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ABSTRACT

One of the leading causes for the failure of dental composite restorations is secondary caries. Effectively inhibiting cariogenic biofilms and reducing secondary caries could extend the service life of composite restorations. Dental composites releasing antibacterial agents such as chlorhexidine (CHX) have shown biofilm-inhibitory efficacy, but they usually have poor physical and mechanical properties. Herein, we present a study of a new method to encapsulate and release CHX from dental composite using mesoporous silica nanoparticles (MSNs). SBA-15 MSNs were synthesized according to a reported procedure. CHX (62.9 wt%) was encapsulated into dried MSN from 0.3 M CHX ethanol solution. The dental composites containing 0% (control), 3%, 5%, and 6.3% CHX or the same amounts of CHX entrapped in MSN (denoted as CHX@MSN) were fabricated with methacrylate monomers and silanized glass fillers (CHX or CHX@MSN + glass filler particle = 70 wt%). The monomer mixture consisted of bisphenol A glycidyl methacrylate (BisGMA), hexanediol dimethacrylate (HDDMA), ethoxylated bisphenol A dimethacrylate (EBPADMA), and urethane dimethacrylates (UEDMA) at a weight ratio of 40:30:20:10. The composites were tested for CHX release and recharge, flexural strength and modulus (at 24 hr and 1 mo), surface roughness, *in vitro* wear, and antibacterial activity against *Streptococcus mutans* and *Lactobacillus casei* (in both planktonic growth and biofilm formation). The results showed that the composites with CHX@MSN largely retained mechanical properties and smooth surfaces and showed controlled release of CHX over a long time. In contrast, the composites with directly mixed CHX showed reduced mechanical properties, rough surfaces, and burst release of CHX in a short time. The composites with CHX either directly mixed or in MSN showed strong inhibition to *S. mutans* and *L. casei*. This research has demonstrated the successful application of MSNs as a novel nanotechnology in dental materials to inhibit oral biofilm without sacrificing materials' mechanical properties and surface integrity.

KEY WORDS: controlled release, surface roughness, bacteria, biofilm, mechanical properties, *Streptococcus mutans*.

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Antibacterial Dental Composites with Chlorhexidine and Mesoporous Silica

INTRODUCTION

Resin-based dental composites have been widely used in dentistry to restore decayed teeth. These composites have sufficient flexural strength (from 80 to 140 MPa) and outstanding esthetics. However, dental composite restorations have limited service life (average, 7 yrs) due to fracture of the restoration itself or to secondary caries developed at the restoration margins caused by accumulation of cariogenic biofilms (Mjör *et al.*, 2000; Deligeorgi *et al.*, 2001; Leung *et al.*, 2005). The subsequent replacement of restorations by drilling and filling in the United States alone costs more than \$5 billion annually (Jokstad *et al.*, 2001). Strategies are needed to extend the service life of the dental composite restorations by effectively inhibiting cariogenic biofilms and thus reducing secondary caries, especially in high-caries-risk populations (Imazato, 2009; Bekhuis, 2011). Dental composites that can inhibit cariogenic biofilms while maintaining their mechanical properties are highly desirable.

Chlorhexidine (CHX), with its broad-spectrum antibacterial activity and low cytotoxicity, has been widely used in oral infection control as a mouthrinse, dental coating (Prevora[®]), and denture wash (Achong *et al.*, 1999; Slot *et al.*, 2011) and is considered a “gold standard” for the evaluation of antimicrobial agents. CHX-containing dental composites have been studied (Jedrychowski *et al.*, 1983; Riggs *et al.*, 2000; Leung *et al.*, 2005; Anusavice *et al.*, 2006; Cheng *et al.*, 2012), and the CHX release rate has been controlled by the degree of cross-linking, which also contributed to a balance between swelling induced by water sorption of hydrophilic composition and polymerization shrinkage (Riggs *et al.*, 2000; Leung *et al.*, 2005). The presence of CHX, however, incurred detrimental mechanical or physical properties of the composites, including decreased strength (Jedrychowski *et al.*, 1983; Slot *et al.*, 2011), porous surface (Anusavice *et al.*, 2006), and increased water sorption (Deligeorgi *et al.*, 2001). The main reason is that CHX (a salt) is immiscible with dental monomers. It forms aggregates in the resin matrix of the composite, and the dissolution of CHX aggregates leads to the formation of a porous surface, which has poor wear resistance. It also increases the potential for staining and bacterial biofilm accumulation. Therefore, simply mixing CHX into a dental composite will produce an inferior material that does not meet the requirements for dental applications (Deligeorgi *et al.*, 2001).

