Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Novel protein-repellent dental adhesive containing 2-methacryloyloxyethyl phosphorylcholine

Ning Zhang [a,b], *Mary Anne S. Melo* [b], *Yuxing Bai* [a,**], *Hockin H.K. Xu* [b,c,d,e,*]

[a] *Department of Orthodontics, School of Stomatology, Capital Medical University, Beijing, China*
[b] *Biomaterials & Tissue Engineering Division, Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Dental School, Baltimore, MD 21201, USA*
[c] *Center for Stem Cell Biology & Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[d] *Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[e] *Department of Mechanical Engineering, University of Maryland, Baltimore County, MD 21250, USA*

ARTICLE INFO

Article history:
Received 22 June 2014
Received in revised form
11 July 2014
Accepted 18 July 2014

Keywords:
Protein repellent
Bacteria repellent
Dental adhesive
Dentine bond strength
Human saliva microcosm biofilm
Caries inhibition

ABSTRACT

Objectives: Biofilms at tooth-restoration margins can produce acids and cause secondary caries. A protein-repellent adhesive resin can potentially inhibit bacteria attachment and biofilm growth. However, there has been no report on protein-repellent dental resins. The objectives of this study were to develop a protein-repellent bonding agent incorporating 2-methacryloyloxyethyl phosphorylcholine (MPC), and to investigate its resistance to protein adsorption and biofilm growth for the first time.

Methods: MPC was incorporated into Scotchbond Multi-Purpose (SBMP) at 0%, 3.75%, 7.5%, 11.25%, and 15% by mass. Extracted human teeth were used to measure dentine shear bond strengths. Protein adsorption onto resins was determined by a micro bicinchoninic acid (BCA) method. A dental plaque microcosm biofilm model with human saliva as inoculum was used to measure biofilm metabolic activity and colony-forming unit (CFU) counts.

Results: Adding 7.5% MPC into primer and adhesive did not decrease the dentine bond strength, compared to control ($p > 0.1$). Incorporation of 7.5% of MPC achieved the lowest protein adsorption, which was 20-fold less than that of control. Incorporation of 7.5% of MPC greatly reduced bacterial adhesion, yielding biofilm total microorganism, total streptococci, and mutans streptococci CFU that were an order of magnitude less than control.

Conclusions: A protein-repellent dental adhesive resin was developed for the first time. Incorporation of MPC into primer and adhesive at 7.5% by mass greatly reduced the protein adsorption and bacterial adhesion, without compromising the dentine bond strength.

Clinical significance: The novel protein-repellent primer and adhesive are promising to inhibit biofilm formation and acid production, to protect the tooth-restoration margins and prevent secondary caries.

* *Corresponding author at*: Biomaterials & Tissue Engineering Division, Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Dental School, Baltimore, MD 21201, USA. Tel.: +1 410 706 7047; fax: +1 410 706 3028.
** *Corresponding author*.
E-mail addresses: byuxing@263.net (Y. Bai), hxu@umaryland.edu (Hockin H.K. Xu).
http://dx.doi.org/10.1016/j.jdent.2014.07.016
0300-5712/

1. Introduction

Dental caries is a prevalent disease which results in a heavy financial burden worldwide.[1,2] Nearly 200 million tooth cavity restorations are performed in the United States each year.[3] The demand for tooth restorations is increasing rapidly with an ageing population and increased tooth retention in seniors.[4] The fact that more teeth are retained into an elderly age has resulted in more occurrences of dental caries.[5] Composites are the principal material for cavity restorations due to their excellent aesthetics and direct-filling capability.[6,7] The compositions and properties of resin matrices, fillers and composites have been significantly improved in previous studies.[8–13] Nonetheless, approximately half of all restorations fail within 10 years, and the replacement of failed restorations accounts for more than half of all restorations performed.[14] Previous studies showed that dental resins *in vivo* tend to accumulate more biofilms and plaques than other restorative materials.[15,16] Furthermore, microgaps can be observed at the tooth-restoration interfaces.[17,18] Microleakage can occur and biofilms at the restoration margins can produce acids and cause secondary caries. Secondary caries has been suggested in previous studies as a primary reason for restoration failure.[7,19,20]

Bonding agents enable the composite restoration to be adhered to the tooth structure.[21–23] Extensive studies have been performed to improve, characterize and understand enamel and dentine bonding.[24,25] It is beneficial for the bonding agent to be antibacterial, to combat biofilms and secondary caries at the margins. Efforts have been made to develop antibacterial primers and adhesives that could kill bacteria,[26–31] and several different compositions of quaternary ammonium methacrylates (QAMs) were synthesized.[26–31] For example, 12-methacryloyloxydodecyl-pyridinium bromide (MDPB) was incorporated into primer and adhesive to combat bacteria and biofilm growth.[26,27] Recently, a quaternary ammonium dimethacrylate (QADM) was synthesized and incorporated into primer[28] and adhesive[29] which reduced biofilm viability and acid production.

