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Dental plaque microcosm biofilm behavior on calcium phosphate nanocomposite with quaternary ammonium

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Abstract

Objectives—Half of dental restorations fail in 10 years, with secondary caries as the main reason. Calcium phosphate composites could remineralize tooth lesions. The objectives of this study were to: (1) Impart antibacterial activity to a composite with nanoparticles of amorphous calcium phosphate (NACP); and (2) investigate the effect of quaternary ammonium dimethacrylate (QADM) on mechanical and dental plaque microcosm biofilm properties for the first time.

Methods—The NACP and glass particles were filled into a dental resin that contained bis(2-methacryloyloxy-ethyl) dimethyl-ammonium bromide, the QADM. NACP nanocomposites containing 0%, 7%, 14%, and 17.5% of QADM by mass, respectively, were photo-cured. A commercial composite with no antibacterial activity was used as control. Mechanical properties were measured in three-point flexure. A human saliva microcosm model was used to grow biofilms on composites. Live/dead assay, metabolic activity, colony-forming unit (CFU) counts, and lactic acid production of biofilms on the composites were measured.

Results—Increasing QADM mass fraction monotonically reduced the biofilm viability, CFU and lactic acid. Biofilms on NACP nanocomposite with 17.5% QADM had metabolic activity that was 30% that on a commercial composite control ($p < 0.05$). Total microorganisms, total streptococci, and mutans streptococci CFU counts (mean $\pm$ sd; $n=6$) on composite control was 6-fold those on NACP+17.5% QADM nanocomposite. Composite control had long strings of cells with normal short-rod shapes, while some cells on NACP-QADM nanocomposites disintegrated into pieces.

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Adding QADM to NACP did not decrease the strength and elastic modulus, which matched ($p>0.1$) those of a commercial composite without Ca-PO_4 or antibacterial activity.

Significance—A dental plaque microcosm model was used to evaluate the novel NACP-QADM nanocomposite. The nanocomposite greatly reduced the biofilm viability, metabolic activity and lactic acid, while its mechanical properties matched those of a commercial composite. NACP-QADM nanocomposite with calcium phosphate fillers, good mechanical properties and a strong antibacterial activity may have potential for anti-biofilm and anti-caries restorations.

Keywords

Antibacterial nanocomposite; amorphous calcium phosphate nanoparticles; quaternary ammonium; dental plaque microcosm biofilm; stress-bearing; dental caries

1. Introduction

Dental caries is a dietary carbohydrate-modified bacterial infectious disease, and is one of the most common bacterial infections in humans [1–3]. Demineralization of enamel and dentin is caused by acid generated by bacterial biofilms (dental plaque) in the presence of fermentable carbohydrates [1,2,4]. Nearly 200 million dental restorations are placed annually in the U.S. [5]. Resin composites are popular filling materials due to their esthetics and direct-filling capabilities [6–13]. Composite filler particles, resin compositions, and handling and polymerization properties have all been greatly improved [14–21]. However, studies have found that composites had more biofilms and plaques than other restorative materials [22,23]. These plaques could lead to secondary caries, which was found to be a main cause for restoration failure [24]. Currently, half of all restorations fail within 10 years, and replacing them consumes 50% to 70% of the dentist's time [25,26]. In the U.S. alone, replacement dentistry costs \$5 billion annually [27].

Therefore, antibacterial composites are developed with the purpose to inhibit secondary caries [28–30]. Quaternary ammonium salts (QAS) are antibacterial and used in water treatment, surface coatings, and the food industry [31]. Previous studies incorporated polymerizable QAS monomers into resins by forming a covalent bond with the polymer network thus immobilizing the QAS in the composite [28–30,32–35]. Resins with 12-methacryloyloxydodecylpyridinium bromide (MDPB) effectively reduced the *Streptococcus mutans* (*S. mutans*) viability and growth [28–30]. Other antibacterial agents, including methacryloyloxyethyl cetyl dimethyl ammonium chloride and cetylpyridinium chloride, were also added into dental resins [33,36].

