Published in final edited form as:

Biomed Mater. 2012 August ; 7(4): 045014. doi:10.1088/1748-6041/7/4/045014.

BisGMA/TEGDMA dental nanocomposites containing glyoxylic acid modified high-aspect ratio hydroxyapatite nanofibers with enhanced dispersion

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Abstract

The purpose of this research was to investigate the influence of the glyoxylic acid (GA) modification of hydroxyapatite (HAP) nanofibers on their dispersion in bisphenol A glycidyl methacrylate (BisGMA)/triethylene glycol dimethacrylate (TEGDMA) dental composites and also investigate the mechanical properties, water absorption, and water solubility of the resulting dental resins and composites. Scanning/Transmission electron microscopy (STEM) images showed that micro-sized HAP nanofiber bundles could be effectively broken down to individual HAP nanofibers with an average length of ~15 μm after the surface modification process. Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS) and thermal gravimetric analysis (TGA) characterization confirmed glyoxylic acid was chemically grafted on the HAP nanofiber surface, hypothetically by reacting with the amine group on HAP nanofiber surface. The enhanced dispersion of HAP nanofibers in dental matrix led to increased biaxial flexural strength (BFS) compared with the corresponding dental resins and composites filled with untreated HAP nanofibers. In addition, impregnation of small mass fractions of the glyoxylic acid modified HAP nanofibers into the BisGMA/TEGDMA dental resins (5wt%, 10wt%) or composites (2wt%, 3wt%) could also substantially improve the BFS in comparison with the controls (pure resins or dental composites filled with silica particles alone). Larger mass fractions could not further increase the mechanical property or even degrade the BFS values. Water behavior testing results indicated that the addition of glyoxylic acid modified HAP nanofibers resulted in higher water absorption and water solubility values which is not preferred for clinical application. In summary, well dispersed HAP nanofibers and their dental composites with enhanced mechanical property have been successfully fabricated but the water absorption and water solubility of such dental composites need to be further improved.

1. Introduction

Since the debut in 1960s, BisGMA based dental restorative composites have received widespread clinical acceptance as alternative restorative materials to dental amalgam in terms of better aesthetic properties and less safety concern [1–3]. However, clinical data shows that these composites possess much shorter average lifespan (6 to 8 years) on posterior restoration than dental amalgam restoration (about 13 years)[1, 3–5]. Considering

composite fracture, besides secondary caries, is another significant reason for the replacement of dental composites, it is anticipated to improve the mechanical strength and toughness of dental composites which could eventually lower the failure rate and extend the longevity [6, 7]. Research on dental composites mainly includes several topics such as development of new resins or techniques to reduce shrinkage stress and rate [8–11], surface modification of filler to improve the filler-resin bonding [12–14], the composite/adhesive/filler interface [15–19], and development of new fillers [20–26]. It is generally accepted that in comparison with particles, fibers are preferred reinforcing materials because fibers can provide much higher load transfer and they can also make composites with improved toughness. As a result, more efforts have been focused on incorporating fiber fillers into dental composites since the late of 1990s. Reinforcement with either high-strength inorganic fibers or relatively low-strength polymer fibers has been shown to lead to significant improvement on the mechanical properties of dental composites [27–31]. Xu and his colleagues reported that the impregnation of extremely strong ceramic whiskers (Si_3N_4 or SiC) could lead to two-fold increase in composite strength and toughness [32–34]. Fong et al. also showed the impregnation of 5 wt% electrospun polymer (Nylon 6) nanofibers could result in 36% and 26% increase in flexural strength and modulus, respectively [31]. Besides the reinforcing effects, it has also been revealed that fibers have the potential to reduce the polymerization shrinkage rate, which is another critical issue on current clinical restoration [35–37]. Recently, considering HAP based materials exhibit good biocompatibility, HAP fibers were selected as dental fillers with the hope to develop mineral releasing dental composites with enhanced mechanical properties. Investigators from Hong Kong reported that the incorporation of silanized HAP microwhiskers (about 1 μm in diameter and $116 \pm 23 \mu\text{m}$ in length) into BisGMA based dental matrix could only slightly improve the flexural strength. In our previous work, we observed that the addition of small mass fraction (3 wt%) of high aspect-ratio (the ratio of fiber length to diameter) HAP nanofibers (about 100 nm in diameter and 60–80 μm in length) into the BisGMA/TEGDMA composites could significantly increase the biaxial flexural strength by 29% [38]. However, it is very easy for the HAP nanofibers to aggregate in bundles both in the water solution and dental resin matrix. If the bundles are very thick with low aspect-ratio, they may work as mechanically weak points and lead to the substantial degradation on mechanical strength.