In the oral environment with salivary flow, a clean dental resin is quickly coated with a salivary pellicle that comprises a layer of selectively adsorbed salivary proteins.[32] It is through this protein layer that oral bacteria attach to the resin and to tooth surfaces.[33,34] The adherence of early colonizers, for example, mutans streptococcus, to the salivary pellicle is an initial step in biofilm formation.[33,34] Biofilm formation is the source of infection and a prerequisite for the occurrence of dental caries.[35] Therefore, it would be highly desirable to develop a new adhesive resin that can repel proteins, to inhibit protein adsorption and hence bacterial adhesion at the tooth-restoration margins and at the eventual microgaps in the margins. A previous study immobilized a protein-repellent material, poly(ethylene glycol) (PEG) and two pyridinium group-containing methacrylate monomers, to silicon wafer surfaces to investigate the influence of prior protein adsorption on bactericidal activity.[36] The results showed that the PEG-modified surfaces had substantially less adsorbed proteins.[36] However, to date there has been no report on dental adhesive resins that possess protein-repellent capability.

It has been demonstrated that hydrophilic material surfaces are usually more resistant to protein adsorption and bacterial adhesion than hydrophobic surfaces.[37,38] 2-Methacryloyloxyethyl phosphorylcholine (MPC) is a methacrylate with a phospholipid polar group in the side chain, and is one of the most common biocompatible and hydrophilic biomedical polymers.[39] MPC shows excellent resistance to protein adsorption and bacterial adhesion,[40,41] and has been used in artificial blood vessels,[42] artificial hip joints,[43] and microfluidic devices.[44] The MPC polymer coating renders the surfaces extremely hydrophilic, prevents the adhesion of proteins, and inhibits the adhesion of bacteria.[39–41] Various medical devices using MPC have already been developed and clinically used with the approval of the United States Food and Drug Administration.[45,46] Previous study evaluated the durability and antiadhesive action of MPC grafting on an acrylic resin denture base material.[47] The results demonstrated that graft polymerization of MPC on denture surfaces contributed to the durability of the coating and prevented microbial retention. However, there has been no report on the application of MPC to the dentine bonding agents.

Accordingly, the objectives of this study were to develop protein-repellent dental adhesive resin incorporating MPC and to investigate the resistance of protein adsorption and oral bacterial adherence for the first time. It was hypothesized that: (1) incorporating MPC into primer and adhesive would not compromise the dentine bond strength; (2) MPC-containing primer and adhesive would have much less protein adsorption than that of commercial bonding agent control; and (3) protein-repellent MPC-containing primer and adhesive would greatly reduce biofilm growth than commercial bonding agent control.

2. Materials and methods

2.1. *Fabrication of protein-repellent bonding agent*

Scotchbond Multi-Purpose (SBMP, 3M, St. Paul, MN) was used as the parent system. According to the manufacturer, SBMP adhesive contained 60–70% of bisphenol A diglycidyl methacrylate (BisGMA) and 30–40% of 2-hydroxyethyl methacrylate (HEMA), tertiary amines and photo-initiator. SBMP primer contained 35–45% of HEMA, 10–20% of a copolymer of acrylic and itaconic acids, and 40–50% water.

MPC (Sigma–Aldrich, St. Louis, MO) was commercially available which was synthesized *via* a method reported by Ishihara et al.[39] The MPC powder was mixed with SBMP primer at MPC/(SBMP primer + MPC) mass fractions of 0%, 3.75%, 7.5%, 11.25% and 15%. The 7.5% was selected following previous studies.[43,44] The other mass fractions enabled the investigation of the relationship between MPC mass fraction and protein-repellent efficacy in a dental resin. MPC mass fractions higher than 15% were not included due to a decrease in the dentine bond strength. Each batch of primer was magnetically stirred with a bar at a speed of 150 rpm (Bellco Glass, Vineland, NJ) for 24 h to completely dissolve the MPC in the SBMP primer. Similarly, MPC was mixed into SBMP adhesive at the same mass fractions. Hence, five bonding agents were tested: