Colloids and Surfaces B: Biointerfaces 173 (2019) 52–57

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Zeolite-embedded silver extends antimicrobial activity of dental acrylics

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ARTICLE INFO

Keywords:
Dental acrylic resins
Zeolites
Ag ion-exchange
Long-term antimicrobial dentures
Candida albicans

ABSTRACT

The insertion of prosthetic devices into the oral cavity affects the oral microflora and results in accumulation of microorganisms on the prosthetic surface. Such fouling of denture surfaces can lead to a number of oral diseases and consequently to the replacement of the denture. Here, we report the post-synthesis introduction of silver in zeolite-loaded dental acrylic (DAZ) resins that does not influence the mechanical or aesthetic properties of the DA resins, and provides them with a long-term antimicrobial activity. Na-FAU zeolite (2 wt%) was incorporated into DA resin, which was conventionally processed and cut into 10 mm × 20 mm × 3 mm coupons. The Na^+ in the zeolite was then exchanged with Ag^+ via immersion of the DAZ coupons in 0.01 M $AgNO_3$ solution to obtain DAZ/Ag-treated coupons used in antimicrobial tests. Antimicrobial tests showed that the DAZ/Ag-treated coupons were active against *Candida albicans* (a reference and a clinically relevant strain), *Streptococcus mutans* and *Fusobacterium nucleatum*. Ag leaching tests on the Ag-charged coupons at 1, 2, 3, 4, 7, 14, 30 and 45 days of incubation in distilled water at 37 °C, indicated sustained release of silver. Antimicrobial tests using a reference *Candida albicans* strain showed that the leached coupons retained antimicrobial activity after 45 days immersion in distilled water, but, after 60 days incubation no antimicrobial activity was observed. Cytotoxicity assay results indicated that the DAZ/Ag-treated coupons showed no additional cytotoxicity compared to neat dental acrylic coupons.

1. Introduction

Oral diseases such as dental caries, periodontal diseases and oral candidosis cause significant health problems [1,2]. Management of oral candidosis is limited due to the low number of antifungal drugs available, their relatively high toxicity and the emergence of antifungal resistance [3,4]. Non-rigorous denture hygiene facilitates biofilm build up and initiates local inflammatory responses in the palatal mucosa [5,6]. The denture itself affects salivary flow and allows more niches for microorganisms to flourish, co-aggregate and form biofilms, leading to malodor and a potential reservoir of respiratory pathogens with an increased risk of pulmonary infections [7–10]. It has been estimated that by 2025, in the EU alone, 53% of the population will be over 65 years of age, resulting in significant challenges for oral healthcare delivery [11]. An increasing elderly population will lead to increased demand for dentures. Thus, it would be worthwhile to develop methods for fabrication of dentures that contain controlled release antimicrobials to address the above-mentioned problems.

Heat-cured dental acrylic resin is composed of powder and liquid components (typical ratio; ca. 2.5:1.0 by mass powder to liquid) [12]. The powder component consists of polymethylmethacylate (PMMA) suspension polymer powder, with accessible dibenzoyl peroxide (DBP) initiator; aesthetics related additives such as pigments, opacifiers and flock fibres are also added. Depending on the mode of manufacture, the DBP present is residual from the suspension polymerization and/or is added along with the other additives. The liquid component is composed of inhibited methylmethacrylate (MMA) monomer combined with ethyleneglycoldimethacrylate (EGDMA) crosslinking agent (ca. 8 wt%). Due to some dissolution of the PMMA beads, a dough like mass is formed a short period after mixing the powder and liquid components. The dough is pressed into the mold and the liquid component polymerized by heating the filled mold, following a carefully planned temperature program. The molding surface is finished using a range of abrasives and then polished. Due to the presence of a significant proportion of incompletely dissolved PMMA suspension polymer particles in a matrix that consists of a mixture of uncrosslinked (dissolved from the PMMA powder particles) and EGDMA crosslinked PMMA chains (from the polymerized liquid component), a dental acrylic surface is heterogeneous in nature even before exposed pigment/opacifier particles and flock fibers are considered. The maximum water sorption

