

## Part 5 Ozone in Water

A recent thread on a US dental blog site started a train of commentary about dental filling materials, which lead on to infective organisms, and to round it off, what should we really tell our patients?

The blog started with a comment that someone had found a reference that stated the resins in tooth-coloured composites contained pseudo-hormone regulators. The comment is quite correct – there are pseudo-hormone regulators in composites and resins used in dental care. It is interesting in that this research is not new – the first papers were published in 1996. Bearing in mind that we are now in 2011, how come this important research was ‘missed’ for 15 years or so before becoming a ‘hot’ topic in the dental blogs?

Bisphenol-A (BPA) is one of the components of bonding resins and composites. The first research paper was published in the mid 1990’s when concerns arose regarding BPA. BPA was thought to leak from dental sealants after application. This chemical mimics the oestrogen hormone and is classified as a pseudo-hormone regulator.

Research published by Olea *et al* (1) showed that after applying a commercial dental sealant, detectable levels of BPA ranging from 3.3 - 30 ppm were present in saliva samples. The concern was greatest as the use of sealants in children was an important part of the preventative protocol, and because exposure to such pseudo- hormones could alter normal development and may increase the risk of cancer.

This published research was conducted initially on rodents, and within this study population, the BPA triggered an oestrogen-like activity in rodents and an acute oral toxicity to the sealant material. But when the same test was performed on humans, the amount of BPA found in the saliva of study participants was 50 times lower than the amount seen in the rodent specimens based on the weight of the subjects.

Several studies about BPA and dental sealant materials followed. Arenholt-Bindslev *et al* (2) conducted another study about BPA leakage using two commercial dental sealants and reached an entirely different conclusion to the Olea *et al* (1) study. Arenholt-Bindslev *et al* (2) collected saliva samples immediately following sealant placement, then at 1 and 24 hours afterwards in human subjects. They found that BPA was present only in the saliva sample collected immediately after the application of the dental sealant in amounts ranging from 0.3 ppm to 2.8 ppm—levels approximately 10 times lower than those reported by Olea *et al* (1). More importantly, no BPA was found in saliva samples collected at 1-hour intervals and 24-hour intervals.

Fung *et al* (3) conducted another study that looked at blood samples in addition to saliva samples. Saliva and blood samples were collected in human participants before sealant application and at intervals of 1 hour, 3, hours, and 24 hours, and 3 days and 5 days after application. Some but not all of the saliva samples collected 1 hour and 3 hours after application showed small amounts of BPA. However, no BPA was found in samples collected after 24 hours and none was ever found in the blood samples. And in 2000, Schuurs & Moorer (4) published their paper entitled ‘Hormone dysregulators. Pseudo-estrogens in dental composite resins and sealants?’ Their research came at a time when many dental practices were replacing amalgam with composites as their restorative material of choice.

Composites have their own set of issues with regards to cavity preparation, isolation, bonding to both a dead crystalline product (enamel) and living tissue (dentine), placement, setting and finishing to name but a few. For many dentists, these 'new' materials were problematic – if the protocols were not followed to the line, they failed. Some of the first tooth-coloured products shrunk to such a degree that they caused teeth and cusps to fracture or the margins opened and the restoration failed. The introduction of the concept of 'total-etch' by Dr. Takao Fusayama with the total-etch protocols introduced in the early 1980s, and popularised by John Kanca III and Ray Bertolotti really changed how dentists carried out their restorative care with composites.

However, as composites became more popular and placement was made easier, patients demanded their amalgam fillings to be replaced with a cosmetic alternative. Dentists were only too willing, and the increasing public awareness of the potential dangers of mercury-based fillings fuelled this demand. This was also helped by a number of articles and web sites that exaggerated the dangers of amalgam. I remember at a lecture I gave in the mid 1990's being asked for my opinion on composite products. My reply was that may be in 50 – 60 years we, as the dental profession, could be saying the same about composites as we are currently saying about amalgam. As it turned out, I was not far wrong! There is actually quite a lot of research around the effects of BPA's and pseudo hormone regulators. And it is not new - this goes back to the early 2000's. What surprises me is that no one has picked up on it before!

I remember commenting in a lecture I gave on composites to a group of dentists in the USA way back in the 1990's that may be the dental profession would be saying the same sort of things about composite as we are saying about amalgam. Composite is technique sensitive, and I shudder each time I see a manufacturers advert proclaiming 'A new composite that handles like amalgam!'. Nothing handles like amalgam - you may hate the product, but it sets under water, saliva, blood and pus. No other dental restorative material - well apart from cohesive gold - does the same. And how many of us have put in a cohesive gold restoration in the last 10, 20, or 30 years? My guess is not too many!