For composites, especially for the nanocomposites, surface modification of fillers is a critical step in improving the mechanical properties of the resulted composites. A proper surface modification could effectively break down the filler agglomerates, achieve a more uniform dispersion in the matrix, and generate good interfacial bonding with the matrix. Investigators from University of Toronto [39] pointed out that the chemo-mechanical treatment on cellulose nanofibers extracted from soybean could improve the dispersion significantly and lead to 4 to 5 fold increase on tensile strength compared to the untreated fiber-blend-polyvinyl alcohol (PVA) film. Shi and his colleagues [40] successfully enhanced the carbon fibers dispersion and filler-matrix interfacial bonding in polystyrene based composites by depositing polystyrene coating on the surface of carbon fibers via plasma polymerization approach. To date, significant efforts have been made in breaking down the HAP nanoparticle agglomerates, which were generally attributed to the interparticle Van der Waals force and the hydrogen bonding between the hydroxyl groups on the surface [37, 41–45]. HAP nanoparticles after grating with poly (L-lactide) (PLA) [41, 42] and poly(ϵ -caprolactone) (PCL) [43] could achieve more uniform dispersion and better colloidal stability in organic solvents. Both the HAP reinforced PLA and HAP reinforced PCL nanocomposites containing surface modified HAP nanoparticles have improved mechanical strength compared to the composites containing untreated HAP nanoparticles [41, 43, 46].

In this study, glyoxylic acid, a natural product presented in fruit, leaves and sweet beet, was used to modify HAP nanofiber surface, with an anticipation to achieve improved dispersion

in dental matrix. The hypothesis was that uniform distribution of HAP nanofibers in BisGMA/TEGDMA composites would lead to significant improvement on mechanical properties. To test this hypothesis, photo-cured dental resins/composites filled with various mass fractions of glyoxylic acid modified HAP nanofibers and silica particles were systematically fabricated. The biaxial flexural strength of dry-state dental resins and wet-state dental composites was then tested and analysis of variance (ANOVA) was used for statistically of the acquired data. FT-IR, TGA and XPS were performed to characterize the surface modification of HAP nanofibers. The dispersion and morphology of HAP nanofibers and the fracture surface of resulting dental resins and composites were examined by scanning/transmission electron microscopy. In addition, the investigation of water absorption and water solubility was conducted in this study as well.

2. Materials and methods

2.1 Materials

Camphorquinone (CQ), Ethyl 4-dimethylamino-benzoate (EDMAB), calcium nitrate, sodium dihydrogen phosphate dehydrate, gelatin, urea, acetone, glyoxylic acid (GA) and sodium cyanoborohydride were purchased from Sigma-Aldrich Company (St. Louis, MO, USA) without further purification. BisGMA, TEGDMA and the 0.7 μm silica particles with 1 wt% γ -methacryloxypropyltrimethoxysialne (γ -MPS, V-117-4107) were provided by Esstech Inc (Essington, PA, USA).

2.2 Synthesis of HAP nanofibers

The synthesis procedure is same as our previous method [38], which is modified from an approach reported by Zhan and his colleagues [47]. In a representative experiment, calcium nitrate (0.02mol/L), sodium dihydrogen phosphate dehydrate (0.02mol/L), gelatin (0.4g/L), and urea (0.04mol/L) were dissolved and mixed in an aqueous solution at the room temperature. Then, this mixed solution was heated to 95 °C and kept at such temperature for 72 hours. Finally, the product was filtered, washed with deionized water, and dried at the room temperature.

2.3 Surface modification of HAP nanofibers

5 gram (4.98 mmol) as-fabricated HAP nanofibers were dissolved in 150 ml deionized water and 0.39 gram (5.26 mmol) glyoxylic acid was then added. After stirred for 30 minutes, 0.28 gram (4.46 mmol) of sodium cyanoborohydride (catalyst) was added. After being stirred for another 24 hours, the fibers were filtered and washed with deionized water for three times so as to remove the residual catalyst. Then, surface modified HAP nanofibers were dried in oven at about 100 °C.

2.4 Dental resin/composite samples preparation

The organic matrix contained 49.5 wt% BisGMA and 49.5 wt% diluent comonomer TEGDMA. CQ (0.5 wt%) and EDMAB (0.5 wt%) were added as the initiator and co-initiator, respectively. Various mass fractions of untreated and GA modified HAP nanofibers were added into the 20 ml glass vial to mix with the dental resins (without silica particles) and composites (with silica microparticles). When silica microparticles are added, the total inorganic filler mass fraction was 60%, including both silica and HAP. 3–5 drops of acetone were added to reduce the viscosity of the mixture. Then the mixed materials (5 gram in total) were further stirred with the magnetic stir for about 2 hours. The glass vials were then placed in the vacuum oven (Napco model 5851) for 24 hours at 40 °C to remove the residual acetone in the well-mixed dental mixture. After thoroughly mixing and acetone removing process, the high viscosity mixture were added carefully to a circular shaped Teflon mold (diameter 12 mm, thickness 1.5 mm) with glass slide cover and photo-cured by Triad 2000

with 250 Watt 120 Volt Tungsten Halogen lamp (Dentsply, York, PA, USA) for 2 minutes on each side. Please note that all the sample preparation procedures were carried out on yellow light environment in order to prevent the unexpected photo-curing.