Mesoporous silica nanoparticles (MSNs) with high pore volume and surface area (Zhao *et al.*, 1998) have attracted increasing attention as reservoirs to encapsulate and release disparate molecules including fenbufen (Carriazo *et al.*, 2010), silver (Liong *et al.*, 2009), CHX (Izquierdo-Barba *et al.*, 2009; Raso *et al.*, 2010), gene delivery system (Petkar *et al.*, 2011), etc. However, studies of applications of MSN in dental materials have been rare. So far only one report (Carpenter *et al.*, 2013) has described the release of nitric oxide as

an antibacterial agent from the dental composite containing MSN modified by O^2 -protected *N*-diazoniumdiolate-based silanes. Two other reports showed that a combination of MSN with non-porous fillers seems to increase the mechanical properties of the composites (Praveen *et al.*, 2006; Samuel *et al.*, 2009). There are also major differences in the design and applications of MSN between dental composites and other drug-releasing materials or biomaterials. The objective of this study was to investigate the effects of MSN (SBA-15) on dental composites for the controlled release of CHX and its physical, mechanical, and antibacterial properties. The hypothesis was that the dental composite containing MSNs with entrapped CHX can provide sustainable release and recharge of CHX while maintaining its physical and mechanical properties.

MATERIALS & METHODS

Chlorhexidine diacetate salt (CHX) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Mesoporous silica nanoparticles (SBA-15) were synthesized following the reported procedure (Zhao *et al.*, 1998). Prior to CHX loading, mesoporous SBA-15 was vacuum-dried at 75°C overnight to remove moisture residue. The dried SBA-15 (about 1 g) was dispersed into 15 mL absolute ethanol containing 0.3 M CHX and sonicated for 10 min, vacuum-infiltrated at room temperature 3 times, and vigorously stirred for 3 days. The mixture was filtered, briefly rinsed with ethanol, and vacuum-dried before use. The CHX loading capacity on SBA-15 was determined by means of a thermogravimetric analyzer (TGA) (Q600, TA Instruments, New Castle, DE, USA) at 10°C/min to 800°C.

The resin monomer mixture consisted of bisphenol A glycidyl methacrylate (BisGMA) (Sigma-Aldrich), hexanediol dimethacrylate (HDDMA), ethoxylated bisphenol A dimethacrylate (EBPADMA) (both from Esstech, Essington, PA, USA), and urethane dimethacrylate (UEDMA) (Sigma-Aldrich) at a weight ratio of 40:30:20:10. A 1% photo-initiator [camphorquinone: phenyl bis(2,4,6-trimethyl benzoyl)phosphine oxide: ethyl 4-dimethylaminobenzoate = 1:2:4 by weight] (Xu *et al.*, 2006, 2012) was added to the mixture as well. CHX at 0%, 3%, 5%, and 6.3%, or the same amounts of CHX entrapped in MSN (denoted as CHX@MSN), was mixed as filler with resin monomers by replacing the same amount of silanized glass fillers (Dentsply/Caulk, Milford, DE, USA) with total filler load maintained at 70 wt%. All components were blended in a SpeedMixer™ (FlackTek, Inc., Landrum, SC, USA) for 3 × 30 sec to form a resin paste, which was stored in the dark at room temperature for 4 hr to allow sufficient time for the monomers to infiltrate MSN mesopores. Bar specimens for flexural strength testing (2 mm × 2 mm × 25 mm, n = 12 each group) were prepared by being light-cured (6 × 40 sec on both sides) with an Optilux 501 curing light (Kerr Corp., Orange, CA, USA) in stainless steel molds. All specimens were polished with 600-grit SiC abrasive paper and stored in deionized water at 37°C for 24 hr or 1 mo before mechanical testing. Flexural strength and modulus were measured by a three-point-bending fixture on an MTS Landmark machine (MTS Systems Corp., Eden Prairie, MN, USA) with a crosshead speed of 1 mm/min.

