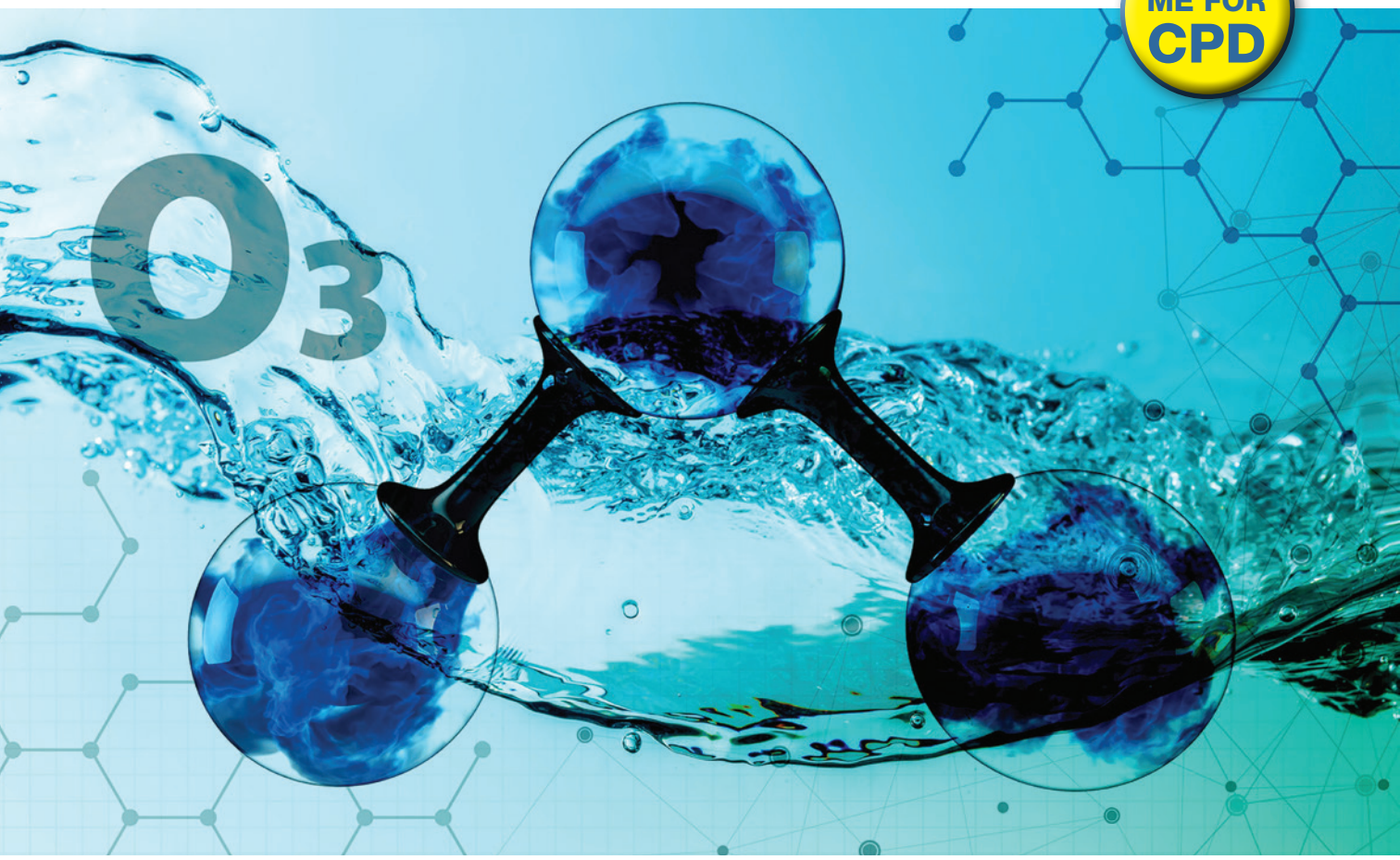

Ozone in water: Let the recent evidence speak

By Emeritus Professor Laurence J. Walsh AO

In recent articles, I've discussed the use of novel oxidant-based technologies in infection prevention and control. This article explores the recent literature on ozonated water, drawing particularly on high level evidence from systematic reviews, meta-analyses and clinical trials that are cited in the PUBMED database.

