The Need for Dental Restorative Materials with Long-term Antibacterial Properties

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Executive Summary

This brief white paper addresses the need for minimal-intervention dentistry that includes dental restorative materials with long-term antibacterial properties. The management of dental caries (tooth decay) by risk assessment is now well established. Patients with existing decay and/or recent restorations as a result of dental caries are at high risk for new caries in the near future. Continuing caries activity around restorations is bacterially generated and leads to the breakdown of the surrounding dentin or enamel and the composite restoration. This is a major problem that requires a solution.

Several groups around the world are researching the possibility of using composite restorative materials that incorporate antibacterial agents to overcome this problem. One such antibacterial agent is quaternary ammonium polyethylenimine (QPEI) in the form of nanoparticles. Dentally related research has been published on this material over the last 12 years. QPEI has been incorporated at low levels of 1% into dental composites and tested in laboratory and in vivo studies.

Laboratory studies have thoroughly assessed the relevant antibacterial properties of QPEI, the effects on the mechanical properties of the composite materials in which QPEI was incorporated, and other relevant topics. In laboratory studies, QPEI nanoparticles were potent antibacterial agents, did not leach out, inhibited breakdown of the composite, and maintained its antibacterial action over time.

Limited *in vivo* studies have supported the laboratory studies by measuring biofilm accumulation and activity over four hours in human mouths and assessing bacterial viability. The QPEI caused cell death throughout the formed biofilm, not just at the restorative material surface.

QPEI incorporated into composite dental restorative materials at 1% shows great promise as an antibacterial agent to combat secondary caries around restorations and maintain the integrity of the restoration. The QPEI antibacterial nanoparticles remain in place and continue their action over time, eliminating the problems of leaching, loss of activity, unnecessary ingestion, and accumulation in the body and the environment.

QPEI is an exciting material with great potential, and it should be further studied in human mouths to verify long-term efficacy, stability, and integrity of the composites. Bond strength studies of the composites with QPEI incorporated are also necessary.

The marketing of such products will require extensive consumer (dentist) education, but should be a major step forward in caries control and management worldwide.

Dental caries management and the need for minimal intervention dentistry

It is well known that dental caries (tooth decay) is bacterially generated and that the so-called cariogenic bacteria that include the *mutans streptococci*, *lactobacillus* species, *bifidobacteria*, and more generate acids when they metabolize fermentable carbohydrates ingested by humans. These acids dissolve the tooth mineral (enamel and dentin), leading to cavities if the process is not halted (Featherstone, 2000). When restorations are placed to fill the cavity, they do nothing to deal with the bacteria and caries generally continues to progress, unless other measures are taken, including remineralization (enhanced by fluoride) and antibacterial therapy (Featherstone et al, 2012). So-called secondary caries occurs around restorations, causing further loss of enamel or dentin mineral and breakdown of the composite restoration.

The management of dental caries (tooth decay) by risk assessment is now well established (Featherstone et al, 2007; Featherstone and Chaffee, 2018). Patients with existing decay or recent restorations as a result of dental caries are at high risk for new caries in the near future. Continuing caries activity around restorations is bacterially generated and leads to the breakdown of the surrounding dentin or enamel and the composite restoration. This is a major problem that requires a solution. Minimal intervention dentistry calls for remineralization and antibacterial therapy (Featherstone and Domejean, 2012; Featherstone and Chaffee, 2018) and the minimal use of appropriate restorative therapy. The commercial availability of composite restorative materials with long-term antibacterial properties is therefore a major unfilled need in the world of minimal intervention dentistry (also known as minimally invasive dentistry).

Several groups around the world are researching the possibility of using composite restorative materials that incorporate antibacterial agents to overcome this problem (Chatzistavrou et al, 2015; Melo et al, 2016; Beyth et al, 2006). One such antibacterial agent is quaternary ammonium polyethylenimine (QPEI) in the form of nanoparticles (Beyth et al, 2006, 2008, 2010A, 2010B; Yudovin-Farber et al, 2008). Dentally related research has been published on this material over the last 12 years. Key papers are reviewed in the present white paper.

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Composite Dental Restorative Materials

Composite resin materials are widely used clinically in dentistry for replacement of decayed hard tissues (enamel and dentin). Although the mechanical properties and wear resistance of these materials have been improved substantially (and continue to improve), their antibacterial properties are essentially non-existent. These resin-based materials accumulate more dental plaque than other restorative materials both *in vitro* and *in vivo*, which often results in secondary caries, as described above. Several investigators have reported studies on composite materials that were impregnated with antibacterial agents such as silver (Melo et al, 2016), and released these agents over time, sometimes resulting in loss of activity and weakening of the remaining composite material. There is, therefore, an obvious need for a composite restorative material that incorporates an antibacterial agent that is not released, but yet retains its antibacterial activity over time.

Quaternary Ammonium Polyethylenimine (QPEI) — Laboratory Studies

For decades, quaternary ammonium compounds have been used as antibacterial agents in solution for many purposes. They are potent antibacterial agents. Relatively recent research led to polymerization with materials that could form nanoparticles with antibacterial activity in the solid form. Work on over 50 such compounds led to a very thoroughly executed research project in which QPEI nanoparticles were incorporated into composite resin restorative materials and tested for antibacterial properties and material properties of the composite over time.

Studies have been performed on the antibacterial activity of QPEI nanoparticles embedded at 1% w/w with clinically used bonding and light cured flowable and hybrid dental composite resins (Beyth et al., 2006). The antibacterial activity was tested with *Streptoccocus mutans* by: (i) the agar diffusion test (ADT); (ii) the direct contact test; (iii) bacterial growth in the materials elute; (iv) and scanning electron microscopy (SEM).

