Available online at www.sciencedirect.com

ScienceDirect

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

In situ antibiofilm effect of glass-ionomer cement containing dimethylaminododecyl methacrylate

Jin Feng[a,d], *Lei Cheng*[a], *Xuedong Zhou*[a,**], *Hockin H.K. Xu*[b], *Michael D. Weir*[b], *Markus Meyer*[c], *Hans Maurer*[c], *Qian Li*[d], *Matthias Hannig*[d], *Stefan Rupf*[d,*]

[a] *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, China*
[b] *Biomaterials & Tissue Engineering Division, Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland Dental School, Baltimore, MD 21201, USA*
[c] *Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, 66421 Homburg/Saar, Germany*
[d] *Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, Saarland University Hospital, Homburg/Saar, Germany*

ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form
18 April 2015
Accepted 15 May 2015

Keywords:
Biofilms
Glass ionomer cements
Dimethylaminododecyl methacrylate
Anti-bacterial agents

ABSTRACT

Objective. The aim of this study was to investigate antibiofilm effects of a recently developed glass ionomer cement (GIC) containing dimethylaminododecyl methacrylate (DMADDM) under oral conditions.

Methods. Biofilms were allowed to form *in situ* on GIC specimens ($n = 216$) which contained DMADDM (1.1 wt.% or 2.2 wt.%). Samples without DMADDM served as control ($n = 108$). GIC specimens were fixed on custom made splints and exposed to the oral cavity in six healthy volunteers for 24, 48 and 72 h, respectively. Biofilm viability and coverage were analyzed by fluorescence microscopy (FM) and evaluated by red/green ratios and an established scoring system. Bacterial morphology and biofilm accumulation were determined by scanning electron microscopy (SEM). Additionally, material properties as surface charge density of quaternary ammonium groups, surface roughness and DMADDM release were recorded.

Results. FM results showed a higher ratio (24 h: 0%: 0.5, 1.1%: 1.2, 2.2%: 2.5) of red/green fluorescence on GIC samples containing DMADDM. Biofilm coverage and viability scores were significantly reduced (24 h: q1/median/q3 for: 0%: 3/4/5, 1.1%: 2/3/3, 2.2%: 1/2/2) on DMADDM containing samples compared to controls after 24 h as well as 48 and 72 h *in situ* ($p < 0.05$). While surface charge density of quaternary ammonium groups and DMADDM release increased with the DMADDM concentration, surface roughness was lowest on specimens containing 2.2 wt.% DMADDM.

Significance. An *in situ* dental biofilm model was used to evaluate the novel GIC containing DMADDM. This material strongly inhibited biofilms *in situ* and is promising to prevent bacterial colonization on the surface of restorations.

* *Corresponding author at*: Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, Saarland University Hospital, Kirrberger Street, Building 73, D-66421 Homburg/Saar, Germany. Tel.: +49 6841 1624968; fax: +49 6841 1624954.
** *Corresponding author at*: West China School of Stomatology, Sichuan University, No. 14, Section 3rd, Renmin South Road, Chengdu, China. Tel.: +86 28 85501481; fax: +86 28 85501481.
E-mail addresses: zhouxd@scu.edu.cn (X. Zhou), stefan.rupf@uks.eu (S. Rupf).

http://dx.doi.org/10.1016/j.dental.2015.05.005
0109-5641/

1. Introduction

Glass ionomer cement (GIC) was invented by Wilson and Kent in 1971 [1]. It is widely used as a dental material, due to its ease of use, low coefficient of thermal expansion, good biocompatibility with dental pulp tissue, and long-term bonding to tooth surfaces and metals [2–4]. In addition, its unique fluoride ion release characteristics are supposed to have antimicrobial and remineralization effects [5,6]. However, clinical systematic review data were not supportive of an anti-caries effect of GICs [7], indicating that the fluoride-release from GICs is not potent enough to inhibit bacterial growth or combat bacterial destruction processes. One of the most common reasons for replacing a dental restoration is recurrent caries around the margins of the biomaterial [8,9]. Therefore, a dental biomaterial which creates a sustained antimicrobial environment around the restoration would be of considerable clinical benefit.