2.5 Characterization

2.5.1 Fourier transform infrared spectroscopy (FT-IR)—Fourier transform infrared spectrophotometer (Perkin-Elmer, Waltham, MA, USA) was used to characterize the functional groups of untreated and surface modified HAP nanofibers. For each spectrum, 32 scans between the wave numbers of 4000 to 400 cm^{-1} were recorded in the absorption mode by a potassium bromide method.

2.5.2 X-ray photoelectron spectroscopy (XPS)—Prior to characterization, untreated and surfaced modified HAP samples were dried in oven at about 100 °C overnight. In order to avoid signals from untreated XPS holder, a RGA procedure-cleaned carbon tip, which is clamped on the stainless steel-made holder, was used as the support to hold a few millimeter thick HAP powder. After samples were loaded into the high vacuum chamber (10^{-8} Torr), Ar ions were then applied to sputter away the surface contamination over HAP samples. Then samples were analyzed by PHI 5400 XPS (Perkin-Elmer, Waltham, MA, USA) with Mg K α source at 15 KV and 20 mA. Sweeping rates were adjusted from 0.1 eV/step to 1 eV/step. Scanning spectrum ranges from 0 eV to 800 eV covering most binding energies of possible elements in HAP. Finally, the data were collected and quantitatively analyzed by AugerScan software.

2.5.3 Thermal gravimetric analysis (TGA)—TGA measurements of untreated and surface modified HAP nanofibers were performed with TA Instruments Q50 (New Castle, DE, USA) from 50 to 900 °C at a heating rate of 10 °C/min.

2.5.4 Morphology—Scanning/Transmission electron microscope (STEM, Quanta 600 FESEM, FEI, Japan) was employed to observe the morphology, size and dispersion of the HAP nanofibers and the fracture surface of as-fabricated dental resins and composites. The average length of the surface modified HAP nanofibers was determined by measuring the lengths of 85 nanofibers in the 645×484 STEM images with Image Pro software.

2.5.5 Biaxial flexural strength (BFS)—As mentioned in Section 2.4, a minimum of six specimens, approximately 12 mm in diameter and 1.5 mm in thickness for biaxial flexural test, were prepared for each dental resin/composite system by photo-curing the specimens for 2 minutes on each side. Prior to mechanical testing, both the dry-state dental resin specimens and the wet-state dental composite specimens (immersed in distilled water for 24 h at 37 °C) were carefully polished with 2400 grit silicon carbide sandpaper. The final dimensions of the specimens were then measured and recorded. Biaxial flexural test was conducted on a computer controlled Instron 3367 universal testing machine at a crosshead speed of 5 mm/min (Instron 3367, Norwood, MA, USA) [48, 49]. The Analyzer recorded the applied load as a function of time. The BFS was calculated according to Timoshenko and Woinowsky-Kreiger (Eq. 1).

$$\sigma = \frac{P}{h^2} \left\{ (1+\nu) \left[0.485 \ln \left(\frac{a}{h} \right) + 0.52 \right] + 0.48 \right\} \quad (1)$$

Where σ was the maximum tensile stress, P the measured fracture load, a was the radius of the knife-edge support, h was the mean specimen thickness measured from fragments at the point of fracture with a screw-gauge micrometer accurate to 10 μm and ν was the Poisson's

ratio. The biaxial flexural test is known to be more clinical relevant compared to the three point flexural test. In addition, disc specimens are photo-cured in one-shot, eliminating an overlapping curing technique associated with bar-shaped specimens, which might reduce the reliability of strength data[50].

2.5.6 Water absorption and solubility—Water absorption rate and solubility rate were measured with five specimens and each of them was stored in 20 ml distilled water for 28 days at 37 °C. The dry weight (M_d) of the specimens was measured with a balance with an accuracy of 0.1mg. During the water immersion, weight of the specimens (M_w) was measured at 1, 2, 3, 7, 14, 21, and 28 days. Solubility weight (M_h) was measured after the dehydration was stabilized at 60 °C in air. The diameter and thickness of dental specimens were measured and the volume (V) was calculated in cubic millimeters. Water absorption and solubility were calculated as follows:

$$\text{Water absorption} = (M_w - M_h) / V$$

$$\text{Water solubility} = (M_d - M_h) / V$$

2.6 Statistical analysis

The data are presented as the mean $\pm$ standard error of mean (SEM) for the indicated number of separate experiments. Post-hoc test was used for statistical analysis by one-way analysis of variance (ANOVA) and P -values less than 0.05 were considered as significant.