For CHX release and recharge, disc specimens containing either 5% CHX or 5% CHX@MSN with a diameter of 10 mm and a thickness of 1.2 mm were light-cured on both sides for 40 sec each. The disc specimens (n = 4 *per* group, weight 0.205 – 0.226 g) were immersed in 2 mL high-purity water (18 MΩ·cm) at 37°C. A 2-mL quantity of equilibrated water was taken at 0, 2, 4, and 8 hr, then every 24 hr up to 10 days and finally at 16 days. The concentration of CHX in the solution was analyzed by high-performance liquid chromatography (HPLC) [HP 1100 (Agilent Technologies, Santa Clara, CA, USA)] with a surfactant column and acetonitrile/water (1:1) mobile phase at 0.5 mL/min. A series of known CHX concentrations (10^{-6} to 10^{-4} M) was prepared for calibration of the HPLC. The analyzed CHX concentration was converted to weight and compared with initial CHX weight in individual discs. For CHX recharge, the disc specimens after release of CHX were further immersed in large amounts of water and sonicated to reduce the amount of CHX. The specimens were then immersed in 10 mL 0.2% CHX aqueous solution for 1 min, then rinsed with deionized water for 10 sec and placed in 5 mL high-purity water at 37°C (replaced every 24 hr); this continued for 5 days and was repeated for 3 cycles. The collected solution was analyzed by HPLC.

The antibacterial activity of the composites against *Streptococcus mutans* and *Lactobacillus casei* was tested in both planktonic growth and biofilm formation. Planktonic growth of two bacteria was tested with Bioscreen C™ (Oy Growth Curves AB Ltd, Helsinki, Finland), which automatically measures the optical density of the bacterial culture in the plates at 600nm (OD_{600nm}). Ring specimens (inner diameter 3.1 mm, outer diameter 5.2 mm, thickness 1.1 mm, n = 3) were made by light-curing the pastes for 40 sec in a Teflon ring mold. After ultraviolet (UV) sterilization, the specimens were laid at the bottoms of 100-well honeycomb microtiter plates of Bioscreen C™. Overnight broth cultures (*S. mutans* and *L. casei*) were transferred to fresh, pre-warmed brain heart infusion (BHI, Becton, Dickinson and Company, Sparks, MD, USA) for *S. mutans* or Lactobacillus MRS (deMan Rogosa, Sharpe) (Difco Laboratories, Detroit, MI, USA) for *L. casei*, and allowed to continue to grow in an aerobic chamber with 5% CO₂ at 37°C (Wen *et al.*, 2010). When reaching mid-exponential phase ($OD_{600nm} = 0.5$), the cultures were diluted 1:100 with proper broth medium, and aliquots (250 μL) were applied to the honeycomb plates. Bacterial growth at 37°C was monitored every half-hour continuously for 24 hr with Bioscreen C™ with moderate shaking for 10 sec prior to OD measurements (Bitoun *et al.*, 2011).

For scanning electron microscopic (SEM) analysis of biofilms, disc specimens were sterilized by UV exposure and placed in 20-mL glass vials, following the same water immersion procedure as for CHX release. Mid-exponential-phase cultures of *S. mutans* were diluted 1:100 into biofilm medium with glucose (18 mM) and sucrose (2 mM) (BMGS), and aliquots (1.5 mL) were added to each glass vial. Biofilms were allowed to grow in a 5% CO₂ incubator at 37°C (Wen and Burne, 2002, 2004). After 24 hr, the specimens from each group were fixed with 2.5% glutaraldehyde overnight at 4°C, dehydrated in 25, 50, 75, 90, and 100% ethanol, and then critical-point-dried in a CO₂ critical point dryer (Electron Microscopy Sciences,

Hatfield, PA, USA) (Wen and Burne, 2004; Wen *et al.*, 2010). After carbon coating, the biofilm morphology on disc surfaces was observed by field emission scanning electron microscopy (FE-SEM, Hitachi 4800).

Disc specimens ($n = 4$) of the composites were mounted on a SABRI Oral Simulating Posterior Composite Wear Test Apparatus (Downer's Grove, IL, USA) and subjected to wear under a 2-N load for 5,000 cycles. The worn specimens were scanned by means of the 3D profilometer scanner TalyScan 150 (Taylor Hobson, West Chicago, IL, USA), and maximum wear depth was measured with the associated image-processing software.