Efforts were made to synthesize quaternary ammonium methacrylates (QAMs) for use in antibacterial dental materials [10–16]. Quaternary ammonium salts (QAS) can cause bacteria lysis by binding to cell membrane to cause cytoplasmic leakage [17,18]. When the negatively charged bacteria contact the positive quaternary amine charge (N^+), the electric balance is disturbed and the integrity of the bacterial cell wall is damaged under the osmotic pressure [19]. Long cationic polymers can penetrate bacterial cells disrupting the membranes [20,21]. The primer incorporating 12-methacryloyloxydodecylpyridinium bromide (MDPB) demonstrated cavity-disinfecting effects, and the world's first antibacterial adhesive system employing the MDPB-containing primer was successfully commercialized [22]. Recently, a new quaternary ammonium monomer, dimethylaminododecyl methacrylate (DMADDM) has been synthesized. *In vitro* studies have shown a strong antibacterial effect on a DMADDM-containing adhesive without compromising its physical characteristics [23,24]. However, the potential of DMADDM for the prevention of biofilm formation and viability *in vivo* has not been proven, yet.

Being an important factor in the occurrence of dental caries and periodontal diseases, dental biofilm comprises complex three-dimensional structures consisting of diverse communities of microbial multispecies complexes formed on oral tissue [25,26]. To evaluate the antibacterial activity of a material, an *in situ* model needs to be established in order to investigate the material properties under realistic conditions.

The current study investigated antibacterial activities of a GIC containing DMADDM on biofilm formation *in vivo*. The null hypothesis tested was that biofilm formation on GIC surfaces under oral conditions is independent from the incorporation of DMADDM into the material.

2. Materials and methods

2.1. Study design and subjects

Biofilms were formed intra-orally on a total of 324 GIC specimens in a prospective, double-blind *in situ* trial. The study protocol was approved by the ethical committee of the Saarland Medical Association (vote number: 193/08). Six healthy volunteers were involved after signing an informed consent form. Inclusion criteria were: full dentition, sufficient compliance, no periodontal or restorative treatment needs, no local or systemic hypersensitivity to the materials used (splints, silicone impression material, resin composite, antimicrobial agent), no systemic disease(s), no pregnancy, no smokers and, no antibiotic treatment in the last six months. The volunteers received detailed information on the handling of the intraoral splints containing the specimens (see below).

2.2. Specimen preparation

Dimethylaminododecyl methacrylate (DMADDM) was synthesized *via* a modified Menschutkin reaction method. Briefly, 10 mmol of 1-(dimethylamino)docecane (DMAD) (Tokyo Chemical Industry, Tokyo, Japan) and 10 mmol of 2-bromoethyl methacrylate (BEMA) (Monomer-Polymer and Dajac Labs, Trevose, PA) were added in a 20 mL vial with a magnetic stir bar. The vial was capped and stirred at 70 °C for 24 h. After the reaction was complete, the ethanol solvent was removed *via* evaporation, yielding DMADDM as a clear, colorless, and viscous liquid [24].

The glass ionomer cement chosen for the current study was a conventional GIC (Fuji IX GP, GC Corporation, Tokyo, Japan). The novel material was modified by adding 5%, 10% DMADDM (w/w) to the liquid of the GIC while keeping the original powder/liquid ratio of 3.6:1.0 g, thus achieving finial mass fractions of 1.1 wt.% and 2.2 wt.% DMADDM in GIC. GIC without DMADDM (0 wt.%) served as control. Specimens with nominal dimensions of 5 mm diameter and 1 mm thickness were formed by mixing the GIC according to the manufacturers' instructions and packing into silicon molds covered by a mylar strip and glass plate under hand pressure. The mixing was carried out by one individual with extensive experience in GIC handling. Specimens were removed from the molds and coated with a thin layer of adhesive. They were placed for 1 day at 37 °C in a chamber that contained wet tissue paper not in direct contact with specimen, to achieve an atmosphere of 100% humidity but to prevent the specimen from coming in contact with water which could result in dissolution during the critical early phases of setting [27,28]. After this, the specimens were polished by wet SiC paper (grit size 2500) at 300 rpm (Phoenix 3000, Buchler, Braunschweig, Germany) and disinfected in ethanol (70%) for 30 min and subsequently washed several times in distilled water.

2.3. In situ formation of oral biofilms

Alginate impressions (Blueprint cremix®, Dentsply DeTrey, Konstanz, Germany) were made from the upper jaw of the six volunteers. Transparent custom made acrylic splints (Thermoforming foils®, Erkodent, Pfalzgrafenweiler, Germany) were fabricated as carrier of the GIC specimens. Six samples were fixed in the left and right buccal position in the molar and premolar regions with silicon impression material (President light body®, Coltène, Altstaetten, Switzerland) onto the splints [29] (Fig. 1). The splints were exposed intraorally for 24, 48 and 72 h, respectively. During meals or for tooth brushing, splints were removed and stored in a wet chamber. Tooth brushing