3. Results

3.1 Surface modification of HAP nanofibers

As shown in figure 1a, the untreated HAP nanofibers tend to form large bundles with size of 1 μm to several μm . After GA modification, microsized HAP nanofiber bundles (figure 1a) were effectively broken down to individual HAP nanofibers with relatively uniform diameter (figure 1b), which ranges from 50 to 100 nm. By measuring the fiber length with Image Pro software, it was found that the mean length of HAP nanofibers after modification is approximately 15 μm with relatively narrow size distribution (figure 1c).

FT-IR spectroscopy is a convenient and effective tool to identify the functional groups on molecular level. The absorption bands of both the untreated HAP nanofibers and surface modified HAP nanofibers are shown in figure 2. All the major characteristic absorption bands of HAP such as stretching vibration mode of lattice OH^- (3457 cm^{-1}), stretching mode of phosphate (1094 , 1035 and 965 cm^{-1}), bending mode of phosphate (565 , 604 and 840 cm^{-1}) and carbonate group (1368 cm^{-1}) could be seen on both the untreated and surface modified samples. In addition, both samples have almost the same intensities at those characteristic peaks except for OH^- . Moreover, the characteristic absorption bands of gelatin, which include C=O stretching (1640 cm^{-1}), C-H stretching (2918 , 2850 cm^{-1}), C-H bending (1470 cm^{-1}), and C-N stretching (1230 cm^{-1}) were also observed on both samples. Please note that the intensity of C=O stretching (1640 cm^{-1}), C-H stretching (2918 , 2850 cm^{-1}), C-H bending (1470 cm^{-1}) and OH^- stretching (3457 cm^{-1}) characteristic peaks of surface modified HAP sample are significant stronger than the corresponding bands on the untreated HAP nanofiber sample, which indicates there are more C-H , C=O and OH^- bands on the modified sample.

Besides FT-IR spectrum, XPS and TGA were performed to characterize the modification as well. The XPS spectrum (figure 3) confirmed the presence of calcium, carbon, oxygen, phosphorus and nitrogen elements on both untreated and GA modified HAP nanofibers. From the quantitative analysis of surface atomic composition (table 1), it can be observed

that more oxygen element was detected after the modification and the relative amount of calcium, phosphorus, and nitrogen all decreased, which is in good agreement with FT-IR characterization. As shown in the TGA curves (figure 4), the untreated HAP nanofiber and GA modified HAP nanofiber reached the stable stage at about 790 °C and 810 °C, respectively. In addition, both samples have very similar TGA curves and almost the same weight loss which is about 3.7 wt%.

3.2 Mechanical properties and water behavior of dental resins and composites containing untreated or glyoxylic acid modified HAP nanofibers

Table 2 shows the biaxial flexural strength (BFS) of BisGMA/TEGDMA dental resins containing various mass fractions of untreated or GA modified HAP nanofibers in dry state. Photo-cured pure resins were used as the control and the BFS of such unfilled dental resin was 100.3 ± 2.4 MPa. Both the untreated and GA modified HAP nanofibers reinforced dental resin reached the maximum BFS value at the filler mass fraction of 10 wt%. The strength values of BFS of GA modified HAP nanofibers reinforced dental resins as a function of HAP mass fraction showed the similar trend with the corresponding dental resins containing untreated HAP nanofibers. The dental resins filled with 10 wt% untreated and GA modified HAP nanofibers reached their maximum BFS at 103.5 ± 5.4 MPa and 114.6 ± 7.8 MPa, respectively. In comparison with the control, no significant difference could be observed on BFS after incorporating 10 wt% untreated HAP nanofibers, while the BFS was improved by 14.3% with 10 wt% of GA modified HAP nanofibers. Table 2 also revealed that both the BFS of dental resins filled with 20 wt% untreated HAP nanofibers (80.4 ± 5.7 MPa) and GA modified HAP nanofibers (82.4 ± 6.2 MPa) were substantially lower than the control ($P < 0.05$). The result indicated that further increasing the mass fractions of both the untreated and GA modified HAP nanofibers did not improve the mechanical strength further and may even significantly degrade the mechanical properties. Additionally, our results showed that with the same mass fraction of filler, the BFS of composites containing GA modified HAP nanofibers were always significantly higher than that of composites containing untreated HAP nanofibers.

Figure 5 shows the SEM images of fracture surface of the dental resins containing various mass fractions of untreated or GA modified HAP nanofibers. As shown in figure 5a (filled with 5 wt% of untreated HAP nanofibers) and 5b (filled with 5 wt% of GA modified HAP nanofibers), many white spots are observed on the fracture surface, which are in general well dispersed HAP nanofibers. In figure 5c (filled with 10 wt% of untreated HAP nanofibers) and 5d (filled with 10 wt% of GA modified HAP nanofibers), besides the well dispersed HAP nanofibers, HAP bundles are also observed with the bundle density is higher and the bundle size is much larger in untreated HAP nanofiber sample than in the GA modified HAP nanofiber sample.

