Published in final edited form as:

Dent Mater. 2018 September ; 34(9): 1310–1322. doi:10.1016/j.dental.2018.06.001.

Novel magnetic nanoparticle-containing adhesive with greater dentin bond strength and antibacterial and remineralizing capabilities

Yuncong Li^{a,b,c,#}, Xiaoyi Hu^{a,d,#}, Yang Xia^{c,e,#}, Yadong Ji^c, Jianping Ruan^a, Michael D. Weir^c, Xiaoying Lin^f, Zhihong Nie^f, Ning Gu^g, Radi Masri^{c,*}, Xiaofeng Chang^{a,*}, and Hockin H.K. Xu^{c,h,i,*}

^aClinical Research Center of Shaanxi Province for Dental and Maxillofacial Diseases, Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China

^bDepartment of Prosthodontics, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China

^cDepartment of Advanced Oral Sciences & Therapeutics, University of Maryland School of Dentistry, Baltimore, MD 21201, USA

^dDepartment of Oral Maxillofacial Surgery, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China

^eJiangsu Key Laboratory of Oral Diseases, Nanjing Medical University, Nanjing, Jiangsu 210029, China

^fDepartment of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA

^gJiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu 210096, China

^hCenter for Stem Cell Biology and Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

ⁱUniversity of Maryland Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Abstract

Objectives—A nanoparticle-doped adhesive that can be controlled with magnetic forces was recently developed to deliver drugs to the pulp and improve adhesive penetration into dentin.

*Corresponding authors: Radi Masri, rmasri@umaryland.edu. Xiaofeng Chang, changxf@xjtu.edu.cn. Hockin Xu, hxu@umaryland.edu.

#Co-first authors.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

However, it did not have bactericidal and remineralization abilities. The objectives of this study were to: (1) develop a magnetic nanoparticle-containing adhesive with dimethylaminohexadecyl methacrylate (DMAHDM), amorphous calcium phosphate nanoparticles (NACP) and magnetic nanoparticles (MNP); and (2) investigate the effects on dentin bond strength, calcium (Ca) and phosphate (P) ion release and anti-biofilm properties.

Methods—MNP, DMAHDM and NACP were mixed into Scotchbond SBMP at 2%, 5% and 20% by mass, respectively. Two types of magnetic nanoparticles were used: acrylate-functionalized iron nanoparticles (AINPs); and iron oxide nanoparticles (IONPs). Each type was added into the resin at 1% by mass. Dentin bonding was performed with a magnetic force application for 3 minutes, provided by a commercial cube-shaped magnet. Dentin shear bond strengths were measured. *Streptococcus mutans* biofilms were grown on resins, and metabolic activity, lactic acid and colony-forming units (CFU) were determined. Ca and P ion concentrations in, and pH of biofilm culture medium were measured.

Results—Magnetic nanoparticle-containing adhesive using magnetic force increased the dentin shear bond strength by 59% over SBMP Control ($p < 0.05$). Adding DMAHDM and NACP did not adversely affect the dentin bond strength ($p > 0.05$). The adhesive with MNP+DMAHDM +NACP reduced the *Streptococcus mutans* biofilm CFU by 4 logs. For the adhesive with NACP, the biofilm medium became a Ca and P ion reservoir. The biofilm culture medium of the magnetic nanoparticle-containing adhesive with NACP had a safe pH of 6.9, while the biofilm medium of commercial adhesive had a cariogenic pH of 4.5.

Conclusions—Magnetic nanoparticle-containing adhesive with DMAHDM and NACP under a magnetic force yielded much greater dentin bond strength than commercial control. The novel adhesive reduced biofilm CFU by 4 logs and increased the biofilm pH from a cariogenic pH 4.5 to 6.9, and therefore is promising to enhance the resin-tooth bond, strengthen tooth structures, and suppress secondary caries at the restoration margins.

Keywords

Magnetic nanoparticles; magnetic force; dental adhesive; dentin bond strength; anti-biofilm; remineralization

1. Introduction

Dental adhesives and resin composites are the first choice for tooth defect restorations due to their excellent esthetics and direct-filling capabilities [1–4]. Unfortunately, composite restorations have a relatively higher failure rate with an average replacement time of only 5.7 years [5, 6]. Secondary caries at the tooth-restoration interface has been suggested in previous studies as one of the primary reasons for restoration failures [7, 8]. Despite great improvements in dental adhesives, the dentin-resin interface is still the weakest area of the composite restorations due to dentinal bond degradation [9–13]. Microleakage and gap formation in this weakened interface provide an effective pathway for invasion of oral plaque biofilms and the development of secondary caries around the tooth-restoration margins. Therefore, improving the bond durability and preventing bacterial invasion are pivotal issues for inhibiting secondary caries and increasing the restoration longevity [11, 14, 15].

Dentin bonding relies on a micromechanical interlocking mechanism that involves the infiltration and subsequent entanglement of adhesive resin into the dentin collagen matrix to form the hybrid layer [16]. A major concern with contemporary adhesives is their limited ability to infiltrate into the collagen fibril network of the demineralized dentin exposed by acid-etching or self-etch processes [14, 15]. Incomplete infiltration of resin into the demineralized dentin and hydrolysis of the polymerized resin result in the exposure of the denuded dentin collagen matrix along the dentin-resin interface. Acids and enzymes produced by bacteria, as well as activated host-derived proteases, further deteriorate this defective bonded interface, thereby compromising the longevity of the resin-dentin bond [17, 18]. Several methods were developed to facilitate the optimal infiltration of resin into the collagen matrix of the demineralized dentin, including the use of hydrophilic resin monomers, catalysts, various solvents, and the ethanol wet-bonding technique [19–23]. The use of hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) can improve the resin wettability, promote the re-expansion of the dried dentin collagen, help displace water in the bonded interface, and facilitate the subsequent resin infiltration [24]. However, adhesives rich with hydrophilic monomers exhibit greater water sorption from the host dentin and are consequently susceptible to hydrolytic degradation [25–27]. This shortcoming was described by many studies showing increased interfacial nanoleakage and lower long-term bonding effectiveness [28, 29]. In addition, adhesive resin polymerization in such a moist environment is a challenge. The compromise in the degree of conversion of adhesive monomers may allow water permeation along the bonded interface, which in turn would result in the formation of a porous hybrid structure with reduced sealing ability [30]. Furthermore, incomplete polymerization of adhesive monomers may also reduce the mechanical properties, which may shorten the durability of the adhesive restoration [31, 32]. The ethanol wet-bonding technique is based on the theory that ethanol dehydration renders the acid-etched dentin less hydrophilic and maintains the dehydrated collagen matrix in an extended state to facilitate the relatively hydrophobic monomers to infiltrate by water replacement from the interfibrillar and intrafibrillar spaces [20]. Although better stability of the resin-dentin bonds has been created, ethanol wet-bonding is highly technique sensitive and difficult for clinical applications.

To solve the limited resin penetration problem, designing a novel adhesive to be forced to infiltrate and penetrate the interfibrillar spaces in the acid-etched dentin would be highly desirable. Unlike self-diffusion which is a passive process, magnetic forces can potentially enhance the adhesive penetration. Under the guidance of an external magnetic force, magnetic nanoparticles (MNP) can transport more drugs to a target than either diffusion or iontophoresis [33, 34]. However, besides our recent study [35], there has been no other report on the application of magnetic forces in dental bonding.

Novel magnetic nanoparticles were recently developed with an iron core and a silica coating shell, which were silanized with a monolayer of vinyl groups on the surface to covalently bond to the resin matrix [35]. The purpose was for the magnetic nanoparticle-doped resin to be actively pulled into the demineralized dentin tissue using a brief application of a magnetic force. Our preliminary study showed that incorporation of the magnetic nanoparticles at 5% by mass in the adhesive had no negative effect on the degree of conversion, and reduced the polymerization shrinkage stress, compared to the control. Application of a magnetic field

force with this magnetic nanoparticle-doped adhesive at 2% by mass enhanced the dentin bond strength with greater resin tag density and depth of penetration into the demineralized dentin[35].

Another aspect to combat caries is to develop antibacterial adhesives to suppress biofilm growth and acid production at the restoration margins [36–38]. Various quaternary ammonium methacrylates (QAMs) were developed and incorporated into resins for antibacterial activities, including 12-methacryloyloxydodecyl-pyridinium bromide (MDPB), methacryloxyethyl cetyltrimethyl ammonium chloride (DMAE-CB), 2-methacryloxyethyl hexadecyl methyl ammonium bromide (MAE-HB), and dimethylaminododecyl methacrylate (DMADDM) [39–43]. These polymerizable cationic monomers covalently bonded within polymer matrix and killed bacteria upon contact. Another study developed quaternary ammonium silane to formulate a cavity disinfectant [44]. Recently, dimethylaminohexadecyl methacrylate (DMAHDM) was synthesized and showed a strong anti-biofilm function [45–49], which may help protect the dentin collagen from the acidic and enzymatic attacks of oral bacteria and enzymes.

Another approach for the bonded interface to resist degradation is to incorporate mineral particles into the demineralized dentin matrix to promote remineralization [11, 50, 51]. Previous studies showed that bonding agents containing nanoparticles of amorphous calcium phosphate (NACP) released high levels of calcium and phosphate ions to induce remineralization [43, 52]. Due to their small particle sizes, the NACP readily flowed with adhesive into the demineralized dentin to deliver calcium and phosphate ions to promote the regrowth of remnant apatite crystals in interfibrillar regions via classic nucleation [53–55]. Thus, NACP-containing adhesive may be highly beneficial to protect the exposed collagen and facilitate remineralization in the bonded interface. A literature search revealed no report on a dental adhesive containing magnetic nanoparticles and antibacterial and remineralizing agents.

The objectives of this study were to develop the first adhesive containing magnetic nanoparticles as well as DMAHDM and NACP, and to investigate the effects on dentin bond strength and anti-biofilm properties. It was hypothesized that: (1) Magnetic nanoparticle-containing adhesive with external magnetic field force would yield greater dentin bond strength than control adhesives without magnetic field force; (2) Incorporating DMAHDM and NACP into the magnetic nanoparticle-containing adhesive would not compromise the dentin bond strength; (3) Incorporating DMAHDM into the magnetic nanoparticle-containing adhesive would greatly decrease biofilm growth and acid production; (4) Incorporating NACP in the magnetic nanoparticle-containing adhesive would neutralize the acid, increase the calcium and phosphate ion concentrations in the biofilm culture medium and increase the biofilm pH.

2. Materials and methods

2.1. Incorporation of DMAHDM into bonding agent

A commercial bonding agent Scotchbond™ Multi-Purpose Adhesive and Primer (referred as “SBMP”, 3M, St. Paul, MN) was used as the parent system for antibacterial and

remineralizing functionalization. According to the manufacturer, SBMP adhesive contained 60–70% of bisphenol A diglycidyl methacrylate (BisGMA) and 30–40% of 2-hydroxyethyl methacrylate (HEMA), tertiary amines and photo-initiator. SBMP primer contained 35–45% of HEMA, 10–20% of a copolymer of acrylic and itaconic acids, and 40–50% water.

DMAHDM, with an alkyl chain length of 16, was synthesized using a modified Menschutkin reaction method, in which a tertiary amine group was reacted with an organo-halide [56]. The reaction was verified via Fourier transform infrared spectroscopy in our previous study [45]. A benefit of this reaction is that the reaction products are generated at virtually quantitative amounts and require minimal purification. Briefly, 10 mmol of 2-(dimethylamino) ethyl methacrylate (DMAEMA, Sigma-Aldrich, St. Louis, MO) and 10 mmol of 1-bromohexadecane (BHD, TCI America, Portland, OR) were combined in 20 mL tared scintillation vial. The vial was stirred at 70 °C for 24 h. The solvent was then removed via evaporation, yielding DMAHDM as a clear, viscous liquid. DMAHDM was incorporated into SBMP primer at a mass fraction of DMAHDM/(SBMP primer + DMAHDM) = 5%. The 5% was selected following previous studies [48, 49]. Similarly, DMAHDM was incorporated into SBMP adhesive at 5% mass fraction.

2.2. Incorporation of NACP into adhesive

NACP [$\text{Ca}_3(\text{PO}_4)_2$] were synthesized via a spray-drying technique and characterized with X-ray diffractometry and transmission electron microscopy, as previously described [54]. Briefly, calcium carbonate (CaCO_3) and dicalcium phosphate anhydrous (CaHPO_4) were dissolved into an acetic acid solution to obtain the final concentrations of Ca and P ions of 8 mmol/L and 5.333 mmol/L, respectively. The solution was sprayed into a heated chamber to evaporate the water and volatile acid. An electrostatic precipitator (Air Quality, Minneapolis, MN, USA) was used to collect the dried NACP powder. This yielded NACP with a mean particle size of 116 nm [46]. NACP were incorporated into the adhesive at NACP/(NACP + adhesive) = 20% mass fraction. Previous studies showed that 20% NACP released high levels of Ca and P ions, without adversely affecting dentin bond strength [43].

2.3. Incorporation of magnetic nanoparticles into bonding agent

Two types of magnetic nanoparticles were used in this study to have a bimodal distribution of small and large particles. The first are iron nanoparticles with acrylate functional groups onto a silica-coated layer on the particles fabricated by a reduction method [57]. These particles were produced by reducing ferrous ion in an aqueous solution of ferrous chloride tetrahydrate by sodium borohydride in a flask. Polyvinylpyrrolidone (PVP, Mw = 40,000) was added as stabilizing ligands. The iron nanoparticles were collected by placing a magnet beneath the flask, and then washed with water to remove the PVP. The nanoparticles were then coated with silica via the hydrolysis of tetraethyl orthosilicate (TEOS) in ethanol. A solution of methacryloxypropyltrimethoxysilane (KH570) in ethanol was then added into the reaction to introduce acrylate functional groups onto silica-coated nanoparticles. A magnet was used to collect the acrylate-functionalized iron magnetic nanoparticles (AINPs).

The second type are magnetic iron oxide nanoparticles (IONPs), or maghemite ($\gamma\text{Fe}_2\text{O}_3$). The $\gamma\text{Fe}_2\text{O}_3$ nanoparticles (γIONPs) were synthesized via chemical co-precipitation using

ferrous chloride hexahydrate and ferrous chloride tetra-hydrate as precursors [58]. Briefly, 0.2 g of polyglucose-sorbitol-carboxy-methyether (PSC) was dissolved in 10 mL of deionized water. Then a mixture of 0.06 g of FeCl_3 and 0.03 g of FeCl_2 in 15 mL deionized water was added. This mixture was cooled to 5 °C, and 1 g of 28% ammonium hydroxide was added with stirring for 2 min. The mixture was heated at 80 °C for 1 h and purified with five cycles of ultrafiltration against deionized water using a 100 kDa membrane. The colloidal suspension of magnetic NPs was examined with transmission electron microscope (TEM; JEOL-7100, Tokyo, Japan). The hydrodynamic diameters of nanoparticles were measured by dynamic light scattering (DLS) with a particle size analyzer (Malvern Zetasizer Nano ZS90, Malvern, UK). 1% by mass of AINPs and 1% by mass of γ IONPs were mixed with the SBMP adhesive via ultrasonic concussion instrument. A previous study showed that 2% magnetic NPs could enhance dentin bond strength under an external magnetic field force [35]. Four groups were investigated:

1. Unmodified SBMP primer and SBMP adhesive (designated "SBMP Control");
2. Unmodified SBMP primer. SBMP adhesive + 1% AINPs + 1% γ IONPs (designated as "SBMP+MNP");
3. SBMP primer + 5% DMAHDM. SBMP adhesive + 1% AINPs + 1% γ IONPs + 5% DMAHDM (designated as "SBMP+MNP+DMAHDM");
4. SBMP primer + 5% DMAHDM. SBMP adhesive + 1% AINPs + 1% γ IONPs + 5% DMAHDM + 20% NACP (designated as "SBMP+MNP+DMAHDM+NACP").

The purpose of testing these groups was to determine the effect of adding MNP coupled with a magnetic force on dentin bond strength; whether adding DMAHDM and NACP would interfere with dentin bonding via a magnetic force; the anti-biofilm properties of the new magnetic bonding agent; and the calcium and phosphate ion releasing properties of group 4.

2.4. Dentins hear bond strength testing

Fifty extracted caries-free human third molars were collected with donor consent, under a protocol approved by the Institutional Review Board (IRB) of the University of Maryland. For the dentin shear bond strength test, group 2 was divided into two subgroups according to whether using a magnetic force or not, in order to investigate the effect of magnetic force on the shear bond strength. The teeth were randomly divided into five groups for shear bond strength test with a magnetic force which is illustrated in Fig. 1 (n = 10/group). Groups 1 and 2 had no magnetic force; groups 3–5 had a magnetic force which was applied as described below.

The teeth were mounted in a PVC tube using dental instant tray mix self-curing acrylic resin (REF 1845, Lang, IL, USA). The occlusal enamel of the tooth was removed to expose midcoronal dentin via a water-cooled low-speed cutting saw (Isomet, Buehler, Lake Bluff, IL, USA). The distance from the occlusal surface of the dentin to the bottom of the PVC tube was 25 mm. Each dentin surface was polished with 600-grit SiC paper under running water for 60 s to create a standardized smear layer, which was etched with 37% phosphoric

acid gel for 15 s and rinsed with distilled water [24]. A primer was applied and the solvent was removed with a stream of air for 5s. For groups 1 and 2, an adhesive was applied and light-cured with continuous output using a conventional intensity light at 430 mW/cm², with curing distance of 5 mm and lasting for 10 s (Optilux VCL 401, Demetron Kerr, Danbury, CT). For groups 3–5, an adhesive was applied and a magnetic field force was exerted for 3 minutes. This was accomplished by using a cube-shaped magnet (maximum internal field 1.4 T, surface field 0.54 T; BX0X0X0-N52, K&J Magnetics, Pipersville, PA, USA), which was placed below the tooth to produce the magnetic field force (Fig. 1). Each tooth was positioned on the center of the magnet to align the magnetic force with the dentinal tubule axis direction [35]. After 3 minutes of magnet application, the magnet was removed, and the adhesive was photo-cured as described above.

For each group, a cylindrical mold (inner diameter = 4 mm, thickness = 1.5 mm) was placed on the adhesive-treated dentin surface. A composite (Filtek Supreme Ultra Universal, 3M, St. Paul, MN, USA) was filled into the mold and light-cured following the same aforementioned photo-cure method for 60 s. The bonded specimens were stored in distilled water at 37°C for 24 h and then tested for bond strength [59]. A chisel on a Universal Testing Machine (MTS, Eden Prairie, MN, USA) was aligned to be parallel to the composite-dentin interface. Load was applied at a cross-head speed of 0.5 mm/min until the bond failed. Dentin shear bond strength = $4P/(\pi d^2)$, where P is the load at failure, and d is the diameter of the bonded area [43]. Failure modes were examined using a stereoscopic microscope and classified as adhesive failure (failure along the adhesive interface), and mixed mode failure (failure within the adhesive joint mixed with failure within the resin composite or within the dentin).

2.5. Preparation of bonding agent resin specimens and *S. mutans* inoculation

The cover of a sterile 96-well plate (Costar, Corning Inc., Corning, NY) was used as molds to fabricate resin disks following a previous study [60]. Briefly, 10 µL of a primer was placed in the bottom of each dent of the 96-well plate. After drying with a stream of air, 20 µL of an adhesive was applied to the dent and photo-polymerized for 60 s (Optilux VCL 401), using a Mylar strip covering to obtain a disk of approximately 8 mm in diameter and 0.5 mm in thickness. The cured resin disks were immersed in 200 mL of distilled water and ultrasonically-shaken for 1 h to remove any uncured monomers, following previous studies [43, 46, 61]. Then the disks were sterilized with ethylene oxide (Anprolene AN 74i, Andersen, Haw River, NC) and de-gassed for 3 days following the manufacturer's instructions.

The use of *Streptococcus mutans* (*S. mutans*) (ATCC 700610, UA159, American Type Culture, Manassas, VA) was approved by the University of Maryland Baltimore IRB. *S. mutans* is a cariogenic, aerotolerant anaerobic bacterium and the primary causative agent of dental caries. Brain heart infusion broth (BHI, Becton, Sparks, MD) was supplemented with 1% sucrose and termed the “growth medium”. Ten µL of stock bacteria was added into 10 mL of BHI broth and incubated at 37 °C with 5% CO₂ for 16 h. During this culture, the *S. mutans* were suspended in the BHI broth. Then, this *S. mutans* culture was diluted 10-fold in the growth medium to form the inoculation medium for subsequent experiments.

2.6. Live/dead assay of *S. mutans* biofilms on resins

Each bonding agent disk was placed in a well of a 24-well plate, and 1.5 mL of the inoculation medium was added to each well. The samples were incubated at 5% CO₂ and 37 °C for 24 h. Then each disk with biofilm was transferred to a new 24-well plate containing 1.5 mL of fresh growth medium, and cultured for another 24 h. This total 2 days of culture which was shown in previous studies to form relatively mature biofilms on resins [62]. Specimens with 2-day biofilms were washed with phosphate buffered saline (PBS). Then the biofilms on resin disks were stained using a live/dead bacterial kit (Molecular Probes, Eugene, OR). Live bacteria were stained with Syto 9 to produce green fluorescence, and bacteria with compromised membranes were stained with propidium iodide to produce red fluorescence. The stained disks were imaged using an inverted fluorescence microscope (Eclipse TE2000-S, Nikon, Melville, NY). Three disks were evaluated for each group. Five images were collected at random locations on each disk, yielding 15 images per group.

2.7. MTT metabolic activity of *S. mutans* biofilms on resins

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is a colorimetric assay that measures the enzymatic reduction of MTT, a yellow tetrazole to formazan [62]. Briefly, disks with 2-day biofilms were transferred to new 24-well plate, with 1 mL of 0.5 mg/mL MTT dye in each well. All specimens were incubated at 37 °C in 5% CO₂ for 1 h. During this process, the metabolically active bacteria reduced the MTT to purple formazan. After 1 h, the biofilm specimens were transferred to a new 24-well plate. An aliquot of 1 mL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals. After incubation for 20 min in the dark, 200 µL of the DMSO solution was transferred to a 96-well plate, and the absorbance at 540 nm was measured via a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA). A higher absorbance indicates a higher formazan concentration, which in turn indicates a higher metabolic activity for the bacteria. Six replicates were tested for each group (n = 6).

2.8. Lactic acid production of biofilms on resins

Resin disks with 2-day biofilms were rinsed in cysteine peptone water (CPW) to remove loose bacteria, and then placed in a new 24-well plate. 1.5 mL of buffered peptone water (BPW) and 0.2% sucrose was added to each well. Samples were incubated at 5% CO₂ and 37 °C for 3 h to allow the bacteria to produce acid. After 3 h, the BPW solutions were stored for lactate analysis. Lactate concentrations were determined using an enzymatic method [63]. The microplate reader was used to measure the absorbance at 340 nm for the collected BPW solutions. Standard curves were prepared using a lactic acid standard (Supelco Analytical, Bellefonte, PA) [62].

2.9. Colony-forming units (CFU) of biofilms on resins

CFU were measured to quantify the total number of viable bacteria present in the 2-day biofilms on each disk. The bacteria in the biofilms on the disks were harvested by sonication (3510R-MTH, Branson, Danbury, CT) for 3 min and vortexing (Fisher, Pittsburgh, PA) for 20 s. The bacterial suspensions were serially diluted and spread onto BHI agar plates for CFU analysis (n = 6) following previous studies[43, 46, 62].

2.10. Calcium (Ca) and phosphate (P) ion concentration and pH in biofilm medium

The biofilm culture medium after 48 h of incubation was collected and centrifuged at 12000 rpm for 5 min (Eppendorf Centrifuge 5415, Brinkmann, Westbury, NY, USA). Then, the supernatant was analyzed for Ca and P ion concentrations via a spectrophotometric method (SpectraMax M5) using known standards and calibration curves [52, 54].

The pH of the biofilm medium was measured using a pH meter (Accumet Excel XL25, Fisher, Pittsburgh, PA) at 48 h of culture. The pH was not measured during the first 24 h of culture, because the non-adherent planktonic bacteria in the medium could contribute to the pH changes [62]. In this way, the measured pH was solely related to the relatively mature 2-day biofilm on the resin.

2.11. Statistical analyses

Statistical analysis was conducted with one-way analysis of variance to detect the significant effects of the variables. Post-hoc pair-wise comparisons were performed using the Tukey's statistic. Parametric statistical methods were employed with the confirmation that the normality and equal variance assumptions of the data sets were not violated. Statistical analyses were performed by SPSS 17.0 software (SPSS, Chicago, IL) at $p < 0.05$.

3. Results

Typical TEM images of magnetic nanoparticles were showed in Fig. 2 for (A) AINPs, and (B) γ IONPs. In (A), the iron core of AINPs was black and opaque, and had a spherical shape with an average diameter of approximately 900 nm. In (B), the γ IONPs had a relatively uniform, spherical morphology. The average size of the nanoparticles was approximately 8 nm.

The dentin shear bond strengths are plotted in Fig. 3 (mean $\pm$ sd; $n = 10$). Adding 1% AINPs and 1% γ IONPs into the adhesive yielded a bond strength of 17.46 ± 3.77 MPa, similar to 17.80 ± 3.96 MPa for SBMP Control ($p > 0.1$). With the magnetic force, the bond strength was increased to 28.42 ± 3.83 MPa for SBMP+MNP with magnetic force group ($p < 0.001$). Incorporation of 5% DMAHDM and 20% NACP caused no significant difference with SBMP+MNP with magnetic force group ($p > 0.1$). Thus, the adhesive with 5% DMAHDM and 20% NACP did not weaken the effect of the magnetic force in enhancing the dentin bond strength, compared to that without DMAHDM and NACP ($p > 0.1$). Failure modes were examined using a stereoscopic microscope, and the main failure mode was failure along the adhesive interface in all groups.

Representative live/dead staining images of 2-day biofilms on resins are shown in Fig. 4. In (A) and (B), SBMP Control and SBMP+MNP adhesive disks were nearly fully covered by live bacteria with green staining. In contrast, images (C) and (D) showed much less bacterial adhesion, and the biofilms consisted of primarily compromised bacteria with red staining. Adding 20% NACP did not appear to significantly alter the biofilm appearance.

Metabolic activity of 2-day biofilms on resins is plotted in Fig. 5(A) (mean $\pm$ sd; $n = 6$). SBMP Control and SBMP+MNP had biofilms with a similarly high metabolic activity ($p >$

0.1). Adding 5% DMAHDM into the bonding agent greatly reduced the biofilm metabolic activity by more than 80% ($p < 0.001$). Adding 20% NACP to the adhesive did not significantly change the antibacterial activity ($p > 0.05$). Lactic acid production by biofilms in Fig. 5(B) showed a similar trend with the MTT assay. Biofilms on SBMP Control and SBMP+MNP disks produced the most acid production. Incorporation of DMAHDM and NACP dramatically decreased the acid production, to less than 1/25 that of SBMP Control and SBMP+MNP ($p < 0.05$). Fig. 5(C) plots the CFU of 2-day biofilms on resins (mean $\pm$ sd; $n = 6$). SBMP Control and SBMP+MNP had similar CFU values ($p > 0.05$). Resins containing DMAHDM had CFU almost 4 logs less than those of controls. Adding NACP had no significant effect on CFU ($p > 0.05$).

Ca and P ion concentrations in the 2-day biofilm medium are plotted in Fig. 6(A) and (B) (mean $\pm$ sd; $n = 6$). Ca and P concentrations of SBMP+MNP+DMAHDM+NACP were significantly higher than other groups without NACP ($p < 0.05$), demonstrating that the NACP-containing resin could provide a reservoir of Ca and P ions to promote remineralization. The pH values of the 2-day biofilm medium are plotted in Fig. 6(C) (mean $\pm$ sd; $n = 6$). The pH in biofilm medium of SBMP Control and SBMP+MNP was much lower, at around 4.5, due to acid production by the biofilms ($p < 0.001$). In contrast, the pH of SBMP+MNP+DMAHDM (6.75 ± 0.05) was much higher ($p < 0.05$). With the addition of NACP, the pH in biofilm medium for SBMP+MNP+DMAHDM+NACP was further increased to (6.96 ± 0.06) ($p < 0.05$).

4. Discussion

A critical challenge in dentin bonding is the limited durability of the resin-dentin bond, which is caused by incomplete infiltration of resin monomers into the demineralized collagen fibrils [11], as well as bacterial invasion into the margins and demineralization. To date, there has been only one report that tested magnetic nanoparticle-doped adhesive with a magnetic force which improved the penetration of adhesive into dentinal tubules, thereby enhancing the bond strength of composites to dentin [35]. However, that report did not include antibacterial and remineralizing agents in the bonding agent to combat biofilm acids and recurrent caries at the restoration margin, which is a primary reason for restoration failures [8, 26]. Recent studies demonstrated that resins containing DMAHDM and NACP had potent antibacterial activity and Ca and P ion release to suppress human dentin demineralization and promote remineralization [46, 48, 49]. Therefore, the present study developed a novel Magnetic nanoparticle-containing adhesive system with antibacterial and remineralization functions for the first time. The novel adhesive SBMP+MNP+DMAHDM+NACP: (1) greatly enhanced the dentine bond strength, (2) reduced biofilm metabolic activity, CFU counts and lactic acid production, and (3) released high levels of Ca and P ions with remineralization potential and increased the biofilm pH from low cariogenic pH to a safe pH. Thus, the hypotheses could not be rejected.

During resin-dentin bonding, mineral ions are removed to expose the collagen through acid etching. Hybridization occurs by resin inter-diffusion into the exposed dentinal collagen layer, combined with resin tags into the opened dentinal tubules to form the bonded interface [64]. The quality of the hybrid layer is essential for dentin bonding [14, 50]. To date, the

infiltration efficacy of the resin monomers is not satisfactory for contemporary adhesive dentistry [14]. This problem may be solved by using a magnetic force to steer magnetic nanoparticles in the bonding agent to pull the resin deeper into dentin, creating a stronger hybrid layer. Recent study showed, for the first time, that the actively guided magnetic nanoparticle-doped adhesive substantially enhanced the bond strength of composite to dentin [35]. Furthermore, antibacterial and remineralizing functions are also highly desirable for a bonding agent to suppress biofilm acids and recurrent caries at the margins. However, no report exists on a magnetic nanoparticle-doped adhesive that has antibacterial and remineralizing capabilities.

In the present study, a dentin bond strength that was 59% greater than the SBMP Control adhesive was achieved in the magnetic nanoparticle-containing adhesive groups with an external magnetic field force. Regarding the mechanism of enhancing the dentin bond strength, using the magnetic nanoparticle-doped adhesive and magnetic field increased the penetration of resin into the dentin. This was demonstrated in a recent study which produced an extensive network of resin tags, with substantial increases in the average resin tag length and resin tag density [35]. The homogeneity, continuity and length of resin tags reflected the infiltration efficacy of the resin [65]. The benefit of the magnetic nanoparticles is that they can readily flow with the bonding agent into the dentinal tubules which are only 1–2 μm in diameters, while traditional micron-sized particles will have difficulty moving through the tubules. For γIONPs , their small sizes of 7–8 nm (Fig. 2B), under the pulling of a magnetic force, may help the resin to infiltrate and fill the interfibrillar spaces which in turn will help reduce or eliminate nanoleakage in the hybrid layer [14]. Further studies are needed to determine the ability of this method to penetrate the interfibrillar spaces in the dentin. In addition, a recent study found that γIONPs had the potential to enhance the performance of dental pulp stem cells [65]. This benefit may be applicable to a wide range of dental conservation treatments which also warrant further study.

Dental resin materials with antibacterial functions can kill the residual bacteria in the prepared tooth cavity, and inhibit new bacterial invasion at the tooth-restoration interfaces, thus improving the bond durability [36, 67–69]. Incorporation of QAMs into resins enables the antibacterial agent to be immobilized in the resin matrix, and not released or lost over time [67, 70]. DMAHDM was a recently developed with an alkyl chain length of 16 carbons and with methacrylate functional groups on one end of the molecule [45, 49]. The present study showed that the DMAHDM-containing magnetic nanoparticle-containing adhesive substantially reduced the metabolic activity, lactic acid production, and the CFU of biofilms. Furthermore, the incorporation of DMAHDM did not adversely influence the ability of magnetic nanoparticle-doped adhesive on enhancing the dentin bond strength. These results indicate the feasibility of delivering antibacterial and other therapeutic agents via the magnetic nanoparticle-containing adhesive and the magnetic force, which warrants further study.

Reincorporation of mineral into the demineralized dentin matrix and the formation newly remineralized tissues are beneficial to resist degradation at the bonded interface [11]. Remineralization could be facilitated by the use of bioactive materials [71–73]. NACP was demonstrated to have high levels of Ca and P ion release and effective remineralization

capabilities [54]. Indeed, significant remineralization in enamel and dentin lesions via NACP resins was shown using quantitative transverse microradiography [74, 75]. In addition, NACP resin could substantially increase the Ca and P ion release at a low cariogenic pH when these ions would be most needed to combat caries [52]. Another benefit was acid neutralization, because NACP resin could neutralize acid challenges (which otherwise would demineralize tooth structures) by raising the pH from a cariogenic pH of 4 to a safe pH of 6.5 [75]. Therefore, it would be highly beneficial to incorporate NACP into the magnetic nanoparticle-containing adhesive. The present study showed that incorporating NACP into the magnetic nanoparticle-containing adhesive did not compromise the dentin bond strength; in fact, the SBMP+MNP+DMAHDM+NACP group had much greater dentin bond strength than that of commercial control. Therefore, the pulling efficacy of the magnetic force on the NACP Magnetic nanoparticle-containing adhesive was the same as that on the magnetic nanoparticle-containing adhesive without NACP. In addition, the present study showed that SBMP+MNP+DMAHDM+NACP released high levels of Ca and P ions in the biofilm culture medium. The BHI medium itself contained Ca and P ions, manifested by the measured Ca and P concentrations in the culture medium for the three groups without NACP. However, the SBMP+MNP+DMAHDM+NACP biofilm medium had significantly higher Ca and P ion concentrations than the other three groups, indicating contributions of Ca and P ion release from the SBMP+MNP+DMAHDM+NACP resin. Higher Ca and P ion concentrations are beneficial in tipping the balance toward favoring remineralization for tooth structures [76, 77].

Another important aspect in favoring remineralization and inhibiting demineralization is to control the local biofilm plaque pH. In the present study, the SBMP+MNP+DMAHDM adhesive with biofilms had a pH of 6.76, much higher than that of the SBMP Control (4.53) and SBMP+MNP adhesive (4.47). This was because the DMAHDM resin suppressed biofilm growth, leaving less biofilm on the resin to produce acids. Maintaining a pH close to neutral in the local plaque is highly beneficial in protecting the tooth structures to avoid demineralization [52]. Furthermore, the SBMP+MNP+DMAHDM+NACP adhesive yielded an even higher pH of 6.96, higher than that of SBMP+MNP+DMAHDM; this was likely because the NACP-containing resin had an acid-neutralization effect to neutralize the remaining acid produced by the bacteria. Therefore, incorporation of DMAHDM and NACP together in the Magnetic nanoparticle-containing adhesive is more beneficial than using DMAHDM alone.

The present study demonstrated the feasibility and beneficial effects of delivering DMAHDM and NACP via the magnetic nanoparticle-containing adhesive for the first time. Besides improving the infiltration of resin to provide a stronger and more durable link between the demineralized tooth tissues and the restoration, the magnetic nanoparticles could serve as carriers to deliver drugs, cytokines, hormones and other molecules through dentin into the pulp to treat pulpal diseases. Since there is an increased interest in less removal of tooth structures and minimally-intervention dentistry, dentists attempt to preserve the vitality of the pulp in clinical treatments [78]. This will leave behind more carious tissues in the prepared tooth cavity with bacterial infections and inflammation. The magnetic nanoparticle delivery method with magnetic forces can be especially beneficial in

minimally-intervention dentistry. This magnetically active delivery method is simple and less invasive.

This study has several limitations. First, the shear bond strength was evaluated with a chisel. Further study should measure the dentin microtensile bond strength using a magnetic force. Second, long-term aging of the bonded samples before evaluations should be performed to determine the bond strength durability. Third, the 3 minutes of magnet application are too long for clinical applications. The effects of the magnitude and direction of the magnetic force on the dentin bond strength need to be determined. Extra-oral magnets are currently being designed to deliver perpendicular forces to the teeth, regardless of their position in the dental arch, in order to save clinical time and reduce technique-sensitivity. Further efforts are needed to investigate the incorporation of “drug-loaded” magnetic nanoparticles into bonding agents to help establish a new and effective strategy in conservative dentistry.

5. Conclusion

This study developed a novel magnetic nanoparticle-containing adhesive for the improvement of dentin bonding and prevention of secondary caries. The new bonding system contained magnetic nanoparticles for enhancing the dentin bond strength, DMAHDM for antibacterial activity, and NACP for remineralization. The novel adhesive greatly enhanced the dentin bond strength, substantially reduced the biofilm metabolic activity and lactic acid, and decreased the biofilm CFU by 4 logs. The new adhesive neutralized biofilm acids and raised the biofilm pH to above 6.5, while biofilms on commercial control adhesive had a cariogenic pH of 4.5. Therefore, the novel magnetic nanoparticle-containing adhesive with DMAHDM and NACP offers multiple benefits and is promising for a wide range of dental applications to enhance the bonded restorations and inhibit secondary caries.

Acknowledgments

We thank Drs. Suping Wang, Haohao Wang, Mary Anne S. Melo and Chao Ji for discussions and experimental help. This study was supported by NIH R01 DE17974 (HX), DE024227 (RM and DD), National Natural Science Foundation of China 81600913 (YL), Shanxi Natural Science Foundation 2013JQ4036 (YL), University of Maryland seed grant (HX) and University of Maryland School of Dentistry bridge fund (HX).

References

1. Ferracane JL. Resin composite—state of the art. *Dent Mater.* 2011; 27:29–38. [PubMed: 21093034]
2. Drummond JL. Degradation, fatigue, and failure of resin dental composite materials. *J Dent Res.* 2008; 87:710–9. [PubMed: 18650540]
3. Randolph LD, Palin WM, Leprince JG. *Dental Composite Materials for Direct Restorations.* Springer; 2018. Composition of Dental Resin-Based Composites for Direct Restorations; 11–24.
4. Lynch CD, Opdam NJ, Hickel R, Brunton PA, Gurgan S, Kakaboura A, et al. Guidance on posterior resin composites: Academy of operative dentistry-European section. *J Dent.* 2014; 42:377–83. [PubMed: 24462699]
5. Garcia I. NIDCR's 2009–2013 Strategic Plan. *Am Dent Hyg Assoc.* 2009; 83:153–4.
6. Jokstad A, Bayne S, Blunck U, Tyas M, Wilson N. Quality of dental restorations FDI Commission Project 2–95. *Int Dent J.* 2001; 51:117–58. [PubMed: 11563679]
7. Ferracane JL. Resin-based composite performance: are there some things we can't predict? *Dent Mater.* 2013; 29:51–8. [PubMed: 22809582]

8. Alhareky M, Tavares M. Amalgam vs Composite Restoration, Survival, and Secondary Caries. *J Evid-Based Dent Pr.* 2016; 16:107–9.
9. Krithikadatta J. Clinical effectiveness of contemporary dentin bonding agents. *J Conserv Dent: JCD.* 2010; 13:173. [PubMed: 21217944]
10. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/dentin interface: the weak link in the composite restoration. *Ann Biomed Eng.* 2010; 38:1989–2003. [PubMed: 20195761]
11. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J Dent Res.* 2011; 90:953–68. [PubMed: 21220360]
12. Silikas N, Masouras K, Satterthwaite J, Watts DC. Effect of nanofillers in adhesive and aesthetic properties of dental resin-composites. *Int J Nano and Biomater.* 2007; 1:116–27.
13. Furuse AY, Gordon K, Rodrigues FP, Silikas N, Watts DC. Colour-stability and gloss-retention of silorane and dimethacrylate composites with accelerated aging. *J Dent.* 2008; 36:945–52. [PubMed: 18778884]
14. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater.* 2011; 27:1–16. [PubMed: 21112620]
15. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt K. State of the art of self-etch adhesives. *Dent Mater.* 2011; 27:17–28. [PubMed: 21109301]
16. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *J Biomed Mater Res Part A.* 1982; 16:265–73.
17. Pashley DH, Tay F, Yiu C, Hashimoto M, Breschi L, Carvalho R, et al. Collagen degradation by host-derived enzymes during aging. *J Dent Res.* 2004; 83:216–21. [PubMed: 14981122]
18. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, Dorigo EDS. Dental adhesion review: aging and stability of the bonded interface. *Dent Mater.* 2008; 24:90–101. [PubMed: 17442386]
19. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials.* 2007; 28:3757–85. [PubMed: 17543382]
20. Sadek FT, Castellan CS, Braga RR, Mai S, Tjäderhane L, Pashley DH, et al. One-year stability of resin–dentin bonds created with a hydrophobic ethanol-wet bonding technique. *Dent Mater.* 2010; 26:380–6. [PubMed: 20083304]
21. Sadek F, Braga R, Muench A, Liu Y, Pashley DH, Tay F. Ethanol wet-bonding challenges current anti-degradation strategy. *J Dent Res.* 2010; 89:1499–504. [PubMed: 20940353]
22. Ye Q, Wang Y, Spencer P. Nanophase separation of polymers exposed to simulated bonding conditions. *J Biomed Mater Res Part B: Appl Biomater.* 2009; 88:339–48. [PubMed: 18335432]
23. Besinis A, van Noort R, Martin N. The use of acetone to enhance the infiltration of HA nanoparticles into a demineralized dentin collagen matrix. *Dent Mater.* 2016; 32:385–93. [PubMed: 26822965]
24. Carvalho RM, Mendonca J, Santiago S, Silveira R, Garcia F, Tay F, et al. Effects of HEMA/solvent combinations on bond strength to dentin. *J Dent Res.* 2003; 82:597–601. [PubMed: 12885842]
25. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. *Dent Mater J.* 2005; 24:1–13. [PubMed: 15881200]
26. Hashimoto M. A Review—Micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. *J Biomed Mater Res Part B Appl Biomater.* 2010; 92:268–80. [PubMed: 19904824]
27. Tjäderhane L. Dentin bonding: can we make it last? *Oper Dent.* 2015; 40:4–18. [PubMed: 25615637]
28. Tay FR, King NM, Chan K-m, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *J Adhes Dent.* 2002; 4:255–69. [PubMed: 12666745]

29. Suppa P, Breschi L, Ruggeri A, Mazzotti G, Prati C, Chersoni S, et al. Nanoleakage within the hybrid layer: a correlative FEISEM/TEM investigation. *J Biomed Mater Res Part B Appl Biomater*. 2005; 73:7–14. [PubMed: 15678495]
30. Tay FR, Pashley DH. Have dentin adhesives become too hydrophilic? *J Can Dent Assoc*. 2003; 69:726–32.
31. Reis A, Ferreira SQ, Costa TR, Klein-Júnior CA, Meier MM, Loguercio AD. Effects of increased exposure times of simplified etch-and-rinse adhesives on the degradation of resin–dentin bonds and quality of the polymer network. *Eur J Oral Sci*. 2010; 118:502–9. [PubMed: 20831585]
32. Cadenaro M, Antonioli F, Sauro S, Tay FR, Di Lenarda R, Prati C, et al. Degree of conversion and permeability of dental adhesives. *Eur J Oral Sci*. 2005; 113:525–30. [PubMed: 16324144]
33. Komae A, Lee R, Nacev A, Probst R, Sarwar A, Depireux DA, et al. 16 Putting Therapeutic Nanoparticles Where They Need to Go by Magnet Systems Design and Control. *Magnetic Nanoparticles: From Fabrication to Clinical Applications*. 2012:419.
34. Kulkarni S, Ramaswamy B, Horton E, Gangapuram S, Nacev A, Depireux D, et al. Quantifying the motion of magnetic particles in excised tissue: Effect of particle properties and applied magnetic field. *J Magn Magn Mater*. 2015; 393:243–52. [PubMed: 26120240]
35. Ji Y, Choi SK, Sultan AS, Chuncal K, Lin X, Dashtimoghadam E, et al. Nanomagnetic-mediated drug delivery for the treatment of dental disease. *Nanomed-Nanotechnol*. 2018; 14:919–27.
36. Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. *Dent Mater J*. 2009; 28:11–9. [PubMed: 19280964]
37. Cocco AR, da Rosa WLdO, da Silva AF, Lund RG, Piva E. A systematic review about antibacterial monomers used in dental adhesive systems: Current status and further prospects. *Dent Mater*. 2015; 31:1345–62. [PubMed: 26345999]
38. Lynch CD, Frazier KB, McConnell RJ, Blum IR, Wilson NH. Minimally invasive management of dental caries: contemporary teaching of posterior resin-based composite placement in US and Canadian dental schools. *J Am Dent Assoc*. 2011; 142:612–20. [PubMed: 21628682]
39. Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. *Dent Mater*. 2003; 19:313–9. [PubMed: 12686296]
40. Imazato S, Kinomoto Y, Tarumi H, Torii M, Russell R, McCabe J. Incorporation of antibacterial monomer MDPB into dentin primer. *J Dent Res*. 1997; 76:768–72. [PubMed: 9109826]
41. Li F, Chai Z, Sun M, Wang F, Ma S, Zhang L, et al. Anti-biofilm effect of dental adhesive with cationic monomer. *J Dent Res*. 2009; 88:372–6. [PubMed: 19407160]
42. Huang L, Yu F, Sun X, Dong Y, Lin P-t, Yu H-h, et al. Antibacterial activity of a modified unfilled resin containing a novel polymerizable quaternary ammonium salt MAE-HB. *Sci Rep*. 2016; 6:33858. [PubMed: 27659279]
43. Zhang K, Cheng L, Wu EJ, Weir MD, Bai Y, Xu HH. Effect of water-ageing on dentine bond strength and anti-biofilm activity of bonding agent containing new monomer dimethylaminododecyl methacrylate. *J Dent*. 2013; 41:504–13. [PubMed: 23583528]
44. Daood D, Yiu C, Burrow M, Niu L-N, Tay F. Effect of a novel quaternary ammonium silane cavity disinfectant on durability of resin–dentine bond. *J Dent*. 2017; 60:77–86. [PubMed: 28315376]
45. Li F, Weir MD, Xu HH. Effects of quaternary ammonium chain length on antibacterial bonding agents. *J Dent Res*. 2013; 92:932–8. [PubMed: 23958761]
46. Zhang N, Melo MA, Chen C, Liu J, Weir MD, Bai Y, et al. Development of a multifunctional adhesive system for prevention of root caries and secondary caries. *Dent Mater*. 2015; 31:1119–31. [PubMed: 26187532]
47. Zhou H, Liu H, Weir MD, Reynolds MA, Zhang K, Xu HH. Three-dimensional biofilm properties on dental bonding agent with varying quaternary ammonium charge densities. *J Dent*. 2016; 53:73–81. [PubMed: 27472954]
48. Xie X, Wang L, Xing D, Zhang K, Weir MD, Liu H, et al. Novel dental adhesive with triple benefits of calcium phosphate recharge, protein-repellent and antibacterial functions. *Dent Mater*. 2017; 33:553–63. [PubMed: 28356216]

49. Melo MA, Orrego S, Weir MD, Xu HH, Arola DD. Designing Multiagent Dental Materials for Enhanced Resistance to Biofilm Damage at the Bonded Interface. *ACS Appl Mater Interfaces*. 2016; 8:11779–87. [PubMed: 27081913]
50. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability—a literature review. *Dent Mater*. 2016; 32:e41–e53. [PubMed: 26743967]
51. Imazato S, Ma S, Chen J-h, Xu HH. Therapeutic polymers for dental adhesives: loading resins with bio-active components. *Dent Mater*. 2014; 30:97–104. [PubMed: 23899387]
52. Chen C, Weir MD, Cheng L, Lin NJ, Lin-Gibson S, Chow LC, et al. Antibacterial activity and ion release of bonding agent containing amorphous calcium phosphate nanoparticles. *Dent Mater*. 2014; 30:891–901. [PubMed: 24954647]
53. Xu HH, Weir MD, Sun L. Nanocomposites with Ca and PO₄ release: effects of reinforcement, dicalcium phosphate particle size and silanization. *Dent Mater*. 2007; 23:1482–91. [PubMed: 17339048]
54. Xu HH, Moreau JL, Sun L, Chow LC. Nanocomposite containing amorphous calcium phosphate nanoparticles for caries inhibition. *Dent Mater*. 2011; 27:762–9. [PubMed: 21514655]
55. Weir MD, Ruan J, Zhang N, Chow LC, Zhang K, Chang X, et al. Effect of calcium phosphate nanocomposite on in vitro remineralization of human dentin lesions. *Dent Mater*. 2017; 33:1033–44. [PubMed: 28734567]
56. Antonucci JM, Zeiger DN, Tang K, Lin-Gibson S, Fowler BO, Lin NJ. Synthesis and characterization of dimethacrylates containing quaternary ammonium functionalities for dental applications. *Dent Mater*. 2012; 28:219–28. [PubMed: 22035983]
57. Huber DL. Synthesis, properties, and applications of iron nanoparticles. *Small*. 2005; 1:482–501. [PubMed: 17193474]
58. Chen B, Li Y, Zhang X, Liu F, Liu Y, Xiong F, et al. An efficient synthesis of ferumoxytol induced by alternating-current magnetic field. *Mater Lett*. 2016; 170:93–6.
59. Antonucci JM, O'Donnell J, Schumacher GE, Skrtic D. Amorphous calcium phosphate composites and their effect on composite–adhesive–dentin bonding. *J Adhes Sci Technol*. 2009; 23:1133–47. [PubMed: 19696914]
60. Zhang N, Melo MAS, Bai Y, Xu HH. Novel protein-repellent dental adhesive containing 2-methacryloyloxyethyl phosphorylcholine. *J Dent*. 2014; 42:1284–91. [PubMed: 25234652]
61. Imazato S, Ehara A, Torii M, Ebisu S. Antibacterial activity of dentine primer containing MDPB after curing. *J Dent*. 1998; 26:267–71. [PubMed: 9594480]
62. Cheng L, Weir MD, Xu HH, Kraigsley AM, Lin NJ, Lin-Gibson S, et al. Antibacterial and physical properties of calcium–phosphate and calcium–fluoride nanocomposites with chlorhexidine. *Dent Mater*. 2012; 28:573–83. [PubMed: 22317794]
63. Van Loveren C, Buijs J, Ten Cate J. The effect of triclosan toothpaste on enamel demineralization in a bacterial demineralization model. *J Antimicrob Chemo*. 2000; 45:153–8.
64. Nakabayashi N, Nakamura M, Yasuda N. Hybrid Layer as a Dentin-Bonding Mechanism. *J Esthet Restor Dent*. 1991; 3:133–8.
65. Langer A, Ilie N. Dentin infiltration ability of different classes of adhesive systems. *Clin Oral Investig*. 2013; 17:205–16.
66. Xia Y, Chen H, Zhang F, Wang L, Chen B, Reynolds MA, et al. Injectable calcium phosphate scaffold with iron oxide nanoparticles to enhance osteogenesis via dental pulp stem cells. *Artif Cells Nanomed Biotechnol*. 2018:1–11.
67. Melo MAS, Weir MD, Fakhari F, Cheng L, Zhang K, Li F, et al. Antibacterial Polymers for Dental Adhesives and Composites. *Adhesion in Pharmaceutical, Biomedical, and Dental Fields*. 2017:301.
68. Farrugia C, Haider J, Camilleri L, Camilleri J. Clinical relevance of antimicrobial testing results for dental restorative materials. *J Appl Biomater Funct Mater*. 2017; 15:e153–61. [PubMed: 28256700]
69. Nedeljkovic I, Van Landuyt KL. *Dental Composite Materials for Direct Restorations*. Springer; 2018. Secondary Caries; 235–43.

70. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater.* 2003; 19:449–57. [PubMed: 12837391]
71. de Moraes RC, Silveira RE, Chinelatti M, Geraldini S, Pires-de FdCP. Bond strength of adhesive systems to sound and demineralized dentin treated with bioactive glass ceramic suspension. *Clin Oral Investig.* 2017;1–9.
72. Fernando D, Attik N, Pradelle-Plasse N, Jackson P, Grosgeat B, Colon P. Bioactive glass for dentin remineralization: A systematic review. *Mater Sci Eng C Mater Biol Appl.* 2017; 76:1369–77. [PubMed: 28482504]
73. Melo MAS, Cheng L, Zhang K, Weir MD, Zhou X, Bai Y, et al. Novel Nanostructured Bioactive Restorative Materials for Dental Applications. *Biological and Pharmaceutical Applications of Nanomaterials.* 2015:151.
74. Weir MD, Ruan J, Zhang N, Chow LC, Zhang K, Chang X, et al. Effect of calcium phosphate nanocomposite on in vitro remineralization of human dentin lesions. *Dent Mater.* 2017; 33:1033–44. [PubMed: 28734567]
75. Weir M, Chow L, Xu H. Remineralization of demineralized enamel via calcium phosphate nanocomposite. *J Dent Res.* 2012; 91:979–84. [PubMed: 22933607]
76. Dickens SH, Flaim GM, Takagi S. Mechanical properties and biochemical activity of remineralizing resin-based Ca–PO₄ cements. *Dent Mater.* 2003; 19:558–66. [PubMed: 12837405]
77. Skrtic D, Antonucci JM, Eanes E. Amorphous calcium phosphate-based bioactive polymeric composites for mineralized tissue regeneration. *J Res Natl Inst Stand Technol.* 2003; 108:167.
78. Jingrwar M, Bajwa N, Pathak A. Minimal intervention dentistry—a new frontier in clinical dentistry. *J Clin Diagn Res: JCDR.* 2014; 8:ZE04.

Fig. 1.

Schematic showing the dentin bonding and magnetic force procedures. Human molar teeth were sectioned to expose the dentin. They were divided into four groups according to the different components of bonding agent. Then, the SBMP+MNP group was divided into two subgroups according to whether applying a magnetic force or not. After a magnetic nanoparticle-doped adhesive was placed, the magnetic force was applied for 3 minutes to pull the adhesive deep into dentinal tubules before photo-polymerization.

Fig. 2. Representative TEM images of magnetic nanoparticles: (A) Acrylate-functionalized iron magnetic nanoparticles (AINPs); (B) $\gamma\text{Fe}_2\text{O}_3$ nanoparticles (γIONPs). The AINPs had a spherical shape with approximately 900 nm in average diameter. γIONPs had a relatively uniform, spherical morphology, with an average size of about 8 nm.

Fig. 3. Dentin shear bond strength using extracted human teeth, tested after storage in water for 24 h (mean $\pm$ sd; n = 10). Bars with dissimilar letters indicate values that are significantly different from each other ($p < 0.05$). The shear bond strength was significantly increased by incorporating MNPs in adhesive and using a magnetic force during bonding ($p < 0.05$).

Fig. 4. Representative live/dead staining images of 2-day biofilms on adhesive resins: (A) SBMP Control, (B) SBMP+MNP, (C) SBMP+MNP+DMAHDM, (D) SBMP+MNP+DMAHDM+NACP, Live bacteria were stained green, and bacteria with compromised membranes were stained red.

Fig. 5.

Two-day biofilm viability on resins: (A) MTT metabolic activity, (B) lactic acid production, (C) Colony-forming units (CFU) (mean $\pm$ sd; $n = 6$). In each plot, values with dissimilar letters are significantly different from each other ($p < 0.05$). Note the log scale in (C), where SBMP+MNP+DMAHDM and SBMP+MNP+DMAHDM+NACP reduced the biofilm CFU by 4 orders of magnitude ($p < 0.05$)

Fig. 6. Calcium (Ca) and phosphate (P) ion concentrations and pH of 2-day biofilm culture medium: (A) Ca ion concentration, (B) P ion concentration, (C) pH of the biofilm culture medium (mean $\pm$ sd, n = 6). In each plot, values with dissimilar letters are significantly different from each other ($p < 0.05$).