Synthesis of Antimicrobial Monomers Using Ciprofloxacin

Yasaman Delaviz[1,2], Michael W. Laschuk[2], Meilin Yang[2], J. Paul Santerre[1,2]
Institute of Biomaterials and Biomedical Engineering, University of Toronto[1]
Faculty of Dentistry, University of Toronto[2]

Statement of Purpose: Composite resins have become the most widely used dental restorative materials due to their superior aesthetics and ease of handling. Composite resins however experience significant biological breakdown in the oral cavity that is believed to contribute to facilitating marginal breakdown and biofilm formation at the tooth margins.[1] Given the importance of biofilms[2] and their relevance to oral disease pathogenesis[1] it is warranted to advance the chemistry of dental biomaterials beyond the current chemistries in order to achieve better control over biofilm formation. A potential approach to addressing this problem could focus on the development of composites and adhesive systems that release antimicrobial agents as degradation byproducts under the attack of salivary enzymes. It is hypothesized that covalently bound pharmaceutical agents incorporated into the backbone of biodegradable oligo-urethanes as part of main chain monomers used during resin polymerization, will allow for the delivery of free antibiotic drug from composite resins, when such resins are degraded by salivary enzymes. The primary objective of this study was to incorporate the antibiotic ciprofloxacin (CF) into the backbone of a novel monomer that can be substituted into the formulation of restorative polymer systems. Further, it is an aim to evaluate the drug release characteristics of the system in the presence of salivary-like enzymes.

Methods: Micro-broth dilution assays were used to determine the minimum inhibitory concentration (MIC_{90}) of CF against *Streptococcus mutans (*UA159*)*, a primary mediator of oral caries formation.[3] A novel antimicrobial monomer has been synthesized using CF, referred to as Ester Drug Vinyl (EDV) monomer. This monomer was synthesized by coupling CF with triphenyl chloride in the presence of triethylamine to protect the amine functional groups. After deprotecting the carboxylic acid by *in situ* methanolysis, the compound was then coupled with triethylene glycol in the presence of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and 4-dimethylamino pyridine (DMAP). The isolated product was reacted with trifluoroacetic acid in the presence of water to deprotect the secondary amine groups. The deprotected product was then coupled with methacrylic acid in the presence of EDC and DMAP. The monomer was purified and assessed for drug release characteristics and bio-stability in the presence of cholesterol esterase (CE), a model enzyme used to assess the degradation of resin monomers, at concentrations comparable to CE-like activity in human saliva.[4] The enzyme activity was maintained over 3 days and degradation by-products were quantified using high performance liquid chromatography (HPLC), UV and mass spectroscopy (MS). Polymerization of the monomer was conducted over 30 seconds using a camphorquinone/ 2-(dimethylamino) ethyl methacrylate (CQ/DMAEMA) photo-initiator system. **Results:** CF had a well-defined MIC_{90} (0.5 µg/mL) against *S. mutans* (UA159). The final di-vinyl drug monomer (off white powder) was successfully synthesized (~ 55% yield) and structures confirmed by ^{1}H-NMR (Figure 1), ^{19}F NMR (chemical shift between -125.80 ppm to -125.96 ppm), MS (912.4 g/mol) and FTIR (not shown). Successful polymerization of the monomer was confirmed by FTIR analysis of converted vinyl groups. Monomer was found to have superior stability in the presence of simulated human salivary esterase (i.e. CE) in comparison to the commercially available monomer, Bisphenol A-glycidyl methacrylate (BisGMA) (Figure 2). Terminal BisHPPP, the degradation product of BisGMA, was isolated after incubation with CE.

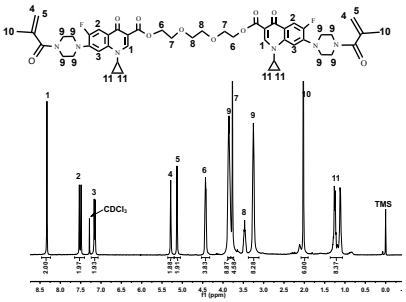

Figure 1. ^{1}H-NMR ($CDCl_3$, 298k, 400MHz) of EDV.

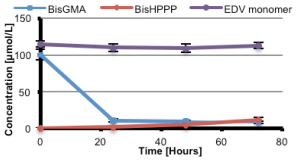

Figure 2. Biodegradation analysis of BisGMA, its derived end product BisHPPP, and EDV in the presence of 5units/mL of CE activity. One unit of CE activity is defined as the amount required to generate 1nmol of para-nitrophenol from para-nitrophenyl butyrate per minute at pH 7.0 and 37°C. Data are reported with S.D. (n=3). HPLC was used to quanitfy the concentration of the monomers and degradation by-products, all compound structures were confirmed by MS.

Conclusions: The synthesis of a novel CF monomer was completed. Stability of the monomer is suggesting that premature drug release may not be a concern. Future work will assess the long-term drug release characteristic and the MIC_{90} of the synthesized polymer against *S. mutans* (UA159). In addition, drug monomer will be substituted at different concentrations into triethylene glycol dimethacrylate/BisGMA formulations to assess for mechanical properties, biodegradation and drug release.

References: [1] Singh J. J Biomed Mater Res A. 2009;88(2):551-560. [2] Khalichi P. Biomaterials. 2009;30:452-459. [3] Kermanshahi S. J Dent Res. 2010;89(9):996-1001. [4] Finer Y. J Dent Res. 2004;83:22-26.

Acknowledgement: NSERC Discovery Grant # 360520.