Another approach to combat caries is to develop calcium phosphate (CaP)-resin composites [37–40]. CaP composites could release supersaturating levels of calcium (Ca) and phosphate (PO_4) ions and remineralize tooth lesions [38,40]. Traditional CaP composites contained particles of about 1 to 55 μm [38,40]. Recent studies developed nanocomposites using calcium phosphate and calcium fluoride nanoparticles with sizes of 50 to 100 nm [39,41]. In one study, nanoparticles of amorphous calcium phosphate (NACP) of 116 nm were incorporated into a composite [42]. The advantage of NACP nanocomposite was that it released Ca and PO_4 similar to, but its mechanical properties were 2–3 fold higher than, those of traditional CaP composites [42]. The NACP nanocomposite was “smart” and greatly increased the ion release at acidic pH, when these ions are most needed to combat caries [42]. Another study showed that, when placed in a lactic acid solution with a cariogenic pH of 4, the NACP nanocomposite quickly neutralized the acid and increased the pH to a safe level of above 6 [43]. The pH stayed at about 4 when commercial restorative controls were tested in the same manner [43].

There has been little report on imparting an antibacterial activity to CaP composites. Recently, a quaternary ammonium dimethacrylate (QADM) was incorporated into the NACP nanocomposite to combine remineralizing and antibacterial properties in the same composite [44]. A single bacterial species, *S. mutans*, was tested in that study [44]. However, dental plaque is a complicated ecosystem with about 1,000 different bacterial species [45]. Therefore, several previous studies used dental plaque microcosm models, which are laboratory models inoculated with saliva from human donors, to examine antibacterial dental materials [3,46–48].

Accordingly, the objective of this study was to investigate the effect of QADM mass fraction in NACP nanocomposite on dental plaque microcosm biofilms for the first time. It was hypothesized that: (1) The capability of the NACP nanocomposite to hinder microcosm biofilm growth would be directly proportional to the QADM mass fraction; (2) The NACP-QADM nanocomposites would have much lower biofilm viability, metabolic activity and lactic acid, than those of a commercial control composite; (3) The NACP-QADM nanocomposite would have mechanical properties matching those of the commercial control composite.

2. Materials and Methods

2.1. NACP and Glass Filler Particles

NACP [$\text{Ca}_3(\text{PO}_4)_2$] was synthesized via a spray-drying technique as recently described [42]. Briefly, calcium carbonate and dicalcium phosphate anhydrous were dissolved into an acetic acid solution to obtain Ca and PO_4 ionic concentrations of 8 mmol/L and 5.333 mmol/L, respectively, yielding a Ca/P molar ratio of 1.5. This solution was sprayed into a heated chamber, and an electrostatic precipitator was used to collect the dried particles. This yielded NACP with a mean particle size of 116 nm [42]. As a co-filler to reinforce the composite, a barium boroaluminosilicate glass of a mean particle size of 1.4 μm (Caulk/Dentsply, Milford, DE) was silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% n-propylamine. These fillers were used in the composites as described below.