Using the direct contact test, antibacterial activity was found in all three types of composite resins incorporated with the synthesized QPEI nanoparticles. The effect lasted for at least one month. SEM demonstrated bacterial debris and no streptococcal chains at 24 hours of bacterial contact. The addition of 1% w/w of nanoparticles did not affect the flexural modulus and the flexural strength of the dental composite materials. The results indicate that QPEI nanoparticles immobilized in resin-based materials have strong antibacterial activity upon contact without the nanoparticles leaching and without

compromise in mechanical properties. Complete inhibition of bacterial growth was shown in the one-month aged samples.

A further exhaustive laboratory study was conducted by the same group to assess the effect of incorporated QPEI over time on the bacterially generated changes in composite materials, including surface roughness (Beyth et al, 2010B). Composite resin-incorporating QPEI nanoparticles were characterized using contact angle goniometry, X-ray photoelectron spectroscopy (XPS), and SEM. Six-month aged samples were tested for antibacterial effect against *S. mutans* using the direct contact test. Surface roughness following one month of bacterial challenge was assessed using atomic force microscopy (AFM). Contact angle increased following QPEI incorporation, and XPS revealed surface iodide and nitrogen elements proving the nanoparticles were available at the surface. Direct contact test results showed that six-month aged composite resins incorporating QPEI nanoparticles completely inhibited the growth of *S. mutans*. AFM analysis showed an increase in root mean square roughness following bacterial challenge in composite resin samples. No such effect was observed in samples incorporating QPEI.

Therefore, changing the surface properties of composite resins by incorporating QPEI antibacterial nanoparticles may improve their clinical performance both by inhibiting bacterial growth and by preventing changes in the surface roughness.

Quaternary Ammonium Polyethylenimine (QPEI) — *In vivo* Studies

The next obvious studies were to investigate the properties of QPEI *in vivo* when incorporated into dental composite restorative material. The same group hypothesized that QPEI nanoparticles incorporated into a resin composite have a potent antibacterial effect *in vivo* and that this stress condition triggers a suicide module in the bacterial biofilm (Beyth et al, 2010A). Ten human volunteers wore a removable acrylic appliance in which two control resin composite specimens and two resin composite specimens incorporating 1% w/w QPEI nanoparticles were inserted to allow the buildup of intraoral biofilms. After four hours, the specimens were removed and tested for bacterial vitality and biofilm thickness, using confocal laser scanning microscopy. The vitality rate in specimens incorporating QPEI was reduced by >50% ($p < 0.00001$), whereas biofilm thickness was increased ($p < 0.05$). The ability of the biofilm supernatant to restore bacterial death was tested *in vitro*. The *in vitro* tests showed a 70% decrease in viable bacteria ($p < 0.05$). Biofilm morphological differences were also observed in the scanning electron microscope micrographs of the resin composite versus the resin composite incorporating QPEI. These results strongly suggest that QPEI nanoparticles incorporated

at a low concentration in resin composite exert a significant *in vivo* antibiofilm activity and exhibit a potent broad-spectrum antibacterial activity against saliva-borne bacteria.

Another similar study was conducted by impregnation of QPEI nanoparticles in wound dressing material for orofacial cancer patients (Atar-Froyman et al, 2015). QPEI nanoparticles were found to have strong bactericidal activity against a wide variety of microorganisms, rapidly killing bacterial cells when incorporated at small concentrations into soft lining materials without compromising mechanical and biocompatibility properties. This appears advantageous over conventionally released antimicrobials with regard to *in vivo* efficacy and safety, and may provide a convenient platform for the development of non-released antimicrobials. This is a crucial issue when it comes to giving an answer to the serious and life-threatening problems of contamination in immunocompromised patients such as orofacial cancer patients.

Overall Summary and Assessment

In laboratory studies, QPEI nanoparticles were potent antibacterial agents, did not leach out, inhibited breakdown of the composite, and maintained their antibacterial action over time.

Limited *in vivo* studies have supported the laboratory studies by measuring biofilm accumulation and activity over four hours in human mouths and assessing bacterial viability. The QPEI caused cell death throughout the formed biofilm, not just at the restorative material surface.

QPEI incorporated into composite dental restorative materials at 1% shows great promise as an antibacterial agent to combat secondary caries around restorations and maintain the integrity of the restoration. The QPEI antibacterial nanoparticles remain in place and continue their action over time, eliminating the problems of leaching, loss of activity, unnecessary ingestion, and accumulation in the body and the environment.

QPEI is an exciting material with great potential, that should be further studied in human mouths to verify long-term efficacy, stability, and integrity of the composites.

Future Studies

Although the laboratory studies on QPEI incorporated at 1% into dental composite resins are very thorough and convincing, the *in vivo* studies are very limited. The *in vivo* studies clearly showed a dramatic antibacterial effect in the mouth against whatever bacteria were present. However, they were very short term. In the opinion of the author of this review, the evidence is very strong to support longer term and more extensive *in vivo*

studies. Ideally, at least one long-term dental caries study is desirable, depending on what claims are to be made. However, *in vivo* antibacterial studies over a month would be easy to do and would most likely provide evidence to pursue utilizing such products as commercially available materials. Bond strength studies are also essential on the composites with QPEI added.

The marketing of such products will require extensive consumer (dentist) education, but should be a major step forward in caries control and management worldwide.

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