Journal of the Mechanical Behavior of Biomedical Materials 80 (2018) 42–50

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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

Effects of water and microbial-based aging on the performance of three dental restorative materials

Xinxuan Zhou[a,1], Suping Wang[a,b,1], Xian Peng[a], Yao Hu[a,b], Biao Ren[a], Mingyun Li[a], Liying Hao[a], Mingye Feng[a], Lei Cheng[a,b,*], Xuedong Zhou[a,b,*]

[a] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, Sichuan University, Chengdu 610041, China
[b] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Dept. of Cariology and Endodonics West China Hospital of Stomatology, Sichuan University, Chengdu 610041, China

ARTICLE INFO

Keywords:
Dental restorative materials
Water aging
Microbial-based aging
Material performance

ABSTRACT

The objective of this study was to evaluate the performance changes of three restorative materials before and after three different aging treatments: storage in distilled water, *Streptococcus mutans* (*S. mutans*) and oral salivary microbes suspensions for one month. Resin composite (RC), giomer and glass ionomer cement (GIC) were chosen for aging procedures. Surface morphology, roughness average (Ra), color changes and mechanical properties were all determined before and after aging respectively. Biomass and metabolism difference of early attached biofilm on the material surface were tested through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactic acid measurement. The results showed that after *S. mutans* or salivary microbes aging treatments, GIC group displayed significant morphology changes, with Ra value significantly higher than that before aging ($p < .001$). Color changes of giomer and GIC group after *S. mutans* aging were not clinically acceptable. All materials after two microbial-based aging treatments had higher flexural strength than that before aging ($p < .05$). Giomer after salivary microbes aging had higher elastic modulus than the initial values ($p < .05$). Additionally, early attached biofilm biomass and lactic acid production in GIC group after *S. mutans* or salivary microbes aging were higher than that before aging ($p < .05$). While one-month water aging showed less influences on material performance to some extent. In conclusion, to better simulate the harsh oral environment, in vitro microbial-based aging models showed more advantages in evaluating dental restorative materials' degradation over time.

1. Introduction

Dental caries remains among the most prevalent chronic diseases in the world (Pitts et al., 2017). Although it is largely preventable, statistics showed that among adults aged from 20 to 64 years old in America, the populations suffering from dental caries accounted for 90%, while among adults aged more than 65 years old, this number went up to 96.2% (Dye et al., 2015). Filling treatment is still the most effective way to treat dental caries, achieved by many restorative materials including resin composite (RC), giomer, glass ionomer cement (GIC), and so on (Farrugia and Camilleri, 2015; Ferracane, 2011; Gordan et al., 2014). Nevertheless, the complex oral environment provides constant challenges to any restorative material due to the presence of ions, enzymes, bacteria, pH and temperature fluctuations, et al. This may result in degradation of these filling materials, progressively yielding increase of wear and roughness, decrease of mechanical properties, changes of color, or microleakage, which seriously affect the service life of filling materials (Valinoti et al., 2008).

Restorative dentistry, provided with a wide range of dental restorative materials, helps restore the tooth anatomical structure after suffering from caries, erosion, traumatic fracture, et al. However, the deficiencies in physical properties of restorative materials or failure in restorations are still big challenges to their clinical applications (Drummond, 2008). Recurrent caries and fractures are two major reasons for restoration failure after long-term use in the mouth (Mjor et al., 2000; Sakaguchi, 2005; Sarrett, 2005). Furthermore, the replacement of failed restorations accounts for 50–70% of all restorations that are placed, and that replacement dentistry costs $5 billion annually in the USA (Deligeorgi et al., 2001; Frost, 2002). For dentists and patients alike, longevity is thought as the most important considerations when choosing restorative materials. Therefore, dental restorative materials must be evaluated to determine if they are susceptible to degradation

* Correspondence to: State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, China.
E-mail addresses: chenglei@scu.edu.cn (L. Cheng), zhouxd@scu.edu.cn (X. Zhou).
[1] Co-first author.

https://doi.org/10.1016/j.jmbbm.2018.01.023
Received 12 November 2017; Received in revised form 18 January 2018; Accepted 22 January 2018

during the long-term use (D'Alpino et al., 2014). Previous studies investigated these restorative materials using various biodegradation models for aging: e.g., solvents such as distilled water, artificial saliva (without salivary microbes), or alcohol, were chosen to simulate the chemical environment in the mouth (Deepa and Krishnan, 2000; Okte et al., 2006); thermal cycling was used to mimic the temperature changes in oral cavity experienced by restorative materials when used clinically (Chen et al., 2014; Hahnel et al., 2010); UV-accelerated aging simulated UV exposure during outdoor activities (Catelan et al., 2010).

However, there was not much information about comparing the different effects of oral microbial-based aging and water aging on the degraded features of restorative materials. Oral microbes could be considered as harsh environment for restorative materials, in that oral microbes can degrade the polymeric components, thus greatly compromising the marginal integrity and leading to the recurrent caries around restorations (Bourbia et al., 2013; Chen et al., 2014). Within the complex oral salivary microbes, *Streptococcus mutans* (*S. mutans*) is believed to be the chief etiological agent responsible for dental caries, and widely used in vitro studies (Costalonga and Herzberg, 2014). Acid produced by these oral bacteria could not only promote demineralization of the tooth but also contribute to degradation of the resin-based dental composites (Gonzalez-Bonet et al., 2015). Thus, *S. mutans* (cariogenic bacterial species) and oral salivary microbes suspensions (multispecies microcosm inoculums) were introduced into the present study as two microbial-based aging methods, using traditional distilled water aging as control.