2.2. QADM Resin Composites

BisGMA (bisphenol glycidyl dimethacrylate) and TEGDMA (triethylene glycol dimethacrylate) (Esstech, Essington, PA) were mixed at a mass ratio of 1:1, and rendered light-curable with 0.2% camphorquinone and 0.8% ethyl 4-N,N-dimethylaminobenzoate. The QADM was bis(2-methacryloyloxy-ethyl) dimethyl-ammonium bromide, which was synthesized as described recently [44,49]. Its synthesis employed a modified Menshutkin reaction in which a tertiary amine group was reacted with an organo-halide. 10 mmol of 2-(N,N-dimethylamino)ethyl methacrylate (DMAEMA, Sigma, St. Louis, MO) and 10 mmol of 2-bromoethyl methacrylate (BEMA, Monomer-Polymer and Dajec Labs, Trevose, PA) were combined with 3 g of ethanol in a 20-mL scintillation vial. After stirring at 60 °C for 24 h, the solvent was removed and the QADM was obtained in the form of a clear, colorless and viscous liquid. This method is desirable because the reaction products were generated at quantitative amounts and required no further purification. The QADM was mixed with the photo-activated BisGMA-TEGDMA (referred to as BT). Previous studies used a single QADM mass fraction of 20% [44,49]. The present study tested the following QADM/(BT + QADM) mass fractions: 0%, 20%, 40%, and 50%. Each resin was mixed with 30% NACP and 35% glass fillers, with a total filler level of 65%, to yield a cohesive paste. Because there was 35% resin in the composite, the QADM mass fractions in the composite were: 0%, 7%, 14%, and 17.5%.

For mechanical testing, the paste was placed into rectangular molds of 2 x 2 x 25 mm. For biofilm testing, disk molds of 9 mm in diameter and 2 mm in thickness were used. A

commercial composite, Renamel (Cosmedent, Chicago, IL), was also tested. It consisted of nanofillers of 20–40 nm with 60% fillers in a multifunctional methacrylate ester resin. Renamel is designated as “composite control”. All the specimens were photo-polymerized (Triad 2000, Dentsply, York, PA) for 1 min on each side. This yielded five composites: Composite control, NACP + 0% QADM, NACP + 7% QADM, NACP + 14% QADM, NACP + 17.5% QADM. Six specimens per composite were used for each test ($n = 6$).

2.3. Mechanical Properties

A computer-controlled Universal Testing Machine (5500R, MTS, Cary, NC) was used. Composite specimens were immersed in distilled water at 37 °C for 1 d, and then fractured in three-point flexure with a 10 mm span at a crosshead-speed of 1 mm/min. The specimens were wet and not dried, and were fractured within a few minutes after being taken out of the water. Flexural strength (S) was calculated as: $S = 3P_{\max}L/(2bh^2)$, where $P_{\max}$ is the fracture load, L is span, b is specimen width and h is thickness. Elastic modulus (E) was calculated as: $E = (P/d)(L^3/[4bh^3])$, where load P divided by displacement d is the slope in the linear elastic region.

2.4. Saliva Collection for Biofilm Inoculum

The microcosm model of this study was approved by the University of Maryland. Saliva is ideal for growing dental plaque microcosm biofilms *in vitro*, with the advantage of maintaining much of the complexity and heterogeneity of the dental plaque *in vivo* [47]. Saliva was collected from a healthy adult donor, following a previous study [48]. The donor had natural dentition without active caries or periopathology, and without the use of antibiotics within the last 3 months. The donor did not brush teeth for 24 h and abstained from food/drink intake for 2 h prior to donating saliva. Stimulated saliva was collected during parafilm chewing and was kept on ice. The saliva was diluted in sterile glycerol to a concentration of 30%, and stored at –80 °C [48].

2.5. Dental Plaque Microcosm Biofilm Formation

The saliva-glycerol stock was added, with 1:50 final dilution, into the growth medium as inoculum. The growth medium contained mucin (type II, porcine, gastric) at a 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; CaCl₂, 0.2 g/L; cysteine hydrochloride, 0.1 g/L; haemin, 0.001 g/L; vitamin K1, 0.0002 g/L, at pH 7 [50]. Composite disks were sterilized in ethylene oxide (Anprolene AN 74i, Andersen, Haw River, NC). 1.5 mL of inoculum was added to each well of 24-well plates with a composite disk, and incubated in 5% CO₂ at 37 °C for 8 h. Then, the disks were transferred to new 24-well plates filled with fresh medium and incubated. After 16 h, the disks were transferred to new 24-well plates with fresh medium and incubated for 24 h. This totals 48 h of incubation, which was adequate to form plaque microcosm biofilms [48].