Ozone is a well-known and potent oxidant which is a powerful antimicrobial agent in its aqueous form, as it dissolves readily into cold water. Effects of ozone will depend on the concentration and the application time.

As well as common pathogenic oral bacteria (such as *Streptococcus mutans* and *Porphyromonas gingivalis*) and fungi (such as *Candida albicans*), ozone is known to be effective against both enveloped and non-enveloped viruses and amoeba, even at sub-ppm levels such as 0.1-0.4 ppm.[1-6]

In terms of delivery systems, ozone can be delivered as the gas dissolved in water or in oil, or used in its natural gaseous form, which is the most unstable. Most research has examined the use of ozonated water, since this can be produced on demand to give consistent ozone levels and is suitable for common applications such as irrigation, mouth rinsing and dental unit waterline treatment. Hence, the emphasis in this article will be on ozonated water. The special situation of oils is discussed at the end of the article.

Ozonated water in the COVID-19 pandemic

In industry, ozone dissolved in water is used extensively in wastewater treatment and in water recycling, where its powerful oxidizing actions and disinfecting properties can render water free of organic contaminants and microorganisms.

These same features have made it attractive in the COVID-19 pandemic as an antiviral agent for use in pre-procedural rinsing, as recommended in the advice issued in 2020 by the ADA Infection Control Committee for transmission-based precautions. Comparative clinical studies of ozonated water versus chlorhexidine and povidone iodine in terms of their effects on microorganisms in dental aerosols generated by scaling have shown similar effects in terms of reducing both aerobic and anaerobic bacterial colony forming units (CFU). Moreover, ozonated water can be generated by a purpose-built unit and delivered through a tap, making it easy to collect water for mouth rinsing, to perform hand hygiene and to rinse impressions or items of equipment. For the latter, an example is decontamination of the chamber of the ultrasonic cleaner at the end of the working day. Ozonated water is highly effective for this purpose.[7]

A built-in water ozonating system can deliver water directly into the waterlines of dental units, so that the cup fill and coolants delivered into the mouth also can provide some antimicrobial actions. The available ozone level as the water exits the dental chair will depend on the temperature, pH and other parameters that influence the half-life of ozone in water, as well as the flow rate and the tubing volume of the dental chair.[8]

It is well known that sub-ppm levels of dissolved ozone in water exert powerful effects on oral microorganisms, including bacteria and fungi as well as viruses. This is why rinsing the oral cavity with ozonated-water (e.g. 0.1-0.4 ppm) will suppress microbial growth and slow biofilm formation and will also disrupt existing biofilms because of microbial cell lysis.[9]

When used at levels up to 10 ppm, ozone in water does not adversely affect the bonding of adhesive restorative materials or orthodontic brackets to tooth structure.[10,11]

The broad-spectrum actions of ozonated water make it useful for applications where inactivation of viruses is the objective. To this end, ozonated water has been used for the disinfection of contaminated dental casts. It causes less surface changes in micro-roughness than diluted sodium hypochlorite.[12] Likewise, it has been used for disinfection of silicone and alginate impressions, causing less microscopic changes to the impression surface than sodium hypochlorite.[13,14]

Dental unit waterline biofilm control

Contamination of dental unit waterlines (DUWL) with heterotrophic bacteria is an important issue in infection prevention and control in dental practice. Bacteria and other microorganisms in waterline biofilms can cause health problems in elderly and immune compromised patients. Many dental practices now use ozonated water with low levels of ozone to address this issue. Ozone treatment inactivates microbes present in reticulated tap water, making the water entering the chair sterile and hence unable to serve as an inoculum for further biofilm development within the waterlines. Moreover, as freshly ozonated water enters the waterlines, any biofilm remnants will be attacked and broken done.