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https://doi.org/10.1016/j.colsurfb.2018.09.043
Received 24 April 2018; Received in revised form 17 September 2018; Accepted 19 September 2018
Available online 20 September 2018

permissible of dental acrylic according to BS EN ISO 20795-1:2013 is 32 μg mm^{-3} which translates to about 2.7 wt% according to Kusy et al. [13]. Cast acrylic sheet has water sorption values reported to be between 0.2 wt% and 2 wt% after very long exposure periods (Perspex design guide, Darwen, UK). The heterogeneous surface of dental acrylic combined with the relatively high water sorption therefore renders it more vulnerable to surface fouling by microbial species than cast acrylic sheet. These conditions enable the growth of *Candida* which is adept at adhering and forming a biofilm [5,14]. The *Candida* genus, and *C. albicans* in particular, plays a very important part in biofilm formation and denture stomatitis, however, bacteria also coexist within denture biofilms [9,15–20]. *Streptococcus mutans* (*S. mutans*) and *Fusobacterium nucleatum* (*F. nucleatum*) have been found in denture biofilms [9], making them relevant to studies of antimicrobial dental materials.

Zeolites are microporous aluminosilicate materials with regular pore structures and high surface areas, which can slowly release preloaded antimicrobials over long periods of time [21]. Their ion-exchange properties can be used to introduce antimicrobial metals into zeolite-loaded dental acrylics. Silver has been selected in this study because of its broad spectrum of antimicrobial activity [22,23]. The aims of the present work were to develop a method for fabrication of Ag-containing dental acrylic resins with long-term antimicrobial activity and a potential for recharging of Ag upon Ag depletion.

2. Materials and methods

2.1. Preparation of dental acrylics

Dental acrylic (DA) was prepared by gently mixing 30 mL liquid component (Pegasus Plus Heat Cure Monomer, Davis Schottlander & Davis Ltd, UK) and 69 g powder component (Pegasus Plus Denture Base, pink veined, Davis Schottlander & Davis Ltd, UK) in a glass vacuum jar. The powder – liquid mixture was allowed to rest for 6–8 min in order to allow a fraction of the PMMA particles to dissolve in the MMA and so confer a dough-like consistency to the mixture. The mixture was removed from the jar and kneaded, and then packed into eight 10 mm × 110 mm × 3 mm molds. Each mold was screwed down tightly and placed in a hydraulic press, applying 150 bar of pressure. After quickly removing the excess acrylic, the mold was again placed in the hydraulic press and pressed at 200 bar pressure The molding was cured at 95 °C for 9 h in a dry heat-curing unit (Milnes Bros, UK). After cooling to room temperature, the moldings were released from the mold and cut into 10 mm × 20 mm × 3 mm coupons for antimicrobial tests.

Na-FAU zeolite was prepared from a gel with the molar composition $8NaOH : 0.2Al_2O_3 : 1.0SiO_2 : 200H_2O$ following the procedure described in ref. [24]. The zeolite was organically functionalized with 3-(trimethoxysilyl) propylmethacrylate (Alfa Aesar) prior to adding to the DA precursor mixture [25]. Na-FAU zeolite was mixed with 10 wt% silane solution in cyclohexane at a zeolite to solution weight ratio of 1 to 10, stirred for 30 min at room temperature, washed several times with cyclohexane and dried at 50 °C for 72 h. Experiments were also performed using Ag-FAU zeolite prepared by ion-exchange with $AgNO_3$ solution (0.05 M $AgNO_3$ solution added at a zeolite to solution weight ratio of 1 to 20, stirred for 72 h in the dark, rinsed repeatedly with distilled water and dried at 60 °C overnight), followed by organic functionalization [25]. Silane-treated Ag-FAU zeolite was added to uncured DA at 0.2, 0.4, 0.7 and 2 wt%, whereas Na-FAU and silane-treated Na-FAU zeolite was added to uncured DA at 2 wt% for post-synthesis silver introduction.

Na-FAU-loaded (silane-modified Na-FAU) DA coupons were treated with $AgNO_3$ solution to ion-exchange Na with Ag to obtain DAZ/Ag-treated coupons. Individual dental acrylic coupons were transferred into 25 mL universal bottles and 20 mL of 0.01 M $AgNO_3$ ($AgNO_3$, Alfa Aesar) solution was added to each universal. The bottles were covered with aluminum foil, and the samples were incubated on a shaker at 150 rpm at room temperature for 24 h. After incubation, the samples were washed 20 times with distilled water by decanting and were dried overnight at 60 °C. Blank DA coupons were treated in the same way to distinguish between silver ion-exchange in the zeolite present in the composite and silver absorption by the DA.