Mechanical properties of the composites were analyzed by ANOVA and Tukey's *post hoc* HSD test (Statistica 8.0, StatSoft Inc., Tulsa, OK, USA) for comparison of means, with a significance level of 95% ($\alpha = 0.05$).

RESULTS

Mechanical properties of composites containing CHX alone or with CHX@MSN after 24-hour and 1-month water immersion are shown in Fig. 1. Release rates of CHX from composites over time are shown in Fig. 2A. Fig. 2B shows the cumulative CHX release over time as the percentage of the total loaded CHX. Fig. 2C elucidates the recharge and release profiles over 3 repeating cycles. Cumulative CHX release over 3 days after recharge is presented in Fig. 2D.

The composite containing 5% CHX@MSN has similar surface roughness and few visible voids on the surface before and after water immersion (Figs. 3A, 3C). Conversely, composites with directly mixed 5% CHX have rough surfaces and deep voids left by dissolution of CHX aggregates (Figs. 3B, 3D). The maximum depth of *in vitro* wear of the composite with 5% CHX@MSN ($3.41 \pm 1.42 \mu\text{m}$) is significantly smaller than that of the composite with directly mixed CHX (14.47 ± 3.40) ($P < .05$).

The planktonic bacterial growth of *S. mutans* and *L. casei* results is shown in Figs. 4A and 4B, respectively. The control composite had no inhibitory function, leading eventually to *S. mutans* biofilm formation on its surface (Fig. 4C). Conversely, only a few bacteria with deformed membranes accumulated on the surface of the composites containing CHX@MSN (Fig. 4D).

DISCUSSION

In this study, well-dispersed MSNs allowed resin monomers or polymer chains to penetrate mesopores forming a necklace-like physical interlocking structure for improvement of polymers' or

Figure 1. Flexural strength (A) and flexural modulus (B) of dental composites with different amounts of CHX by direct mixing or entrapment in MSN (CHX@MSN) after 24-hour and 1-month water immersion at 37°C. Composites with 70% glass fillers without CHX or CHX@MSN served as controls. Data are shown as mean $\pm$ standard deviation ($n = 12$). The groups with the same label letters have no significant difference ($P > .05$).

composites' mechanical strength. This process may substitute hydrolytically sensitive coupling agents such as silane in the composites (Luo *et al.*, 1998; Praveen *et al.*, 2006; Samuel *et al.*, 2009). Considering CHX@MSN as a whole particle, the CHX loading capacity on MSN is 62.9%, or 3.36 mmol CHX per gram of MSN, determined by thermal gravimetric analysis (TGA). The leftover mesopores may still allow for the infiltration of dental monomers (Bowen and Reed, 1976; Samuel *et al.*, 2009). Incorporation of CHX alone as filler decreased the composites' flexural strength and modulus, similar to previous reports (Jedrychowski *et al.*, 1983; Slot *et al.*, 2011). The composites containing CHX@MSN with the same CHX content had significantly higher flexural strength than those directly mixed with CHX, and even higher flexural modulus than the control containing 70% glass filler. To encapsulate the same amount of CHX, at 5%, for example, in dental composites, 7.5% CHX@MSN particles are needed. Of the composites, there are 2.5% neat MSN and 62.5% glass filler as part of the total 70% filler weight ratio. The rigid MSNs in composites impart higher modulus to the composites than the low-molecular-weight CHX

Figure 2. CHX release and recharge. **(A)** CHX release profiles from composites containing directly mixed CHX or CHX@MSN. (The insert is the release rate ($\mu\text{mol}/\text{cm}^2/\text{hr}$) during the first 72 hr.) **(B)** Cumulative CHX release as the weight percentage of the total loaded CHX. **(C)** CHX recharge profile in 3 repeated recharges. **(D)** Cumulative CHX release over 3 days after recharge.

(Fig. 1B). After 1-month water immersion, all experimental composites showed reduced flexural strength, which is a common phenomenon in dental composites due to sorption of water as a plasticizer, dissolution of uncured monomer, etc. The composites with CHX@MSN after 1 mo displayed better flexural strength than those with directly mixed CHX after 24 hr. This rigid inorganic characteristic of the MSN manifests an improved modulus after water immersion. The more uniform filler

distribution in the composite with CHX@MSN also contributes to improved composite mechanical properties.