So what are the problems with composites and resin bonding systems? The research on BPA's showed that composites and resins contain polluting chemicals, or hormone disruptors. Hormone disruptors have a number of negative effects in the human and animal body, and among others, males appear to become feminized by the action of the so-called pseudo-estrogens. BPA research showed men's' fertility may decrease, and the long-term-effects and synergism with pseudo-estrogens from other sources should prompt further studies in order to verify the safety of the BPA containing products.

One solution would be to minimise the use of the composites and adhesives; and one simple way would be to use lab-made or milled porcelain inlays instead of placing large volumes of composite. The adhesive luting cement would reduce the exposure of our patients to BPA's.

However it may not be necessary to stop using your favourite composite, sealing resins and sealants! Research looking at water pollution published in 2005 showed that the use of ozone could destroy BPA's in liquids. Deborde *et al* (5) were researching the kinetics of aqueous ozone-induced oxidation of some endocrine disruptors, and showed endocrine disruptors were destroyed by ozonated water. In 2009, Broséusa *et al* (6) researched the ozone oxidation of pharmaceuticals, endocrine disruptors and pesticides during the treatment of farm waste water, and that ozone treatment destroyed these chemicals. Both these papers show that the use of ozone in treating water and fluids destroy endocrine disruptors.

There is considerable published research on the role of the use of ozone to purify and sterilise water. In 1954, Dickermann *et al* (7) published their research examining the use of ozone to inactivate and destroy bacteria in the sterilisation of water. And in 1975, Burleson *et al* (8) showed that ozone was effective against both bacteria and viruses. The question dental researcher asked in the late 1990's was what part did ozonated water have to play in the modern dental practice, and more importantly, dental unit water lines (DUWL).

Research looking at the risk of cross infection identified numerous bacterial species from DUWL. Two studies by Szymanska in 2005 showed *Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus* spp. from *Aspergillus glaucus* group, *Aspergillus repens*, *Citromyces* spp., *Geotrichum candidum*, *Penicillium aspergilliforme*, *Penicillium pusillum*, *Penicillium turolense*, *Sclerotium sclerotiorum*; yeast-like fungi: *Candida albicans*, *Candida curvata* and other yeasts (9, 10).

Dental units from clinics in conservation, periodontology, and prosthodontics show the highest micro-organism counts and the risk for cross infection. Other researchers showed that conservative dental units had the highest counts, followed by periodontology and prosthodontics (Al Shorman *et al* (11,12,13), Al-Hiyasat *et al*, 2007 (14)). Research by O'Donnell *et al* in 2006 showed the most common bacterial species in mains water is *Micrococcus luteus* and *Sphingomonas* spp. and are known opportunistic pathogens (15) – important if you have patients who are immune-compromised. This had been commented on previously in 2004 by Montebugnoli (Montebugnoli *et al* 2004) who showed in their study dental units have the potential to transmit periodontal pathogens between patients (16).

When Szymanska *et al* started to look at dental air-water aerosol, they showed that water is the main source of bacterial endotoxin contaminating the aerosol from dental hand pieces, even after sterilisation. (9, 10). Wirthlin *et al* 2003 showed that detachment of microorganisms from dental unit water lines biofilm flushed into the oral cavity can infect patients (18), and this was repeated by Montebugnoli *et al* 2004 (16).

The bio-film is the key to the cross-contamination issue. Stasis in DUWL's during non-working time results in the proliferation of the biofilm and colony forming units (CFU's) (Al-Hiyasat *et al* 2007). In dental air-water aerosols, water is the main source of bacterial endotoxin contaminating the aerosol from dental hand pieces, even after sterilisation. (Szymanska 2005). And some bacteria may be a cause of opportunistic infections in people with immunological disorders. This was proposed by Al Shorman AL *et al* in 2002 (19).

The aim of the Al Shorman AL *et al* study was to apply ozone to control the contamination of DUWL's. In this experiment the water lines in a dental unit with existing biofilm was treated with ozone and subjected to microbiological assessment. The results showed a bacterial reduction from  $5.2 \times 10^3$  CFU/ml before treatment to 300 CFU/ml after the first O<sub>3</sub> application and then to 0 CFU/ml after the second application onwards.