Biaxial flexural testing results of BisGMA/TEGDMA dental composites filled with various mass fractions of untreated or GA modified HAP nanofibers and 0.7 micrometer size silica particles in wet state are shown in table 3. The control samples were the composites with 60 wt% silica particles. It revealed that similar as the aforementioned dental resin samples, GA modified HAP nanofibers reinforced dental composites have the same trend as the related untreated HAP samples in terms of BFS values. The values of BFS of both untreated HAP nanofiber samples and GA modified HAP nanofiber samples were significant higher ($P < 0.05$) than the control, when small mass fraction HAP fillers (2 wt%, 3 wt%, 5 wt%) were added into dental composites. However, a higher mass fraction of 10 wt% HAP nanofibers, either untreated or GA modified, did not change the BFS value significantly ($P < 0.05$, compared the control). Both the untreated and GA modified HAP nanofibers reached the maximum reinforcing effect at mass fraction of 3 wt%. Dental composites containing 3 wt% untreated HAP nanofibers and 57 wt% silica particles, the BFS was

120.7±14.4MPa, improved by 28.9 %, compared to the control. Meanwhile, for the GA modified 3 wt% HAP fibers and 57 wt% silica particles samples, the BFS was 137.2±4.9 MPa, improved by 46.5 %, in comparison with the control. Please note that all the GA modified HAP samples except on 10 wt% have significant higher values on BFS than the associated untreated HAP samples after incorporating HAP nanofibers on same mass fractions. Figure 6 shows the SEM images of fracture surface of dental composites containing silica particles and untreated or GA modified HAP nanofibers. With silica particles, the nanofibers tend to form bundles and aggregates at relatively lower content of HAP. In addition, GA modified HAP nanofibers have much better dispersion in BisGMA/TEGDMA dental matrix than corresponding untreated HAP nanofibers.

The water absorption rate of dental composites containing 60 wt% silica particles, 2 wt% GA modified HAP nanofibers and 58 wt% silica particles, and 5 wt% GA modified HAP nanofibers and 55 wt% silica particles was presented in figure 7. It can be observed that all the three samples reached the stable state after seven days immersion in distilled water. With the addition of GA modified HAP nanofibers to the dental composites, the values of water absorption rate were significantly increased ($P<0.05$). After 28 days immersion, the water absorption rate of 60 wt% silica particles impregnated dental composites was $32.8\pm0.6\text{ }\mu\text{g/mm}^3$. With the addition of 2 wt% and 5 wt% GA modified HAP nanofibers, the water absorption rate values increased to 41.7 ± 2.3 and $43.1\pm1.8\text{ }\mu\text{g/mm}^3$, respectively. Table 4 showed that the water solubility rate was also enhanced substantially after incorporation of GA modified HAP nanofibers into the dental composites. The water solubility values of dental composites containing 60 wt% silica particles, 2 wt% GA modified HAP nanofibers and 58 wt% silica particles, and 5 wt% GA modified HAP nanofibers and 55 wt% silica particles, are 3.36 ± 0.98 , 11.14 ± 2.32 and $10.58\pm1.25\text{ }\mu\text{g/mm}^3$, respectively.

4. Discussion

Our previous investigation[38] revealed that such untreated HAP nanofiber bundles formed very significant filler agglomerations in dental matrix even with low mass fraction incorporation and degraded the reinforcing effect substantially. In this study, we are aimed to break down the large HAP nanofiber bundles and significantly improve the mechanical strength of corresponding dental composites. According to the crystal growth mechanism proposed in Zhan's paper [47] and our previous work[38], the HAP nanofiber surface is uniquely covered by a layer of gelatin, which has similar chemical composition to collagen and contains both amine (NH_2) and carboxyl (COOH) group. It is generally believed that amine group is protonated in water solution at pH 7.0 to form NH_3^+ with positive charge; and the carboxyl group dissociates to proton and COO^- with negative charge in water. These HAP nanofibers are synthesized and washed in water solution and should contain both positive charges and negative charges on the similar quantity. In case the nanofiber surface contains only negative charges or positive charges, repulsive force between nanofibers would be generated from the same type of charges on different nanofibers, which may very likely overcome the interfibers Van der Waal force and the hydrogen bonding on the surface to breakdown the HAP nanofiber bundles and achieve better dispersion in water. Based on this hypothesis, glyoxylic acid, which is able to react with the amine groups on gelatin and meanwhile generate more carboxyl groups on fiber surface [51], was selected to modify the HAP nanofiber. The detailed mechanism is shown in scheme 1.