2.6. Live/dead Assay and Bacterial Viability

After 48 h growth, the plaque microcosm biofilms on the disks were gently washed three times with phosphate buffered saline (PBS), and then stained using the BacLight live/dead bacterial viability kit (Molecular Probes, Eugene, OR). Live bacteria were stained with Syto 9 to produce a green fluorescence, and bacteria with compromised membranes were stained with propidium iodide to produce a red fluorescence. Disks were examined using an inverted epifluorescence microscope (Eclipse TE2000-S, Nikon, Melville, NY).

Disks with 48-h biofilms were rinsed with PBS and then immersed in 1% glutaraldehyde in PBS for 4 h at 4 °C. The specimens were rinsed with PBS, subjected to graded ethanol

dehydrations, and rinsed twice with 100% hexamethyldisilazane. The specimens were then sputter-coated with gold and examined via scanning electron microscopy (SEM, Quanta 200, FEI, Hillsboro, OR).

2.7. MTT Assay of Metabolic Activity

MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to examine the metabolic activity of biofilms [44,49]. MTT is a colorimetric assay that measures the enzymatic reduction of MTT, a yellow tetrazole, to formazan. Each disk with 48-h biofilm was transferred to a new 24-well plate, then 1 mL of MTT dye (0.5 mg/mL MTT in PBS) was added to each well and incubated at 37 °C in 5% CO₂ for 1 h. During this process, metabolically active bacteria reduced the MTT to purple formazan. After 1 h, the disks were transferred to a new 24-well plate, 1 mL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals, and the plate was incubated for 20 min with gentle mixing at room temperature in the dark. After mixing via pipetting, 200 µL of the DMSO solution from each well was transferred to a 96-well plate, and the absorbance at 540 nm (optical density OD₅₄₀) was measured via a microplate reader (SpectraMax M5, Molecular Devices, Sunnvale, CA). A higher absorbance is related to a higher formazan concentration, which indicates a higher metabolic activity in the biofilm on the disk.

2.8. Colony Forming Unit (CFU) Counts and Lactic Acid Production

Each disk with 48-h biofilm was rinsed with cysteine peptone water (CPW) to remove loose bacteria. The disks were transferred to 24-well plates containing buffered peptone water (BPW) plus 0.2 % sucrose, and incubated in 5% CO₂ at 37 °C for 3 h to allow the biofilms to produce acid. Subsequently, the BPW solutions were stored for lactate analysis.

The disks with biofilms were then transferred into tubes with 2 mL CPW, and the biofilms were harvested by sonication and vortexing at the maximum speed for 20 seconds using a vortex mixer (Fisher, Pittsburgh, PA). Three types of agar plates were used to measure the CFU counts to assess the microorganism viability. First, tryptic soy blood agar culture plates were used to determine total microorganisms [48]. Second, mitis salivarius agar (MSA) culture plates, containing 15% sucrose, were used to determine total streptococci [51]. This is because MSA contains selective agents crystal violet, potassium tellurite and trypan blue, which inhibit most gram-negative bacilli and most gram-positive bacteria except streptococci, thus enabling streptococci to grow [51]. Third, cariogenic mutans streptococci is known to be resistant to bacitracin, and this property is often used to isolate mutans streptococci from the highly heterogeneous oral microflora. Hence, MSA agar culture plates plus 0.2 units of bacitracin per mL was used to determine mutans streptococci. The purpose of measuring these three CFU counts was to provide antibacterial information on not only the total microorganisms, but also mutans streptococci, in the plaque microcosm biofilm. The mutans streptococci group consists of *S. mutans* and *S. sobrinus*, both species playing a key role in tooth decay.

Lactate concentrations in the BPW solutions were determined using an enzymatic (lactate dehydrogenase) method, following previous studies [44,52]. The microplate reader was used to measure the absorbance at 340 nm (optical density OD₃₄₀) for the collected BPW solutions. Standard curves were prepared using a lactic acid standard (Supelco, Bellefonte, PA).