The study by Puttaiah *et al* (20) examined the effect of ozone dissolved in water to control microbial contamination. The baseline waterline samples showed viable mature biofilm, while the post study waterline samples showed no viable or non-viable biofilm. The conclusion drawn was that a concentration of 2ppm of dissolved ozone controlled biofilm formation. Further research by Chih-Shan *et al* (21) showed that ozone and ozonated water had a role in surface decontamination and sterilisation. Lastly in this section, Coulter *et al* (22) showed that ozonated water was effective at decontaminating MRSA and *C. difficile*. Other uses for ozone dissolved in water were shown by Holmes in 2002 (23) where it was used to control volatile sulphur compounds that are implicated in bad breath.

More recent research has been examining soft tissue healing. Tranina *et al* 2009 analyzed the biological effects of ozonated water on soft tissue healing (24). In this animal study, the enhanced gradient of oxygen in tissues is postulated as being responsible for the accelerated healing that was reported. Another study by Francino *et al*, 2010 (25) showed 4ppms ozonated water used to irrigate bone wounds accelerated healing with 4ppms of ozone.

What really astounds me is that the public seem to be blissfully aware that the dental practice is probably the worst place to spend any time in. And whilst there is a great deal of negative comment from dental practitioners about the new regulations, may be the dental profession only has itself to blame.

Then I'd say this topic begs the question: How much transparency or information should we tell our patients? Should the dental profession disclose the results of research that shows some materials are dangerous? If the profession fails to do this, what is the status of informed consent that we rely on to start a course of treatment?

From the other side, how much information can patients tolerate before the additional information only causes confusion? And the related concern: What if dental patients actually read the Health and Safety forms, procedure warnings and other legal documents before signing them? Imagine the time it would take; and then if they had additional questions! From both the dentist's side and the patient's side, time has a cost implication. Dentistry is expensive enough! And for those dentists who have UDA's to meet, that time factor has a huge implication in profitability and the ability to meet the UDA demands.

Should we tell our patients about every positive and negative aspect of the procedure and products we are planning to use. Well yes, I think we should. How do you get informed consent with anything less than a full honest disclosure? No wonder the legal profession has such a field day when it comes to consent forms, and the issues surrounding them. There is an expectation that the medical profession should disclose the cost, procedure, materials used, the pros / cons and alternatives.

It is an issue we could spend a lot of time sweating over; - how much information should you give your patients? Or you could take a different tack and be pro-active in preventing the potential problems. Lawyers and regulatory authorities would like complete disclosure - but then we face the issue of what we do not know, and where do we get that information! So to address the topic of BPA's, I give my patients a glass of ozonated water to rinse with and drink - then any residual chemistry is reduced to harmless components. The long term is more of an issue, as the research shows the BPA's leach out for a long time - sound familiar with reference to mercury?

I guess the answer is to place bonded inlays instead of filling, so then the composite volume is reduced as far as possible. Then the patient can be sold a small ozone device to make ozonated water at-home - not only sorting out the pseudo-hormone issue, but also the oral hygiene, health care, etc too - sounds like a win-win for everyone here! As a researcher and developer of ozone systems, I try to bring a bit of reality to these issues, and design simple protocols that work for the dentists as well as the patient!

In a perfect world, patients should be informed of all aspects of irreversible procedures and should be provided access to even the confusing and contradictory information about everything. It's a Hippocratic quandary: Are dental patients more likely to be hurt by what they don't know, or by what they know? We know removing amalgam fillings for fear of toxicity only harms patients

Having said that, in my own practice I will go through the broad outlines of what alternatives are around, and what I believe in, and if it was my tooth, what would I be willing to accept. The really good news is that if you do place a lot of composite, and you want to reduce the risk for these pseudo-hormone regulators, then it is a really easy task; the research from the agricultural industry showed that when waste farm water that was loaded with hormone and pseudo-hormone products was treated

with ozonated water, the ozone destroyed these hormone regulators, and removed them from the system (6).

So if you really want to practice holistic dentistry, reduce the infection opportunity that is your practice and still continue to use composites and bonding system, then invest in a water ozone system, and use it to clean out your dental units, your water lines, as well as protect and enhance the care you give your patients! Grey Cell Enterprises has two units - the PEM-Duo which is designed for the clinic, and the PEM-Probe designed for at-home use. You can get them through [www.greycellenterprises.com](http://www.greycellenterprises.com)