STEM images showed that the GA modification process effectively broke down the micro-sized HAP nanofiber bundles (figure 1a) to individual HAP nanofibers (figure 1b) with average 15 μm in length and relatively uniform diameter (50 to 100 nm). FT-IR spectrum (figure 2) showed that there are more C-H, C=O and OH-bands on the surface modified sample, which could be attributed to the newly grafted CH_2 and COOH groups on

the modified HAP nanofiber surface as shown in scheme 1. In addition, XPS test (figure 3 and table 1) also indicated that more oxygen element was detected on the surface modified HAP nanofibers, which was in good agreement with FT-IR spectrum and the reaction mechanism in scheme 1. The weight loss data from TGA test (figure. 4) excluded the possibility of significant amount of GA that is physically adsorbed on the HAP nanofiber surface. Therefore, the characteristic peaks of GA on FT-IR and XPS spectrums could be attributed to the GA chemically grafted on HAP surface. In addition, the similar TGA curves in figure 4 also suggested that the reaction was on the HAP nanofiber surface and did not influence the intrinsic properties of HAP nanofibers. In summary, the aforementioned STEM, FT-IR, XPS and TGA data indicated that both the modification mechanism in scheme 1 and the hypothesis for achieving good dispersion of HAP nanofibers worked well.

Our previous BFS results showed that the incorporation of a certain amount of untreated HAP nanofibers into dental resins could substantially improve the mechanical strength, while adding too much of untreated HAP nanofibers would not further increase or even decrease the mechanical strength of dental resin.[38]. In that study, the mechanical strength of the dental resin reach to the maximum point when the mass fraction of untreated HAP nanofibers is about 10%. The BFS strength of untreated HAP nanofibers reinforced dental resins obtained in the present study has small difference with the previous results, which could be ascribed to the slight differences on two batches of HAP nanofibers used, such as the size and size distribution. In order to obtain accurate comparison and minimize deviation, the same batch of HAP nanofibers was used to conduct the research for the comparison of untreated HAP nanofibers and GA modified HAP nanofibers in both table 2 and table 3. Results in table 2 indicated that the BFS values of dental resins filled with GA modified HAP nanofibers are significantly higher than those dental resins containing untreated HAP nanofibers with the same mass fraction incorporation. The SEM images of the fracture surface of dental resins (figure 5) showed that the GA modified HAP nanofibers have relatively more uniform dispersion than corresponding untreated HAP nanofibers in the dental resins. It is well known that with enhanced dispersion, more fiber surface become available for bonding with resin matrix which could result in the significant improvement on mechanical strength. In addition, table 2 also revealed that the mechanical strength of dental resin was significantly increased by adding small amount of GA modified HAP nanofiber and the BFS reached the maximum value at 10 wt%. It can be observed from the SEM images in figure 5b and 5d that most of the GA modified HAP nanofibers were uniformly distributed in the dental resin at small mass fraction incorporation (5wt% and 10 wt%). The improvement on mechanical strength could be attributed to the increased mass fraction of well dispersed GA modified HAP nanofibers. When the mass fraction of GA modified HAP nanofibers reached 15 wt%, the resulting dental resins have much lower BFS values, which is similar to the results observed for untreated HAP nanofibers. The mechanism behind the strength degradation is most likely attributed to formation of agglomeration of HAP nanofibers.

It has been known that high resin shrinkage rate is one of the significant reasons for the secondary caries, which is considered as the most common problem on clinical restoration application [1, 6, 7]. In order to minimize micro-leakage and improve the marginal adaption, at least 60 wt% inorganic fillers have to be incorporated into commercial dental composites so as to reduce the total polymerization shrinkage rate to about 2%[1, 52]. Additionally, the incorporation of large mass fraction of inorganic fillers into the dental composites is also preferred since it could provide much higher elastic modulus to prevent or minimize defect formation and reduce the wear generally resulting from the increased surface contact under high stress [6]. Currently most of the commercial universal dental composites contain 75–85 wt% of inorganic fillers (primarily made of glass) while most of the commercial flowable dental composites contain about 60 wt% of inorganic fillers. The mechanical testing results

in table 2 indicated that it was impossible to achieve a high mass fraction of inorganic fillers with untreated or GA modified HAP nanofibers alone, because even the incorporation of 15 wt% of untreated or GA modified HAP nanofibers into dental resins already resulted in significant reduction of mechanical strength. In order to meet the low shrinkage rate requirement and achieve adequate elastic modulus, commercial 0.7 μm sized silica particles with 1 wt% silane provided by Esstech Inc were incorporated into the composites system together with HAP nanofiber in the present study. The dental composites containing 60 wt% inorganic fillers in total with various mass fractions untreated or GA modified HAP nanofibers were systematically fabricated and evaluated for BFS strength and water absorption behavior. The control sample was the resin filled with 60 wt% silica microparticles. In order to acquire more clinical relevant mechanical data, the biaxial flexural testing was performed with the dental composite specimens immersed to distilled water for 24 hours at 37°C.