Light-cured dental composites containing CHX@MSN showed a more sustainable, controlled release of CHX for 16 days as compared with that of the composite with directly mixed CHX. The latter had a high release rate in the initial 2 hr, after which the release quickly diminished (Fig. 2A) and the cumulative release leveled off after one day (Fig. 2B). This may be caused by the quick dissolution and depletion of CHX aggregates on the composite surface. Except for the initial burst release, the composite with directly mixed CHX had a CHX release rate lower than that with CHX@MSN. Furthermore, the CHX aggregates embedded inside the hydrophobic resin matrix were unable to release from the composites. Therefore, the CHX release rate decreased sharply after the first few hours, even though more than 96% CHX remained in the composites. In contrast, MSN seemed to provide a better pathway and reservoir for sustainable release of CHX over a long time. Even after 16 days, about 90% CHX remained within composites. Although we have tested the release of CHX for only a short period of time, we can predict that this release of CHX from MSN-containing composites can last for an extended period of time.

Sustainable release for a prolonged period of time is beneficial to cariogenic biofilm control (Chen *et al.*, 2010). To achieve a continuous sustained release of the antibacterial agent, it is advantageous for an antibacterial dental composite to have the capability of absorbing or recharging CHX from surrounding medium—for example, from a mouthrinse or varnish containing CHX—to re-invigorate its antimicrobial activity once the loaded antimicrobial agents have been exhausted. The specimens were recharged with 0.2% CHX aqueous solution at days 2, 7, and 12. It is clear that composites directly mixed with CHX have rapid release for the first day after each recharge, and lower releasing quantity thereafter (between days 4 and 7, days 9 and 12, and days 14 and 17) than those with CHX@MSN, suggesting that larger surface voids on directly mixed composites have shorter CHX retention time. In all 3 repeating recharges, the composites with CHX@MSN exhibited higher cumulative release of CHX after recharge, indicative of a potential prolonged antimicrobial effect. The mesopores on MSN provide a permanent “sponge” that can be recharged repeatedly with antimicrobial agents, providing a new opportunity for long-term antimicrobial dental composite applications with recharge and release on demand.

Surface integrity of the composite after CHX release is of clinical importance. To test such an effect, we scanned the same disc with CHX or CHX@MSN before and after water immersion. Surface roughness (S_a) is a measure of surface texture. Before water immersion, both composites with CHX and CHX@MSN had similar surface roughnesses. After 2-week water immersion, the surface of the composite with CHX@MSN became only slightly coarser. In contrast, numerous voids with depths of more than 5 μm appeared on the surfaces of composites with directly mixed 5% CHX, causing a dramatic change in surface roughness. A rough surface usually wears more quickly and has higher friction coefficients than a smooth surface. In this study, the composites with CHX@MSN had better wear resistance (less wear), which is in agreement with results reported previously (Luo *et al.*, 1998).

Figure 3. SEM of composites with 5% CHX@MSN (A) and 5% directly mixed CHX (B) after water immersion. Wear grooves and surface roughness on composites with 5% CHX@MSN (surface roughness: $S_a = 0.344 \pm 0.050^a$ before water immersion, and 0.410 ± 0.099^a after water immersion) (C), and directly mixed 5% CHX ($S_a = 0.311 \pm 0.060^a$ before water immersion, and 1.113 ± 0.055^b after water immersion) (D).

The antibacterial efficacy of the composites was evaluated by 2 methods: planktonic bacterial growth and biofilm tests. First, the planktonic bacterial growth of *S. mutans* and *L. casei*, two major cariogenic bacteria, was monitored with Bioscreen C™ (Wen *et al.*, 2010; Xu *et al.*, 2012), with the composite ring specimens placed at the bottoms of honeycomb plate wells. The control composite had significant growth of both bacteria [increasing in optical density (OD)], while little or no growth was measured after 24 hr for the cured composites containing 5% CHX or CHX@MSN for both bacteria.