One-way and two-way analyses of variance (ANOVA) were performed to detect the significant effects of the variables. Tukey's multiple comparison test was used to compare the data at a p value of 0.05.

3. Results

Fig. 1 plots (A) flexural strength, and (B) elastic modulus of the composites (mean $\pm$ sd; $n = 6$). The NACP nanocomposite with various QADM mass fractions had strengths similar to that of composite control ($p > 0.1$). While increasing the QADM content caused a decreasing trend in composite strength, the decrease was not significant ($p > 0.1$). The elastic moduli were also generally similar to each other.

Fig. 2 shows live/dead images of 2-day microcosm biofilms. Biofilms on composite control and NACP+0% QADM were primarily alive, with a mostly continuous coverage. NACP +7% QADM had a noticeable increase in red and yellow/orange staining. When the QADM mass fraction was increased to 14% and 17.5%, the red/yellow/orange staining areas increased. These results demonstrate that adding QADM rendered the NACP nanocomposite antibacterial, and the antibacterial activity became stronger with more QADM.

SEM micrographs of microcosm biofilms on the composites are shown in Fig. 3. Composite control was covered with a thick and dense biofilm, with strings (arrows in A) growing in three-dimensions that formed the biofilm architecture. In (B), NACP+0% QADM nanocomposite had biofilms similar to those of composite control. In (C) and (D) with 7% and 14% QADM, respectively, the biofilms were much less denser, with “P” indicating pores, and the arrow in (C) indicating gaps, in the biofilms. NACP+17.5% QADM (not included) had biofilm features similar to those at 14% QADM. At a higher magnification in (E), the bacterial cells on composite control had a normal short-rod shape, connected with each other to form twisted strings, similar to those in (F). However, as shown in (G) and (H), many cells on nanocomposites with QADM had dissolved into pieces, with long arrows in (G) and (H) indicating cell disintegration. While some cells fell to pieces, other cells with a normal shape were still present on NACP nanocomposites with QADM (short arrows in G and H).

Fig. 4 plots the biofilm metabolic activity (mean $\pm$ sd; $n = 6$). Biofilms on composite control and NACP+0% QADM had similar metabolic activity ($p > 0.1$). Increasing QADM mass fraction significantly decreased the biofilm metabolic activity ($p < 0.05$). At 17.5% QADM, the metabolic activity was approximately 30% that on composite control.

Fig. 5 plots the CFU counts for: (A) Total microorganisms, (B) total streptococci, and (C) mutans streptococci (mean $\pm$ sd; $n = 6$). All three CFU counts showed a similar decreasing trend with increasing QADM mass fraction ($p < 0.05$). All three CFU counts on the NACP +17.5% QADM nanocomposite were reduced to approximately only 1/6 of those on composite control.

The microcosm biofilm acid production data are plotted in Fig. 6. Biofilm on composite control produced the most acid, closely followed by NACP+0% QADM. With increasing QADM mass fraction, the lactic acid production monotonically decreased ($p < 0.05$). Biofilm lactic acid production on NACP+17.5% QADM was about half of that on composite control.

4. Discussion

The present study developed NACP-QADM nanocomposites and investigated the effect of QADM mass fraction on dental plaque microcosm biofilm response for the first time. Little has been reported on the incorporation of antibacterial agents into Ca-PO₄ releasing composites with remineralization capabilities. QADM addition provided a strong antibacterial activity for the NACP nanocomposite, and the microcosm biofilm viability and acid production monotonically decreased with increasing QADM content. CFU counts on

NACP+17.5% QADM were reduced to only 1/6 of those on a commercial composite control. Metabolic activity and lactic acid were also substantially reduced, compared to composite control. The antibacterial activity of NACP nanocomposite was achieved without compromising the mechanical properties. Flexural strength and elastic modulus of NACP nanocomposite were not significantly reduced from 0% to 17.5% of QADM. Mechanical properties are important for composites in load-bearing restorations. NACP-QADM nanocomposites had strength and modulus similar to those of the commercial composite control, which had no Ca-PO₄ or antibacterial activity.

