Synthesis of Antimicrobial Monomers Using Ciprofloxacin

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Introduction

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 degradation 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 oligomeric monomers as part of the resin used during 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.

Materials and 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 at 5units/mL of CE activity over 3 days and degradation by-products were quantified using high performance liquid chromatography (HPLC), UV and mass spectroscopy (MS). 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^{o}C. The monomer's polymerization character was also evaluated. Polymerization of the monomer was conducted over 30 seconds of exposure to blue light at an intensity of approximately 420 mW/cm^2 with 0.4wt% of photo-initiator composed of camphorquinone/2-(dimethylamino) ethyl methacrylate. To characterize the polymer and confirm the conversion of vinyl groups, Fourier Transform infrared spectroscopy (FTIR) analysis was performed. The gel content of the polymerized resin and degree of unreacted monomer are being assessed in the on-going work.

Results and Discussion:

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 with a total yield of approximately 55%, with individual reaction steps ranging between 80 to 97%. The final structure was 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 (data not shown). Polymerization of the monomer by light curing was confirmed by FTIR analysis showing the disappearance of vinyl groups that were converted (data not shown).

The EDV 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). Degradation of BisGMA began after 24 hours of incubation with CE, as confirmed by the isolation of BisHPPP, a terminal degradation product of BisGMA. In contrast, the EDV monomer was found to be stable up to 72 hours without any degradation by-products detected. The unexpected stability may be caused by the inherent ability of CF to self-associate into a π- π stacking configuration.[5] The tendency of CF to aggregate may have resulted in shielding of the susceptible bonds from water or potentially reducing the ability of enzyme-substrate interaction. In order for enzymes to catalyze the hydrolysis of susceptible bonds, it is widely accepted that the enzyme must bind to the substrate.[6]

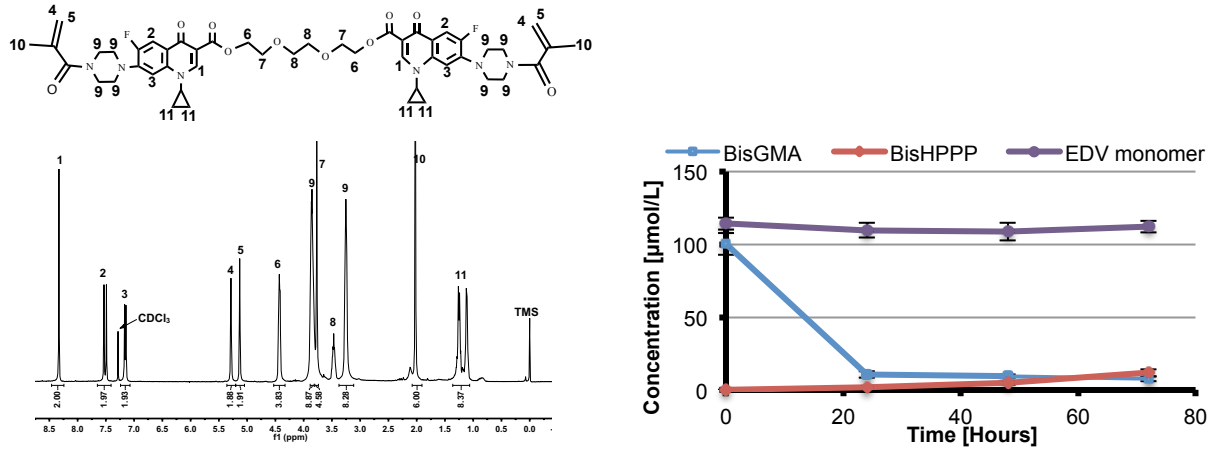

Figure 1. *Left image:* ^{1}H-NMR ($CDCl_3$, 298k, 400MHz) of EDV. *Right image:* Biodegradation analysis of BisGMA, its derived end product BisHPPP, and EDV in the presence of 5units/mL of CE activity. Data are reported with S.D. (n=3).

Conclusion:

The synthesis of a novel CF monomer was completed. Stability of the monomer is suggesting that pre-mature 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, the 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. [5] Turcu I. J Phys Chem B. 2012;116(22):6488-6498. [6] Tang YW. Biomaterials 2003;24:2003-2011.

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