2.2. Characterization of dental acrylics

10 mm × 50 mm × 2 mm test pieces were band sawn from the dental acrylic sheets that were prepared as previously described. The long edges of the test pieces were smoothed using water-cooled metallographic grinding decks fitted with progressively finer silicon carbide paper (240-400–600 grit). Thickness and widths of test pieces were measured using a digital caliper. Flexural properties (modulus and strength) of DA and DA loaded with 2 wt% Na-FAU or 2 wt% 3-(trimethoxysilyl) propylmethacrylate-treated Na-FAU and Ag-FAU were determined using a Hounsfield H10KS; span was 32 mm and cross-head speed was 4 mm min^{-1}. Flexural modulus was determined via linear regression of the load deflection data between limits of 0.3 and 0.8%, respectively. Flexural strength was the maximum flexural stress recorded. Six test pieces of each material were tested and the averages and standard deviations recorded. Statistical analysis was performed with Prism version 7 (GraphPad Software, La Jolla, CA, USA). One-way ANOVA followed by Tueky's multiple comparisons test was used to evaluate statistical differences between the samples at 95% confidence.

Scanning electron microscopy analysis of DA and DAZ/Ag-treated coupons was performed with a Carl Zeiss Ltd 40VP Supra Scanning Electron Microscope. Coupons were mounted on Al stubs and coated with gold prior to analysis. For cross-section images, the coupons were attached perpendicularly to the Al stubs with a cement glue.

2.3. Microorganisms

In total, four microorganisms were used for the antimicrobial testing, namely *C. albicans* NCYC 1363, *C. albicans* 135BM2/94, a clinical strain isolated from a patient attending the dental hospital at Cardiff University (ref. [26]), *S. mutans* NCTC 1083 and *F. nucleatum* KS 515-14. The two *C. albicans* strains were grown on Sabouraud Dextrose Agar (SDA; Oxoid, UK) and then sub-cultured in Sabouraud Dextrose Broth (SAB; Oxoid, UK) for 18 h at 37 °C under aerobic conditions. *S. mutans* NCTC 10832 and *F. nucleatum* KS 515-14 were grown on Fastidious Anaerobe agar (FAA; Lab M Limited, UK) containing 5–10% defibrinated horse blood (TCS Biosciences Ltd, UK) and then sub-cultured in Fastidious Anaerobe broth (FAB; Lab M Limited, UK) at 37 °C under anaerobic conditions.

2.4. Antimicrobial testing

Antimicrobial tests were performed as described in our previous work [25]. Single colonies of *C. albicans* NCYC 1363 and *C. albicans* 135BM2/94 were sub-cultured from SDA in sterile SAB. The broth was incubated for 18 h at 37 °C on an orbital shaker (New Brunswick Scientific, UK; I-26 model) at 150 rpm. The liquid culture was centrifuged at 3000 rpm (1721 *g*; Sigma 3-16 L model, UK) for 10 min. Afterwards, the supernatant was discarded and the pellet re-suspended in sterile ¼ Ringer's solution (Oxoid, UK). An optical density of 1.0 at 540 nm was obtained (Jenway 6305 Spectrophotometer, UK), equaling approximately 10^7 CFU mL^{-1}. A 1:100 dilution was performed in Ringer's solution and the suspension (approximately. 10^5 CFU mL^{-1}) was used to inoculate the dental acrylic surfaces, which were laid flat in individual Petri dishes. 50 μL of the standardized suspension (*C. albicans* NCYC 1363, 5×10^5 CFU mL^{-1}; *C. albicans* 135 BM2/94, 10×10^5 CFU mL^{-1}; *F. nucleatum* KS 515-14, 5×10^5 CFU mL^{-1}; and *S. mutans* NCTC 10832, 3×10^6 CFU mL^{-1}) was pipetted onto each coupon's surface and a 20 mm × 10 mm polyethylene film was gently pressed on top of the droplet to ensure the cell suspension were spread evenly on the surface. Immediately after inoculation, three coupons were placed