Oral biofilm is a heterogeneous structure consisting of clusters of various types of bacteria embedded in an extracellular matrix. Cariogenic bacteria such as *S. mutans* and lactobacilli in the plaque can metabolize carbohydrates to acids, causing caries. In previous studies, three types of biofilm models were used: Single species, defined consortium, and microcosm [2,46–48,53]. *S. mutans* was frequently used in single species biofilm models because it plays a key role in caries [2,28,33]. Since dental plaque is a complicated ecosystem [45], microcosm models were inoculated using saliva from donors to maintain the complexity and heterogeneity *in vivo* [47]. Previous studies either used saliva from a single donor [3,46,48,50], or mixed saliva from several donors [53,54]. In the present study, microcosm biofilm from a single donor was tested. Besides total microorganisms, total streptococci CFU was measured because in the dental plaque, streptococcal species plays an important role in the caries process, especially in the initial microflora [55]. Furthermore, mutans streptococci CFU was measured because it was shown that the mutans group, which includes *S. mutans* and *S. sobrinus*, are the major pathogens of caries [56]. All three CFU counts were markedly reduced on NACP+17.5% QADM. These results indicate that QADM had a strong antibacterial effect against total microorganisms, total streptococci, and mutans streptococci. Hence, the dental plaque microcosm biofilm model is useful in evaluating antibacterial composites.

In previous studies, QAS monomers were incorporated into resins to achieve antibacterial activity [28–30,32–34]. QAS monomers can be copolymerized with resins by forming a covalent bond with the polymer network, leading to QAS immobilization in the composite. Extensive studies were performed on composites containing MDPB where the antibacterial activity was demonstrated [28–30]. Besides composites, QAS bromides and chlorides were incorporated into glass ionomer cements [34]. In addition, QAS monomers were used to develop antibacterial adhesive resins and primers [33,36,57,58]. QAS monomers in previous studies are usually monomethacrylates, including MDPB [28–30] and QAS chloride [33]. Recently, QAS dimethacrylates were synthesized [49]. One study incorporated a QAS dimethacrylate (referred to as QADM) into a CaP composite which greatly reduced the titer counts, metabolic activity, and acid production of *S. mutans* biofilms [44]. A potential benefit of QADM is that it is a dimethacrylate and has reactive groups on both ends of the molecule. The synthetic method for QADM was relatively easy comparing to other QAS monomers [49]. In addition, QADM has a low viscosity and is readily miscible with other dental dimethacrylates.

SEM examination showed that bacterial cells on composite control had normal short-rod shapes connected with each other to form long strings, while many cells on NACP-QADM nanocomposite had disintegrated into pieces. These observations are consistent with a previous study which showed that a portion of *S. mutans* cells dissolved to become debris upon the application of antibacterial agents [59]. Regarding the antimicrobial mechanism, it is suggested that QAS materials can cause bacteria lysis by binding to the cell membrane to cause cytoplasmic leakage [32]. When the negatively charged bacterial cell contacts the positively charged (N⁺) sites of the QAS resin, the electric balance of the cell membrane could be disturbed, and the bacterium could explode under its own osmotic pressure [36].

Based on this mechanism, it could be postulated that increasing the number of positively charged sites of QADM on the resin composite surface would increase its antibacterial potency. Indeed, in the present study, gradually increasing the QADM mass fraction from 0% to 17.5% in the NACP nanocomposite significantly and monotonically decreased the biofilm viability, CFU counts and lactic acid production. Further study is needed to optimize the QADM content in the nanocomposite to maximize its antibacterial capability without compromising its load-bearing capability. The durability of the nanocomposite needs to be investigated by measuring its antibacterial properties against microcosm biofilms after long-term water-aging treatments. In addition, the anti-caries capability of the nanocomposite needs to be evaluated in a tooth cavity model by measuring the mineral content of enamel and dentin near the margin under biofilms.

