Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Short-time dental resin biostability and kinetics of enzymatic degradation

Xiaohong Wang [a], Sheng Song [a], Lei Chen [b], Christopher M. Stafford [c], Jirun Sun [a,*]

[a] Volpe Research Center, American Dental Association Foundation, Gaithersburg, MD 20899, USA
[b] Center for Nanoscale Science and Technology, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
[c] Materials Science and Engineering Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

ARTICLE INFO

Article history:
Received 17 January 2018
Received in revised form 16 April 2018
Accepted 7 May 2018
Available online xxxx

Keywords:
Dental resins
Biostability
Esterase
Lithography
Hydrolysis

ABSTRACT

Resin biostability is of critical importance to the durability of methacrylate-based dental resin restorations. Current methods for evaluating biostability take considerable time, from weeks to months, and provide no short-time kinetics of resin degradation. The objective of this study is to develop a more sensitive method to assess resin biostability over short-time spans (hours to days) that will enhance our understanding of biostability and its resin chemistry. Ultra-flat resin films of equimolar urethane dimethacrylate (UDMA) and triethylene glycol dimethacrylate (TEGDMA) are produced through photocuring between two flat surfaces. Next, metal-covered enclaves and bare-resin channels are generated using stencil lithography to create both degradable and protected (internal reference) regions simultaneously in a single specimen. Resins having three different degrees of vinyl conversion (DC) are compared, and changes of surface roughness and step height in the two regions are monitored by atomic force microscopy (AFM) before and after incubated in enzyme solutions and saline controls. Specimen biostability is ranked based on the topological profile changes when viewed in cross-section before and after enzymatic challenges. In addition, a model is proposed to quantify specimen enzymatic degradation. Based on this model, enzymatic degradation is detected as early as 4 h, and a surge of enzymatic degradation is detected between 4 h and 8 h. The correlation between the DC of resin network and the surge in degradation is discussed. In summary, this new method is effective in ranking biostability and quantifying enzymatic degradation while also reducing labor, time and cost, which lends itself well to materials development and evaluation of dental resins.

Statement of Significance

We report, for the first time, the short-time kinetics of enzymatic degradation of methacrylate dental resins. A nanotechnology based method is developed to accelerate the evaluation of resin biostability. This new method reduces experimental time from weeks to one or two days, which will significantly reduce the costs of labor and enzymes. It also introduces a corresponding parameter (ΔH) and a three-cause model for ranking biostability, which confirms the correlation of chemical structure (DC) and material performance and opens new opportunities for studying the resin biostability and its impact on dental applications. Overall, this is a new tool for evaluating resin biostability and developing new materials.

1. Introduction

Resin biostability is one of the leading concerns regarding the durability of methacrylate-based dental resin restorations [1–4]. The methacrylate functional groups are subjected to hydrolysis by acid, base and enzymes, which split ester function groups into alcohols and acids [2,5]. Consequently, the resin network is degraded and becomes vulnerable to extended mechanical and biological challenges in the oral cavity. Studies have shown that dentin bond strength decreases over time due to degradation of both resin and collagen fibrils [6–8]. Enzymatic challenges to resin biostability are highlighted by the ability of human salivary esterases to degrade methacrylate-based resins and by the potential synergetic degradation effects of cariogenic bacteria,

* Corresponding author at: 100 Bureau Drive, Stop 8546, Gaithersburg, MD 20899-8546, USA.
E-mail address: jsun@nist.gov (J. Sun).

https://doi.org/10.1016/j.actbio.2018.05.009
1742-7061/

e.g., *Streptococcus mutans*, which secrete both acid and esterases that may split ester groups [9,10].

Pseudocholine esterase (PCE) and cholinesterase (CE) are commonly used to evaluate resin biostability due to their existence and activity in human saliva [11–14]. Resin biostability has been characterized directly through changes in chemical, physical, and mechanical properties including surface chemistry [15], mass retention [16], surface roughness and morphology [17,18], hardness [19], and tensile bond strength [20]. Resin biostability has also been ranked by the evolution of hydrolysis products such as methacrylic acid, which in fact is the hydrolytic product of unpolymerized methacrylate functional groups [9,21]. In general, current methods all require more than two weeks of incubation time with esterase, and frequent (at least every two days) refreshing of the expensive esterase. The extended experimental time and high cost of esterases are obstacles in the determination of resin degradation kinetics, which may provide a better understanding of the service life of resin composite restorations. The goal of this study is to develop a method that will significantly shorten the experimental timeframe, enhance the sensitivity and reduce the cost for resin biostability evaluation comparing to the methods mentioned above. With this advancement, we anticipate a better understanding of the kinetics of resin degradation and their correlation with the service life of resin restorations. Additionally, this new method reduces material consumption by scaling down from bulk plaques to a thin film geometry and consequently provides a rapid screening tool for new and possibly limited formulations.