DUWL biofilms can contain not only bacteria, but also fungi and amoeba. Ozonated water is highly effective against both fungi and amoeba when they are present in DUWL, including when in the forms of fungal spores or cysts, which represent the most difficult and resilient forms of these organisms.[15]

Given these microbial challenges, chemical treatments of water entering dental units are commonplace. Consequently, ozone has been used to treat DUWL for more than 30 years.[16,17] Recent studies provide strong support for using ozonated water for overcoming problems associated with DUWL. A 2019 study of the effects of low concentration (0.4 ppm) ozonated water as a means to limit biofilm growth measured bacteria in DUWL exit water and also examined dental unit components to assess if any harmful chemical effects occurred. Ozonated water was found to be highly effective against heterotrophic bacterial biofilms. Moreover, it was not harmful to dental chair internal components. This confirms its value for preventing DUWL contamination over the longer term.[18]

Useful clinical effects on pain and inflammation

As well as being a potent antimicrobial agent, ozone dissolved in water exerts additional actions, including the modulation of pain and inflammation, which make it of interest to dentistry for a wide range of clinical applications where antimicrobial actions and anti-inflammatory or immune modulating effects are desirable.[19,20] A noteworthy example of this is limiting postoperative pain after extractions. Ozonated water used postoperatively as an irrigant over 1 week is superior to both ordinary saline and to povidone iodine in terms of reducing pain and preventing alveolar osteitis (dry socket).[21,22] Ozone in a gel has been shown in another study to reduce postoperative pain, swelling and trismus and hence patients had lower requirements for analgesics.[23]

In medication-induced osteonecrosis of the jaw due to bisphosphonates, ozone combined with debridement using piezosurgery has been shown to give an overall success rate of 64%, with healing of the lesion when multiple applications of ozone were undertaken over 10 weeks.[24,25] Similar positive results have been seen for cases of MRONJ caused by denosumab or bevacizumab.[26,27]

Likewise, topical ozone therapy has been shown to improve healing of the oral mucosa healing in cases of chemotherapy-induced ulcerations and mucositis. This has been attributed to the combination of its antimicrobial and anti-inflammatory actions, which prevent supra-infections and promote healing, respectively.[28]

The benefits of using ozonated water rinses for reducing the bacterial counts and encouraging the healing of chemotherapy-induced oral ulcers has been demonstrated conclusively in animal models of chemotherapy-induced oral mucositis stomatitis.[29] One contributing mechanism to the positive effect of ozone on wound healing is its strong induction of vascular endothelial growth factor (VEGF), which is elevated for 7 days after a single topical application.[30]

Safety for human tissues

All the applications of interest to dentistry involve the use of very low levels of ozone - in the low ppm range for topical use or even in the sub-ppm

range for water treatment. These low levels avoid issues with toxicity. Healthy human cells contain antioxidant systems such as those based on vitamins C and E, which protect cells against harmful effects of oxidation.[31-34]

In cell culture studies, ozone has good biocompatibility with periodontal cells and gingival fibroblasts. In keeping with this, clinical trials have not documented any adverse effects on oral soft tissues. This includes studies in children. A recent study of 10-12 year old children who used ozonated water or chlorhexidine as a mouthrinse for 15 days did not find any issues with tolerance of the ozonated water. The authors concluded that ozonated water was preferred for use in children over chlorhexidine, as it was free of adverse effects such as staining and taste alterations.[35] This finding validated earlier research from 2017 which compared ozonated water to chlorhexidine when used daily over 14 days in patients with high caries rates. Ozonated water was superior to chlorhexidine for suppressing salivary levels of *S. mutans*.[36]

Similar positive conclusions regarding ozonated water as an alternative to chlorhexidine were reached in clinical trials that compared plaque and gingival parameters in orthodontic patients. An ozonated water rinse yielded better outcomes than chlorhexidine in the management of gingivitis in these orthodontic patients.[37]

Ozone in periodontal therapy

Periodontal debridement

Using ozonated water rather than regular water as the coolant for ultrasonic scaling is a simple substitution to make. Studies show that this gives an enhanced reduction in bacterial load and can be of benefit in non-surgical periodontal treatment cases with moderate to severe chronic periodontitis.[38]

Similar benefits have been seen in the recent clinical studies when conventional periodontal debridement (full-mouth scaling and root planning using hand instruments) is combined with ozonated water irrigation using a 22-gauge needle. Ozonated water irrigation improves the clinical outcomes of mechanical debridement.[39,40] A similar study published in 2020 showed that ozonated water used as the coolant for an ultrasonic scaler for the initial therapy of patients with moderate to severe chronic periodontitis eradicated the key periodontal pathogens *T. forsythia* and *T. denticola*.[41]

A 2020 meta-analysis that included data from six studies concluded that ozone therapy was an effective adjunct to scaling and root planing in the treatment of periodontitis.[42] Of note, prior studies up to 2019 have shown more mixed results, with no difference when scaling and root planing plus sulcus irrigation with ozonated water were used, when compared to scaling and root planning plus irrigation with 2% chlorhexidine gluconate. Issues with 12 studies published between 2010 and 2019 on the adjunctive clinical effect of ozone therapy on nonsurgical periodontal treatment include heterogeneity across the studies, confounding factors and short follow-up periods. Hence, additional work is needed to give definitive clinical protocols for the optimal clinical outcomes.[43,44]

Ozonated water and implant dentistry

Implant production

Using ozonated water during production can be useful for decontaminating the implant surface, as it removes oils and other organic contaminants.[45] The same approach of using ozonated water for removal of organic surface deposits has been used with screw- and cement-retained prostheses, to eliminate contamination that has occurred during their fabrication in the dental laboratory.[46]

In the later stages of the production of dental implants, ozonated water containing 20 ppm ozone and 100 mM calcium chloride may be a useful approach for modifying the surface of roughened titanium, since using this approach calcium is deposited on the implant surface and cell attachment is enhanced, while adhesion of bacteria is reduced.[47]

Implant placement

Ozonated water has been used to irrigate implant osteotomy sites as an alternative to saline. A recent study published in April 2021 used ozonated water at 25 μg/mL concentration, with 30 patients in each group. Patients were assessed by evaluating pain, wound healing and levels of C-reactive protein (CRP). Overall, the study demonstrated that using ozonated water decreased pain and inflammation and accelerated wound healing. Pain levels were lower at 2 days and 7 days post-operatively, while soft tissue healing indices were significantly higher at both 7 and 14 days post-operatively, compared to saline. CRP levels were 84% lower. The ozonated water did not cause any side effects.[48]

Treatment of biofilms on implant surfaces

Ozonated saline solution has been considered as a possible irrigant for helping to destroy biofilms on titanium implant surfaces. In a recent study published in November 2020, ozonated saline was used to treat both single and multi-species biofilms of *Porphyromonas gingivalis* that had been grown on titanium for 5 days under anaerobic conditions. Biofilms were treated with an ozonated saline solution at different concentrations for 1 minute. The effect was compared to 0.12% chlorhexidine and to conventional saline solution (0.89% NaCl) without added ozone. The ozonated saline solution containing 80 µg/NmL ozone exerted antibiofilm activity when used for 30 seconds, reducing *P. gingivalis* viability, with 2.78 and 1.7 log reductions in CFU for single and multi-species biofilms, respectively, over ordinary saline. Its effects were far superior to chlorhexidine, which gave log reductions of only 1.4 and 1.2 under the same laboratory conditions.[49]

In light of its antibiofilm activity, ozonated saline is a promising candidate therapy for the treatment of peri-implant diseases. In more severe cases of peri-implantitis where implant surface decontamination procedures are combined with reconstructive surgical treatment (RST), decontamination of the implant surface by irrigation with ozonated water has been suggested. A recent study compared this approach to decontamination of the implant surface using sterile saline. Following surgery, the ozonated water treatment led to significantly higher clinical attachment levels and better radiographic bone fill of the defects at 6 months. Using ozonated water for implant surface decontamination also gave a notable reduction in inflammation.[50]

Endodontics

Endodontic irrigation for vital pulp therapy

Ozonated water (2-4 ppm) may be a suitable irrigant in regenerative endodontics and vital pulp therapy because of its potent antimicrobial actions as well as its favourable effects on vital dental pulp cells and its low cytotoxicity.[51,52]

Irrigation

For conventional anterograde endodontics, useful effects have been documented for ozonated water as an irrigant when activated by laser pulses or by ultrasonics, including on biofilms of *Enterococcus faecalis* in the root canal system.[53,54] There have not as yet been sufficient detailed clinical trials of ozonated water as an irrigant to make valid assessments of its adjunctive use as a terminal disinfection step.[55] A systematic review of 8 studies comparing ozone with sodium hypochlorite concluded that ozonated water should not be used to replace conventional sodium hypochlorite irrigants.[56]

Oral mucosal disease

Oral lichen planus

Ozonated water has been used in the treatment of erosive oral lichen planus (OLP), as an adjunct to traditional topical therapy (such as rinsing with betamethasone). Clinical trials have shown that patients treated with betamethasone and ozonated water as a combined therapy experienced significantly greater improvement in symptoms of pain and had a higher rate of overall improvement than those using the steroid rinse only.[57,58]

A systematic review of 55 trials of treatments for erosive OLP published in March 2021 summarised the benefits of adding ozonated water on to topical corticosteroids. This improved pain resolution compared to placebo from 3.18 for topical corticosteroids to 9.9 fold for topical corticosteroids plus ozonated water. Likewise, rates of clinical resolution versus placebo also improved, from 13.6 fold for topical corticosteroids, to 52 fold for topical corticosteroids plus ozonated water. The authors of this systematic review concluded that topical corticosteroids should be the first-line method for treating oral lichen planus and commented that adding on ozonated water had a very useful benefit. Using ozonated water was free of adverse effects, which distinguishes it from other alternatives such as topical cyclosporine.[59]

Fungal diseases

Candida albicans is a major cause of oral fungal infections. Candida-associated denture stomatitis is common among denture wearers. Ozonated water exerts anti-fungal actions and also disrupts fungal biofilms. Based on these actions, ozonated water is a useful strategy to include in denture stomatitis management.[60,61]

Ozonated oil

Olive oil can be dosed with ozone. Other oils have also been used, including sunflower, castor and almond oil. An important caveat is that the antimicrobial and therapeutic actions of such ozonated oils are not due to ozone. Rather, they are due to products formed by the ozonation of mineral oils, such as formaldehyde and not to the ozone itself, which has a very short half-life. Contact with ozone breaks double bonds between carbon atoms of lipid oil molecules, resulting in unsaturated molecules including formaldehyde and other end products.

A study of ozonated oils found that none contained any free ozone, when tested using the sensitive indigo trisulfonic dye test. However, all the oils were found to contain formaldehyde. The presence of this compound explains why ozonated oils can exert antimicrobial actions, despite having been manufactured several months earlier. Hence, one cannot compare directly the effects of ozonated oils with those of ozonated water, where there is only dissolved ozone gas and ozone radicals as active agents - both of which convert to normal di-atomic oxygen.[62]

Several commercial ozonated olive oil products have been developed for the European market, including Ialozon Blu and Ialozon Rose mouthwash (Gemavip, Cagliari, Italy). As would be expected from their formaldehyde content, these oils exert antimicrobial actions against oral bacteria, including *Streptococcus mutans*, at dilution factors up to 1:32.[63] Likewise, ozonated olive oil also exerts antifungal effects against *Candida albicans*.[64]

The use of an ozonated olive oil-based mouthrinse over 3 months by patients with periodontitis has been shown to improve the results of non-surgical periodontal treatment, with lowered levels of salivary matrix metalloproteinase-8 and reduced bleeding on probing and probing pocket depths when used after periodontal debridement, compared with treatment using debridement alone.[65]

Studies of ozonated oils have given variable outcomes. One study which compared ozonated olive oil as an adjunctive subgingival irrigant to chlorhexidine in the non-surgical treatment of patients with chronic periodontitis found that both had similar effectiveness, with improvements in clinical indices as well as reductions in subgingival levels of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.[66] Given concerns regarding the safety of formaldehyde, long term use of such oils as topically applied oral care products cannot be supported by this author.

Practical applications of ozonated water and current technology

The first major application of ozonated water is point-of-care treatment systems built into the joinery of the dental practice with the ozonated water delivered via a tap. The treated water has multiple applications, including use as a pre-procedural mouthrinse, hand washing (for both staff and patients), feed water for a dental chair with a bottled water system, irrigation for stand-alone devices such as ultrasonic scalers, rinsing instruments, rinsing of S traps and suction systems, decontamination of impressions and environmental cleaning. Mint Devices Australia introduced the Kona Infection Control tap™ (KIC tap) system and this has been installed in many dental clinics as well as in residential aged care facilities. This system has a patented electrically powered ozone gas generator and valve system and a Venturi mixing tube, in a fully contained enclosed under-bench unit that is activated by a sensor to provide treated water on demand. KIC taps give a standardised level of ozone in water. From a regulatory perspective, as a disinfectant treatment, KIC taps are TGA approved (ARTG 315409). For installation in Australia they also are WaterMark™ approved, which means that

they comply with Australian certification requirements for plumbing products (AS/NZS 3718:2005 Water supply - tap ware) and can be connected into normal plumbing as a permanent installation. The rapid viral and bacterial inactivation caused by the treated water (10 seconds) makes this a very practicable approach in dental clinic settings, for enhancing infection prevention and control. These units will produce up to 10,000 continuous hours of ozonated water (0.8 - 1.4 ppm) before the treatment cartridge needs to be replaced.

Figure 1-2. The Kona Infection Control (KIC) Tap and the EnozoPro spray bottle ozonate tap water on an on-demand basis. Both are available from Mint Devices.

A second major way for generating ozonated water is using the Active Diamond Electrolytic Process Technology (ADEPT). The EnozoPro™ system was introduced by Mint Devices Australia. This is an on-demand ozonated water-producing spray bottle device for environmental sanitizing based on ADEPT, with the system built into the top of the spray bottle with a patented ozone genator. Ordinary tap water is added into a 400 mL reservoir in the spray bottle. When the trigger is pressed, a battery-powered micro-pump draws water from this reservoir into a chamber. Here the water comes in contact with a solid diamond plate in the presence of a direct electrical current, supplied by a lithium ion 7.4 volt 1.8 Amp-hr rechargeable battery. The water is broken down by electrolysis. The resulting oxygen is then converted to ozone. The ozonated water (containing 0.5 - 2 ppm ozone) then exits through the spray nozzle. The overall sequence can best be described as "charge the battery, fill the water reservoir, spray the ozonated water, then wipe". The unit is able to dispense at least 10 full reservoirs (a total of 3785 mL) of ozonated water for each charge cycle of the rechargeable battery; and 5000 refills across the life of the battery. The whole device is only slightly larger than a normal spray bottle and so can be taken from room-to-room as needed. The EnozoPro system is Green Seal® certified (under GS standard 37 Cleaning Products for Industrial and Institutional use and GS standard 53 Specialty Cleaning Products for Industrial and Institutional Use), which means, amongst other things, that the undiluted spray is not toxic for humans and that the dissolved ozone is fully biodegradable and leaves no residues. The ozone exposure levels emitted when the water is sprayed onto surfaces are below the allowed peak and short-term exposure limits.

Conclusions

There is a strong evidence base drawn from clinical trials and high-level systematic reviews and meta-analyses to support the safe and effective use of ozonated water for specific applications in modern clinical practice. The most common applications for treatment of dental unit waterlines leverage an extensive literature and decades of experience with this approach in industrial settings and in drinking water treatment and wastewater management. In a similar way, the use of ozonated water as a mouthrinse during the COVID-19 pandemic is built on the known antiviral actions of this agent and its potent actions as an oxidizer.

Clinical applications that exploit the ability of ozonated water to influence cell healing are now attracting considerable interest. Clinical challenges where wound healing is impaired, such as MRONJ or mucositis, or is particularly complex, such as in peri-implantitis, have shown positive results. Further work on these topics will lead to a better understanding of the molecular pathways by which wound healing processes are modulated by sub-ppm levels of ozone delivered in water.

About the author

Emeritus Professor Laurence J. Walsh AO is a specialist in special needs dentistry who is based in Brisbane, where he served for 36 years on the academic staff of the University of Queensland School of Dentistry, including 21 years as Professor of Dental Science and 10 years as the Head of School. Since retiring in December 2020, Laurie has remained active in hands-on bench research work, as well as in supervising over 15 research students at UQ who work in advanced technologies and biomaterials and in clinical microbiology. Laurie has served as Chief Examiner in Microbiology for the RACDS for 21 years and as the Editor of the ADA Infection Control Guidelines for 12 years. His published research work includes over 330 journal papers, with a citation count of over 15,400 citations in the literature. Laurie holds patents in 7 families of dental technologies. He is currently ranked in the top 0.25% of world scientists. Laurie was made an Officer of the Order of Australia in January 2018 and a life member of ADAQ in 2020 in recognition of his contributions to dentistry.

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