Therefore, the objectives of this study were to investigate the properties changes of three commercial restorative materials after water aging and two microbial-based aging treatments for one month for the first time. It was hypothesized that: (1) Water aging, *S. mutans* aging and oral salivary microbes aging treatments have different influences on the material surface appearance, average roughness (RC), color changes and mechanical properties; (2) The changed surface properties after aging would influence the interactions of material surfaces and early attached biofilm biomass or metabolism.

2. Material and methods

2.1. Specimen preparation

Three kinds of common commercial dental restorative materials were used for this study (Table 1), including Group I: Resin composites (RC); Group II: Giomer; Group III: Glass ionomer cement (GIC). Standard bar-shaped or disk-shaped specimens were prepared from these three materials for the following aging procedures. One kind of specimens for three-point bending tests were prepared using a 25 mm × 2 mm × 2 mm stainless steel split mould, according to previous studies (Kadiyala et al., 2016; Park and Ferracane, 2014; Wang et al., 2016); the other kind of specimens were made by the cover of 48-well plates, producing disks with 10 mm in diameter and 1 mm in thickness (Wang et al., 2014). Then all specimens were light cured or self-cured according to the manufacturer's instructions, respectively. Before three aging procedures began, bar-shaped specimens for three-point bending tests were placed into 6-well plates, the other disk-shaped specimens were placed into 24-well plates. After aging treatments, all samples were all naturally dried for the next tests. As for the biofilm experiments, related specimens would be sterilized by ethylene oxide before tests.

2.2. Bacterial strains and culture conditions

S. mutans (ATCC 700610, UA159) was provided by the State Key Laboratory of Oral Diseases (Sichuan University, Chengdu, China), and routinely incubated aerobically at 37 °C with 5% CO_2 in brain heart infusion (BHI) broth (BD, Franklin Lakes, NJ, USA). Oral salivary microbes were collected from ten healthy adult donors having natural dentition without active caries or periodontal disease and without history of taking antibiotics within the last 3 months, which was approved by Ethics Committee of West China Hospital of Stomatology of Sichuan University in Chengdu, China (WCHS-IRB-AF-006). The donors did not brush teeth for 24 h and abstained from food and drink intake for 2 h prior to donating saliva. The collected salivary microbes were diluted and cultured in McBain medium beneficial to salivary microbes growth as reported before (Wu et al., 2015), containing mucin at concentration of 2.5 g/L; bacteriological peptone, 2.0 g/L; tryptone, 2.0 g/L; yeast extract, 1.0 g/L; NaCl, 0.35 g/L; KCl, 0.2 g/L; $CaCl_2$, 0.2 g/L; cysteine hydrochloride, 0.1 g/L; hemin, 0.001 g/L; vitamin K1, 0.0002 g/L and buffer agents (used for maintaining stable pH value at 6–7), then incubated aerobically at 37 °C with 5% CO_2.

2.3. Immersion conditions for aging

All specimens were divided into three aging groups. In water aging group, bar-shaped specimens were placed in each well of 6-well plates with 4 mL distilled water and disk-shaped specimens were placed in each well of 24-well plates with 2 mL distilled water at 37 °C. In *S. mutans* aging group, with the same volume of immersion condition, specimens were stored in BHI medium containing *S. mutans* (10^7 colony forming unit [CFU]/mL) (Arthur et al., 2013). Similarly, some other specimens were immersed in McBain medium with the same volume of salivary bacterial suspension (10^7 CFU/mL), as salivary microbes aging group. The *S. mutans* aging group and salivary microbes aging group were kept in anaerobic condition at 37 °C with 5% CO_2 (Zhang et al., 2015). The immersion media in three aging groups were refreshed every day, which lasted for one month. All specimens before or after aging were collected for the following measurements.

2.4. Scanning electron microscopy (SEM) observation

Morphological characterization of specimens' surfaces were performed using SEM (Quanta 200, FEI, Hillsboro, OR, USA), as previously described (Loomans et al., 2011). For SEM observations, specimens were rinsed twice with phosphate buffered saline (PBS), fixed with 2.5% glutaraldehyde overnight, then serially dehydrated with ethanol (50%, 60%, 70%, 80%, 90%, 95%, and 100%). Finally, all specimens were sputter coated with gold for SEM observation (FEI, Hillsboro, OR, USA). Three random fields were selected to obtain SEM images of each specimen. Six specimens were tested for each group respectively.

Table 1
Materials used in this study.

Material	Product name	Shade	Manufacturer	Composition
Resin composite	Clearfil AP-X	A2	Kuraray Medical Inc., Okayama, Japan	Bis-GMA,TEGDMA, Ba-Si glass filler, camphorquinone and others
Giomer	Beautiful Flow Plus	A2	Shofu Inc.,Kyoto,Japan	Bis-GMA, UDMA,TEGDMA, S-PRG fillers, micro fumed silica and others
Glass ionomer cement	Fuji IX	A2	GC Corp.,Tokyo, Japan	Powder: Alumino-fluoro-silicate glass, polyacrylic acid; Liquid: Polyacrylic acid, polybasic acid, water

Bis-GMA: Bisphenol A glycerolate dimethacrylate; TEGDMA: Triethylene glycol dimethacrylate; UDMA: Urethane dimethacrylate; S-PRG: Surface pre-reacted glass.