Second, the bacterial biofilms were grown on the experimental and control composites. Relative to the control, *S. mutans* on the composite containing CHX@MSN also had an altered surface structure, a likely result of cell membrane damage or cell death caused by CHX. One critical factor contributing to biofilm inhibition is the retention time that antimicrobial agents can stay on the restoration or teeth. The traditional mouthrinse or varnish raises a concern of time interval between applications, which results in reduced concentration of antimicrobial agents over

time between doses. Inconsistent and insufficient application of the antimicrobial agents may allow cariogenic bacteria to recolonize/survive and accumulate on the tooth or restoration surface. Our results suggest that the sustainable release of CHX from the CHX@MSN-loaded composites can provide a consistent amount of CHX—for example, at or above the minimum inhibitory concentration of 0.25–1.0 $\mu\text{g/mL}$ for *S. mutans* (Järvinen *et al.*, 1993)—over a prolonged period of time, which may enhance inhibitory effects against bacterial attachment and biofilm formation and minimize potential side-effects.

In summary, we have presented a type of new dental composite consisting of MSN to encapsulate or recharge and sustainably release antimicrobial agents, such as CHX, without compromising mechanical strength, surface esthetics, and surface integrity of the material. This technique is particularly useful for those antimicrobial agents or drugs that are poorly dispersed in polymers or composites, and for those hydrophobic polymers or composites containing antimicrobial agents or drugs for release. It has opened up opportunity for the application of nanotechnology in developing

Figure 4. Planktonic growth curves of *S. mutans* (A) and *L. casei* (B) in the presence of the composites containing the same amount of CHX. SEM images of *S. mutans* biofilms grown on control composite (C) and composite with 5% CHX@MSN (D).

new and antimicrobial dental composites. We expect this method to be a general approach for antimicrobial agent release in dental materials and other biomaterials where antimicrobial function is required. Furthermore, we must acknowledge that the oral environment is complicated, since temperature, pH values, oral bacteria, and the volume and compositions of fluids fluctuate frequently. The release and antibacterial activity of these materials need to be further optimized under more clinically relevant conditions.

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REFERENCES

Achong RA, Briskie DM, Hildebrandt GH, Feigal RJ, Loesche WJ (1999). Effect of chlorhexidine varnish mouthguards on the levels of

- selected oral microorganisms in pediatric patients. *Pediatr Dent* 21:169-175.
- Anusavice KJ, Zhang NZ, Shen C (2006). Controlled release of chlorhexidine from UDMA-TEGDMA resin. *J Dent Res* 85:950-954.
- Bekhuis T (2011). Chlorhexidine varnish may prevent dental caries in children and adolescents. *J Evid Based Dent Pract* 11:84-86.
- Bitoun JP, Nguyen AH, Fan Y, Burne RA, Wen ZT (2011). Transcriptional repressor Rex is involved in regulation of oxidative stress response and biofilm formation by *Streptococcus mutans*. *FEMS Microbiol Lett* 320:110-117.
- Bowen RL, Reed LE (1976). Semiporous reinforcing fillers for composite resins: I. Preparation of provisional glass formulations. *J Dent Res* 55:738-747.
- Carpenter AW, Reighard KP, Saavedra JE, Schoenfish MH (2013). O²-Protected diazeniumdiolate-modified silica nanoparticles for extended nitric oxide release from dental composites. *Biomater Sci* 1:456-459.
- Carriazo D, Arco MD, Fernandez A, Martin C, Rives V (2010). Inclusion and release of fenbuphen in mesoporous silica. *J Pharm Sci* 99:3372-3380.
- Chen F, Rice KC, Liu XM, Reinhardt RA, Bayles KW, Wang D (2010). Triclosan-loaded tooth-binding micelles for prevention and treatment of dental biofilm. *Pharm Res* 27:2356-2364.
- Cheng L, Weir MD, Xu HH, Kraigsley AM, Lin NJ, Lin-Gibson S, *et al.* (2012). Antibacterial and physical properties of calcium-phosphate and calcium-fluoride nanocomposites with chlorhexidine. *Dent Mater* 28:573-583.
- Deligeorgi V, Mjör IA, Wilson NH (2001). An overview of reasons for the placement and replacement of restorations. *Prim Dent Care* 8:5-11.
- Imazato S (2009). Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. *Dent Mater J* 28:11-19.