The proposed method involves first generating ultra-flat (surface roughness <0.2 nm) resin channels with hydrolytically stable metal-covered enclaves through photocuring between two smooth surfaces [22–25] followed by stencil lithography [26,27]. The width of the channels resembles the thickness of the dental adhesives (20–50 μm) in resin composite restorations [28–30], where the resin-rich dental adhesives are sandwiched between dentin/enamel and filler-rich composites. The resin-rich areas are more prone to hydrolysis than other areas of the composite. Together with the ultra-flat surface, we employ atomic force microscopy (AFM) to determine degradation-induced morphology changes with high resolution and sensitivity that conventional methods are not able to provide. Specifically, we rank the resin biostability according to the nanoscale changes within the resin channels, and the short-time (within 24 h) kinetics of resin degradation is detected and discussed for the first time. For method development and demonstration purposes, PCE and the copolymers of equimolar urethane dimethacrylate (UDMA) triethyleneglycol dimethacrylate (TEGDMA) are used because both monomers are hydrolyzed sequentially under PCE challenges [14,31]. Furthermore, two variables including degree of vinyl conversion (DC) and incubation time are applied for biostability comparison and kinetic studies using this new methodology. Previous studies have also demonstrated that the addition of filler to the resin formulation reduced resin degradation, suggesting that resin-rich dental adhesives are more vulnerable to degradation than filler-rich composites [3,32]. In this study, we focus on the degradation of pure resin. The success of this method and the information gained from this study promise a practical new tool for material evaluation and development.

2. Materials & methods

2.1. Materials

The methacrylate monomers, urethane dimethacrylate (UDMA) and triethyleneglycol dimethacrylate (TEGDMA) were provided by Esstech Inc (Essington, PA, USA). They were mixed in equimolar ratio. The monomer mixtures were activated for photopolymerization by adding 0.2% mass fraction of camphorquinone (CQ; Aldrich, Saint Louis, MO, USA) and 0.8% mass fraction of ethyl 4-N,N-dimethylaminobenzoate (4EDMAB; Aldrich, Saint Louis, MO, USA). Ultrapure water (18.3 MΩ cm) from a Barnstead Easypure UV Purification System (Thermo Fisher Scientific, USA) was used throughout the experiments.

2.2. Preparation of ultra-flat resin surface with reference areas

2.2.1. General description

Fig. 1 illustrates the sample preparation process: photocuring of the dental resin followed by stencil lithography. For directed adhesion, 5 μL of monomer mixture was sandwiched between a hydrophobized silicon wafer (N-type, ⟨1 0 0⟩, 500 μm thick, prime grade, University Wafers Company, Boston MA, USA) and methacrylate-silanized glass substrate. After pressing the assembly together, the resin was photocured. After curing, the resin is covalently attached to the glass substrate, while the hydrophobized silicon wafer can be carefully separated from the resin using a razor blade. The cured resin is ≈50 μm thick. The ultraflat resin surface is then metal-coated using an E-beam evaporator (Infinity 22, Denton Vacuum, Moorestown, NJ, USA), being guided by a stencil mask. As a result, arrays of resin channels (width = 40 μm) with metal-covered enclaves (125 × 125 μm^2) are generated.

2.2.2. Surface treatment of Si-wafers and glass substrates to enhance directed adhesion

Silicon wafers (10 × 10 mm^2 squares) were first treated with Piranha solution (3 parts by volume of conc. H_2SO_4 and 1 part of 30% H_2O_2) at 100 °C for 1 h, then rinsed with copious amounts of water and dried with high purity nitrogen (99.95%). The clean silicon wafers were then placed inside a 60 mL glass vessel filled with 20 μL perfluorodecyltrimethoxysilane [$CF_3(CF_2)_7CH_2CH_2Si(OCH_3)_3$] (FAS17, Gelest Inc., Morrisville, PA, USA). The vessel was sealed with a cap and then heated in an oven at 150 °C for 3 h [33] to complete the hydrophobization. Glass substrates (10 × 10 mm^2 in size) were also treated with Piranha solution, rinsed with copious amounts of water, and dried with nitrogen. The glass substrates were then silanized by immersing in an acetone solution containing 1 vol% 3-methacryloxypropyltrimethoxysilane (MPTMS, Monomer-Polymer and Dajac Labs, Trevose, PA, USA) and 0.2 vol % formic acid for 30 s. The silanized glass substrates were rinsed with ethanol and dried with nitrogen.

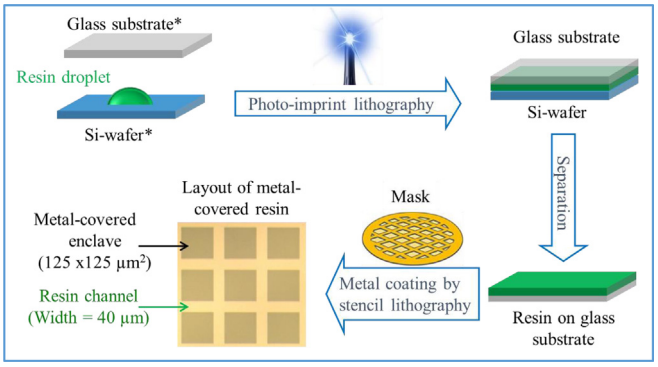

Fig. 1. Preparation process of ultra-flat resin surface with non-hydrolyzable reference areas. *Note:* The glass substrate and the Si-wafer were treated by methacryloxypropyltrimethoxysilane and perfluorodecyltrimethoxysilane to enhance adhesion and release, respectively.