1. Olea N, Pulgar R, Pérez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, Pedraza V, Soto AM, Sonnenschein C. Estrogenicity of resin-based composites and sealants used in dentistry. *Environ Health Perspect.* 1996;104:298-305.
2. Arenholt-Bindslev D, Breinholt V, Preiss A, Schmalz G. Timerelated bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. *Clin Oral Investig.* 1999;3:120-125.
3. Fung EY, Ewoldsen NO, St Germain HA Jr, Marx DB, Miaw CL, Siew C, Chou HN, Gruninger SE, Meyer DM. Pharmacokinetics of bisphenol A released from a dental sealant. *J Am Dent Assoc.* 2000;131:51-58.
4. Schuurs AH, Moorer WR. Hormone dysregulators. Pseudo-estrogens in dental composite resins and sealants? *Ned Tijdschr Tandheelkd.* 2000 Dec;107(12):490-4.
5. Deborde M, Rabouan S, Duguet JP, Legube B. Kinetics of aqueous ozone-induced oxidation of some endocrine disrupters, *Environmental Science and Technology* 39 (16) (2005)
6. Broséusa R, Vincenta S, Aboulfadlb K, Daneshvarc A, Sauvéb S, Barbeaua B, Prévosta M. Ozone oxidation of pharmaceuticals, endocrine disruptors and pesticides during drinking water treatment. *Water Research* Volume 43, Issue 18, October 2009
7. Dickermann J M, Castrabertti AO, Fuller JE. Action of ozone on water-borne bacteria. *J Eng Water Works Assoc* 1954; 68: 11.
8. Burleson GR, Murray TM, Pollard M. Inactivation of viruses and bacteria by ozone, with and without sonication. *Appl Microbiol.* 1975 Mar;29(3):340-4.
9. Szymanska, J. (2005a). Exposure to bacterial endotoxin during conservative dental treatment. *Ann Agric Environ Med* 12, 137–139.
10. Szymanska, J. (2005b). Endotoxin levels as a potential marker of concentration of Gram-negative bacteria in water effluent from dental units and in dental aerosols. *Ann Agric Environ Med* 12, 229–232
11. Al Shorman H, Abu-Naba'a L, Coulter W, Lynch E. Ozone, An Effective Treatment For Dental Unit Water Lines. *J Dent Res.* 2002; 81:112.
12. Al Shorman H, Abu-Naba'a L, Coulter W, Lynch E. Ozone Efficacy in the Treatment of Dental Unit Water Lines. *J Dent Res.* 2002; 81:B-299.

13. Al Shorman H, Coulter W, Abu-Naba'a L, Mahon G, Boyle C, Lynch E. Effect of Ozone on Biofilms in Dental Unit Waterlines. *J Dent Res.* 2002; 81:364
14. Al-Hiyasat AS, Ma'ayeh SY, Hindiyeh MY, Khader YS. The presence of *Pseudomonas aeruginosa* in the dental unit waterline systems of teaching clinics. *International Journal of Dental Hygiene* Volume 5, Issue 1, pages 36–44, February 2007
15. O'Donnell MJ, Tuttlebee CM, Falkiner FR, Coleman, DC. Bacterial contamination of dental chair unit in a modern dental hospital caused by leakage from suction system hoses containing extensive biofilm. *J Hosp Infect* 59, 348–360. 2005
16. Montebugnoli L, Sambri V, Cavrini F, Marangoni A, Testarelli L, Dolci G. (2004) Detection of DNA from periodontal pathogenic bacteria in biofilm obtained from waterlines in dental units. *New Microbiol* 27, 391–39. 2004
17. Szymanska, J. Evaluation of mycological contamination of dental unit waterlines. *Ann Agric Environ Med* 12, 153–155. 2005
18. Wirthlin MR, Marshall GW, Rowland EW. Formation and decontamination of biofilms in dental unit waterlines. *J Periodontol* 74, 1595–160. 2003
19. Al Shorman AL, Abu-Naba L, Coulter WA, Lynch E. Ozone, An Effective Treatment For Dental Unit Water Lines. *IADR Abstract* 0711, 2002
20. Puttaiah R, Lin SM, Cederberg R. Biofilm Control - Ozonation in an Automated Waterline Cleaning Dental Unit. *IADR Abstract* 0081, 2005
21. Chih-Shan L, Yu-Chun W. Surface Germicidal Effects of Ozone for Microorganisms. *IADR* 2006.
22. Coulter J, Fulton CR, Lynch E, Coulter WA. Ozonated water for disinfection of MRSA and *C difficile* *IADR Abstract* 0766, 2008.
23. Holmes, J. Ozone as a control for volatile sulphur compounds. *ISBOR Abstract and Poster*, 2002.
24. Tranina AA, Correa L, Acai R, Urruchi W, Naclerio-Homem MG, Deboni MCZ. Ozonized water irrigations on dermal wound healing in rats. *IADR Abstract* 3466, 2009
25. Francino AVM, Juliana N, Andrea M, Zindel DMC. Bone Repair of Monocortical Wounds after Ozonized Water Irrigation. *IADR Abstract* 1017, 2010.