As shown in Table 3, the GA modified HAP nanofibers reinforced dental composites have significantly greater wet state BFS values than those dental composites filled with untreated HAP nanofiber with the same mass fraction incorporation, which is very similar to the aforementioned dry state BFS data of dental resins reinforced by HAP nanofibers alone. According to the fracture surface SEM images in figure 6, GA modified HAP nanofibers had enhanced dispersion in dental matrix, which was responsible for the improved mechanical strength of corresponding dental composites compared to the untreated HAP nanofibers impregnated dental composites. In addition, the incorporation of small amount (2 wt% and 3 wt%) of GA modified HAP nanofibers into dental composites could lead to significantly improved mechanical strength compared to the control with only 60 wt% of silica filler. While the loading rate reached 5 wt%, the BFS of dental composites started to degrade. It can be observed from SEM images of fracture surface of dental composites that the GA modified HAP nanofibers could be well dispersed in the dental composite matrix at a small mass fraction (2 wt%, figure 6b). As the GA modified HAP nanofibers mass fraction increased to 5 wt%, the relatively lower BFS of dental composites compared to dental composites filled with 2 wt% and 3 wt% GA modified HAP nanofibers could be attributed to the observed bundles on the fracture surface (figure 6d). When the mass fraction of GA modified HAP nanofibers reached 10 wt% (figure 6f), most of nanofibers aggregated to bundles and a greater reduction on BFS was observed. These results indicated that previous conclusion [38] about the double-edged effects of untreated HAP nanofibers on reinforcement could also be drawn on the GA modified HAP nanofibers.

It is well known that low water absorption rate and water solubility are desired for clinical restoration due to the penetration of water molecules into dental composites could not only generate evolution of localized stress and strain but also significantly reduce the mechanical strength of dental composites by breaking the water sensitive silane interphase bonding between fillers and matrix. [53, 54]. The results in table 4 indicated that the addition of GA modified HAP nanofibers could result in much higher water solubility. As stated in Section 2.1, the silica microparticles contained 1wt% γ -MPS silane on the surface which could polymerize with the BisGMA and TEGDMA matrix to form strong chemically interfacial bonding and lead to low water solubility. However, the carboxyl and hydroxyl groups on GA modified HAP nanofiber surface could only generate relatively weak hydrogen or physical bonding with dental matrix, which was responsible for the substantially enhanced water solubility value after adding the GA modified HAP nanofibers into the dental composite system. Water absorption results in figure 7 revealed that with the impregnation of GA modified HAP nanofibers, the water absorption rate increased significantly after 7 days immersion on both 2 wt% and 5 wt% mass fractions. These results and phenomenon could be explained in the following possibilities. First of all, due to the GA modified HAP nanofibers had poor interfacial bonding with dental matrix, more monomers and fillers

leached out and formed porous structure in dental composites, which resulted in the significant increase on water absorption rate in further. In addition, since the GA modified HAP nanofiber surface contained many hydrophilic carboxyl and amine groups, the interface between fillers and dental matrix became more hydrophilic after incorporating GA modified HAP nanofibers into dental composites, which could provide more sites for water molecules and result in the enhancement on water absorption. Herein, we concluded that although the GA modified HAP nanofibers significantly improved the mechanical strength of dental composites, but meanwhile they also brought the concern on water absorption and water solubility.

4. Conclusion

In the present study, the HAP nanofiber bundles were successfully well dispersed in water and dental matrix after a simple chemical surface treatment. The mechanical testing and fractographic analysis indicated that the improved dispersion of HAP nanofibers in dental matrix could lead to significantly enhanced mechanical strength. In addition, similar as untreated HAP nanofibers, incorporation of small mass fraction of GA modified HAP nanofibers into the BisGMA/TEGDMA dental resins (5 wt%, 10 wt%) or into composites (2 wt%, 3 wt%) could substantially improve the biaxial flexural strength. However, compared to dental composites containing only silica particles, GA modified HAP nanofibers reinforced dental composites have higher water absorption rate and water solubility, which is a concern on clinical application. In summary, our results indicated that GA modified HAP nanofiber is a better choice than untreated HAP nanofiber as a promising reinforcing material candidate for dental composites and the following investigation should be targeted on reducing water absorption rate and water solubility rate of such dental composites so as to meet clinical requirements and extend lifespan of resulting dental composites.

Acknowledgments

This work was funded by the National Institute of Health (1R21DE018821-01A2) and National Science Foundation (CMMI-0846744). The authors would also like to thank Esstech Inc. for donating the BisGMA, TEGDMA and silanized barium borosilica particles.

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Figure 1. STEM image of (a) untreated HAP nanofibers, and (b) glyoxylic acid (GA) modified HAP nanofibers. (c) The fiber length distribution of GA modified HAP nanofibers.

