Triethylene glycol derived from composite resins modulates the expression of genes associated with biofilm formation and other virulence factors in *Streptococcus mutans*

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Statement of Purpose: Bacterial microleakage along the tooth/composite resin dental restoration interface contributes to postoperative sensitivity, recurrent caries, pulp inflammation and necrosis[1]. Studies have confirmed that saliva can catalyze the degradation of constitutive monomers in dental restorative composites, forming biodegradation by-products (BBP's) such as methacrylic acid (MA), triethylene glycol (TEG), and bis-hydroxy-propoxyphenyl propane (BisHPPP)[2]. Other work has shown that ethylene glycol-based co-monomers such as EGDMA and TEGDMA may promote the proliferation of important cariogenic micro-organisms[3]. Recently it was demonstrated that TEG can accelerate the growth of select oral micro-organisms, namely *Streptococcus mutans* and *Streptococcus salivarius* strains[4]. It was, therefore, hypothesized that TEG alters gene expression in oral bacterial species. Restriction fragment differential display PCR (RF DDPCR) in conjunction with quantitative real-time PCR techniques were employed to probe for modulations in gene expression of *S. mutans* NG8 strain grown in the presence of TEG.

Methods: Total RNA was isolated from *S. mutans* NG8 liquid cultures grown in TYE medium at pH 5.5 containing 0.1% glucose and TEG concentrations of 0.0 (control), 0.1, 1.0, and 10.0 mM. For each sample, 1 µg of DNase treated RNA was subjected to RT-PCR, digested with *Taq* restriction enzyme, followed by ligation with adaptors and amplified using 16 radiolabeled primers. The fragments were resolved in 7% denaturing polyacrylamide gels, and exposed to autoradiography. Bands that displayed altered expression were eluted, reamplified using radiolabeled dATP, and resolved further in 7% non-denaturing polyacrylamide gels. The major fragments were eluted, reamplified and cloned into pDrive (Qiagen) vector and transformed into *E. coli*. Plasmids harboring gene fragments were isolated and sequenced. For q-RT PCR experiments, 1 µg of DNase treated RNA isolated from *S. mutans* NG8 cells grown planktonically or as a biofilm in the presence of 0.0, $1.0x10^{-3}$, $1.0x10^{-2}$, 0.1, or 1.0 mM TEG at pH 5.5 or 7.0, was subjected to amplification using specific primers designed to amplify a 100-150 bp region of open reading frames of interest. The PCR reactions were performed using SYBR green fluorescence dyes. Gene expression values were quantified using the relative expression method and were normalized and validated using internal housekeeping genes *gyrA* and *16SrRNA*.

Results / Discussion: RF-DDPCR experiments identified *glucosyltransferase B* (*gtfB*) (involved in biofilm formation[5]) and *yfiV* (putative transcription regulator of bacterial cell-surface fatty acid genes[6]) to have altered expression in *S. mutans* cells grown in the presence of TEG at pH 5.5. Further, q-RT PCR experiments revealed that at $1.0x10^{-2}$ mM TEG, *gtfB* was upregulated an average of 2.0 - 2.4-fold for both pH levels in planktonic cells ($p<0.01$). At the same concentration there was an average of 1.5-fold upregulation of *gtfB* in biofilm cells grown at pH 5.5. One of the most widely researched virulence attributes of mutans streptococci (MS) has been its ability to produce extracellular polysaccharides (also known as glucans) from dietary sucrose via glucosyltransferases (*gtfB*, *C*, and *D*), and fructosyl-transferase[7]. Hence, the upregulation of *gtfB* has a particular physiological relevance in this environment.

The consistent and significant 2-fold downregulation of *yfiV* gene ($p<0.05$ at pH 5.5 and $p<0.01$ at pH 7.0) in planktonically grown cells was particularly interesting and conformed well to the differentiating pattern observed in *gtfB* expression at the same TEG concentration. Although the physiological functions of the *S. mutans yfiV* gene is not clear, it has been hypothesized that *yfiV* is a transcriptional regulator of the *Fab* operon, controlling the fatty acid composition of *S. mutans* cell membranes in a pH-dependent manner enabling the cells to better tolerate acidic pH levels. Biofilm cells grown at acidic pH expressed higher levels of *yfiV* in the presence of TEG at the mid-concentration levels of $1x10^{-2}$ and $1x10^{-1}$ mM. The latter findings, therefore, warrant a further investigation of *yfiV* function.

Given that recent work has revealed the presence of BBP's *in vivo* at a concentration range of $0\text{-}1x10^{-1}$ mM post-implementation of composite resins[8], the findings of this work have physiological significance.

Conclusions: The presence of TEG upregulates the expression of *gtfB* and may modulate virulence properties such as biofilm formation and acid tolerance. These are some of the first reports describing the interactions of composite resin-derived BBP's on the physiological functions of oral bacteria at product concentration levels found *in vivo*. The current work highlights the potential for *in vivo* consequences of BBP's with respect to biofilm formation and microbial survival.

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Acknowledgements: This research was supported by NSERC Grant RGPGP 183686-03, the CIHR strategic training fellowship STP-53377, and Ontario Graduate Scholarship in Science and Technology.