- Izquierdo-Barba I, Vallet-Regi M, Kupferschmidt N, Terasaki O, Schmidtchen A, Malmsten M (2009). Incorporation of antimicrobial compounds in mesoporous silica film monolith. *Biomaterials* 30:5729-5736.
- Järvinen H, Tenovou J, Huovinen P (1993). In vitro susceptibility of *Streptococcus mutans* to chlorhexidine and six other antimicrobial agents. *Antimicrob Agents Chemother* 37:1158-1159.
- Jedrychowski JR, Caputo AA, Kerper A (1983). Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. *J Oral Rehabil* 10:373-381.
- Jokstad A, Bayne S, Blunck U, Tyas M, Wilson N (2001). Quality of dental restorations - FDI Commission Project 2-95. *Int Dent J* 51:117-158.
- Leung D, Spratt DA, Pratten J, Gulabivala K, Mordan NJ, Young AM (2005). Chlorhexidine-releasing methacrylate dental composite materials. *Biomaterials* 26:7145-7153.
- Liong M, France B, Bradley KA, Zink JJ (2009). Antimicrobial activity of silver nanocrystals encapsulated in mesoporous silica nanoparticles. *Adv Mater* 21:1684-1689.
- Luo JH, Lannutti JJ, Seghi RR (1998). Effect of filler porosity on the abrasion resistance of nanoporous silica gel/polymer composites. *Dent Mater* 14:29-36.
- Mjör IA, Moorhead JE, Dahl JE (2000). Reasons for replacement of restorations in permanent teeth in general dental practice. *Int Dent J* 50:361-366.
- Petkar KC, Chavhan SS, Agatonovik-Kustrin S, Sawant KK (2011). Nanostructured materials in drug and gene delivery: a review of the state of the art. *Crit Rev Ther Drug Carrier Syst* 28:101-164.
- Praveen S, Sun Z, Xu J, Patel A, Wei Y, Ranade R, et al. (2006). Compression and aging properties of experimental dental composites containing mesoporous silica as fillers. *Mol Cryst Liq Cryst* 448:223-231.
- Raso EM, Cortes ME, Teixeira KL, Franco MB, Mohallem ND, Sinisterra RD (2010). A new controlled release system of chlorhexidine and chlorhexidine:βcd inclusion compounds based on porous silica. *J Incl Phenom Macro* 67:159-168.
- Riggs PD, Braden M, Patel M (2000). Chlorhexidine release from room temperature polymerising methacrylate systems. *Biomaterials* 21:345-351.
- Samuel SP, Li S, Mukherjee I, Guo Y, Patel AC, Baran G, et al. (2009). Mechanical properties of experimental dental composites containing a combination of mesoporous and nonporous spherical silica as fillers. *Dent Mater* 25:296-301.
- Slot DE, Vaandrager NC, Van Loveren C, Van Palenstein Helderman WH, Van der Weijden GA (2011). The effect of chlorhexidine varnish on root caries: a systematic review. *Caries Res* 45:162-173.
- Wen ZT, Burne RA (2002). Functional genomics approach to identifying genes required for biofilm development by *Streptococcus mutans*. *Appl Environ Microbiol* 68:1196-1203; published erratum in *Appl Environ Microbiol* 69:722 (2003).
- Wen ZT, Burne RA (2004). LuxS-mediated signaling in *Streptococcus mutans* is involved in regulation of acid and oxidative stress tolerance and biofilm formation. *J Bacteriol* 186:2682-2691.
- Wen ZT, Yates D, Ahn SJ, Burne RA (2010). Biofilm formation and virulence expression by *Streptococcus mutans* are altered when grown in dual-species model. *BMC Microbiol* 10:111.
- Xu X, Ling L, Wang R, Burgess JO (2006). Formulation and characterization of a novel fluoride-releasing dental composite. *Dent Mater* 22:1014-1023.
- Xu X, Wang Y, Liao S, Wen Z, Fan Y (2012). Synthesis and characterization of antibacterial dental monomers and composites. *J Biomed Mater Res B Appl Biomater* 100:1151-1162.
- Zhao D, Feng J, Huo Q, Melosh N, Fredrickson GH, Chmelka BF, et al. (1998). Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 Ångström pores. *Science* 279:548-552.