Figure 2.
FT-IR spectrum of: (a) untreated HAP nanofibers and (b) GA modified HAP nanofibers.

Figure 3.
XPS spectra of: (a) Untreated HAP nanofibers and (b) glyoxylic acid modified HAP nanofibers.

Figure 4.
The TGA curves of untreated HAP and GA modified HAP nanofibers.

Figure 5. Representative SEM images of fracture surfaces of biaxial flexural test specimens: BisGMA/TEGDMA filled with 5 wt% untreated HAP nanofibers (a) and GA modified HAP nanofibers (b); BisGMA/TEGDMA filled with 10 wt% untreated HAP nanofibers (c) and GA modified HAP nanofibers (d).

Figure 6.

Representative SEM images of fracture surface of biaxial flexural test specimens: BisGMA/TEGDMA filled with 2 wt% untreated HAP nanofibers (a) or GA modified HAP nanofibers (b) and 58 wt% silica particles; BisGMA/TEGDMA filled with 5 wt% untreated HAP nanofibers (c) or GA modified HAP nanofibers (d) and 55 wt% silica particles; BisGMA/TEGDMA filled with 10 wt% untreated HAP nanofibers (e) or GA modified HAP nanofibers (f) and 50 wt% silica particles.

Figure 7.

The water absorption rate ($\mu\text{g}/\text{mm}^3$) of dental composites containing 60 wt% silica particles, 2 wt% GA modified HAP nanofibers and 58 wt% silica particles, and 5 wt% GA modified HAP nanofibers and 55 wt% silica particles after different immersion time in distilled water. Each datum is the mean value of five measurements with standard deviation (SD).

Scheme 1.

Reaction route of modification on HAP nanofiber surface with glyoxylic acid

Table 1

Surface composition by XPS for Untreated HAP nanofibers and glyoxylic acid modified HAP nanofibers.

	<u>Surface composition in at%</u>			
	Ca	P	O	N
Untreated HAP	12.4	9.3	74.6	3.7
Glyoxylic acid modified HAP	7.0	4.4	85.3	3.3

Table 2

Biaxial flexural strength of BisGMA/TEGDMA dental resins filled with various mass fractions of untreated or GA modified HAP nanofibers in dry state. Each datum is the mean value of six measurements with standard deviation (SD).

HAP Mass fraction	Untreated HAP nanofibers		GA modified HAP nanofibers	
	Mean strength $\pm$ SD (MPa)	ANOVA Analysis (* $P<0.05$)	Mean strength $\pm$ SD (MPa)	ANOVA Analysis (* $P<0.05$)
0%	100.3 $\pm$ 2.4	Control	100.3 $\pm$ 2.4	Control
5%	97.9 $\pm$ 5.2		104.9 $\pm$ 6.5	
10%	103.5 $\pm$ 5.4		114.6 $\pm$ 7.8	*
15%	78.9 $\pm$ 6.5	*	95.7 $\pm$ 5.6	
20%	80.4 $\pm$ 5.7	*	82.4 $\pm$ 6.2	*

Table 3

Biaxial flexural strength of BisGMA/TEGDMA dental composites filled with various mass fractions of untreated or glyoxylic acid modified HAP nanofibers and 0.7 micrometer size silica particles (total mass fraction 60 wt%). All the specimens were immersed in distilled water for 24 hours at 37 °C. Each datum is the mean value of six measurements with standard deviation (SD).

Filler Mass fraction	Untreated HAP nanofibers([41])		GA modified HAP nanofibers	
	Mean strength $\pm$ SD (MPa)	ANOVA Analysis (* $P<0.05$)	Mean strength $\pm$ SD (MPa)	ANOVA Analysis (* $P<0.05$)
60% Silica	93.7 $\pm$ 10.2	Control	93.7 $\pm$ 10.2	Control
2% HAP, 58% Silica	115.2 $\pm$ 10.8	*	127.5 $\pm$ 6.9	*
3% HAP, 57% Silica	120.7 $\pm$ 14.4	*	137.2 $\pm$ 4.9	*
5% HAP, 55% Silica	118.4 $\pm$ 16.3	*	130.4 $\pm$ 8.5	*
10% HAP, 50% Silica	95.4 $\pm$ 9.2		94.8 $\pm$ 6.9	

Table 4

The water solubility of dental composites containing different amount of GA modified HAP nanofibers and silica particles after 28 days immersion in distilled water. Each datum is the mean value of five measurements with standard deviation (SD).

Dental composites	Water solubility ($\mu\text{g}/\text{mm}^3$)
60 wt% silica	3.36 $\pm$ 0.98
2 wt% GA modified HAP and 58 wt% silica	11.14 $\pm$ 2.32
5 wt% GA modified HAP and 55 wt% silica	10.58 $\pm$ 1.25