5. Conclusions

This study investigated dental plaque microcosm biofilm behavior on NACP nanocomposites with varying QADM content for the first time. Previous studies showed that composites with calcium phosphate fillers released Ca and PO₄ ions and remineralized tooth lesions; however, little has been reported on rendering these composites antibacterial. In the present study, increasing the QADM mass fraction monotonically decreased the microcosm biofilm viability and lactic acid production. NACP nanocomposite with 17.5% QADM had total microorganisms, total streptococci, and mutans streptococci CFU counts of only 1/6 of those on a commercial composite. Incorporation of QADM for up to 17.5% in NACP nanocomposite did not significantly compromise the strength and elastic modulus, which matched those of a commercial composite without Ca-PO₄ or antibacterial activity. The novel NACP-QADM nanocomposite with calcium phosphate fillers, good mechanical properties and a strong antibacterial activity may be promising to combat secondary caries.

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Figure 1. Mechanical properties of composites: (A) Flexural strength, and (B) elastic modulus (mean $\pm$ sd; n = 6). The composite specimens were immersed in distilled water at 37 °C for 1 d, and were then fractured within a few minutes after being taken out of the water. In each plot, bars with the same letters indicate values that are not significantly different ($p > 0.1$).

Figure 2.

Live/dead staining of dental plaque microcosm biofilms adherent on composites: (A) composite control, (B) NACP+0% QADM, (C and D) NACP+7% QADM, (E) NACP+14% QADM, (F) NACP+17.5% QADM. In (A and B), the microcosm biofilms were primarily alive, with a mostly continuous coverage. The live bacteria were stained green, and the dead bacteria were stained red. However, when live/dead bacteria were in close proximity or on the top of each other, the staining had yellow/orange colors. (C) Example of red staining (arrow), and (D) example of yellow/orange staining (arrow), in different areas on the same composite.

Figure 3.

SEM micrographs of plaque microcosm biofilms: (A-D) Lower magnification, and (E-H) higher magnification. In (A), composite control was covered with dense biofilms consisting of numerous long strings (arrows), similar to those on NACP nanocomposite without QADM in (B). Some areas of the biofilms had bulges of bacteria growth, indicated by “G” in (A). In (C) and (D), NACP-QADM nanocomposites had thinner biofilms with numerous pores “P”, without long strings. The arrow in (C) indicates gaps. In (E and F), the long strings were made of bacterial cells connected with each other, and the cells had a normal, healthy short-rod morphology. However, as shown in (G) and (H), many cells on NACP-QADM nanocomposites had dissolved into pieces, while other cells still had a normal short-rod shape (long arrows indicate cell disintegration, and short arrows indicate normal healthy cells).

Figure 4. MTT assay of metabolic activity of plaque microcosm biofilms adherent on the composites (mean $\pm$ sd; n = 6). Bars with dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

Figure 5.

Colony-forming unit (CFU) counts for: (A) Total microorganisms, (B) total streptococci, and (C) mutans streptococci (mean $\pm$ sd; $n = 6$). All three CFU counts showed a decreasing trend with increasing QADM mass fraction in the NACP nanocomposite ($p < 0.05$). All three CFU counts on NACP+17.5% QADM were reduced to only approximately 1/6 of those on the commercial composite control. In each plot, bars with dissimilar letters indicate values that are significantly different ($p < 0.05$).

Figure 6. Lactic acid production by dental plaque microcosm biofilms adherent on the composites (mean $\pm$ sd; n = 6). Bars with dissimilar letters indicate values that are significantly different ($p < 0.05$).