M.D. Weir¹, L.C. Chow^{1,2},
and H.H.K. Xu^{1*}

¹Biomaterials & Tissue Engineering Division, Department of Endodontics, Prosthodontics and Operative Dentistry, University of Maryland School of Dentistry, Baltimore, MD 21201, USA; and ²Paffenbarger Research Center, American Dental Association Foundation, National Institute of Standards & Technology, Gaithersburg, MD, USA; *corresponding author, hxu@umaryland.edu

J Dent Res 91(10):979-984, 2012

ABSTRACT

Secondary caries remains the main problem limiting the longevity of composite restorations. The objective of this study was to investigate the remineralization of demineralized human enamel *in vitro* via a nanocomposite containing nanoparticles of amorphous calcium phosphate (NACP). NACP were synthesized by a spray-drying technique and incorporated into a dental resin. First, caries-like subsurface enamel lesions were created *via* an acidic solution. Then, NACP nanocomposite or a commercial fluoride-releasing control composite was placed on the demineralized enamel, along with control enamel without a composite. These specimens were then treated with a cyclic demineralization/remineralization regimen for 30 days. Quantitative microradiography showed typical enamel subsurface demineralization before cyclic demineralization/remineralization treatment, and significant remineralization in enamel under the NACP nanocomposite after the demineralization/remineralization treatment. The NACP nanocomposite had the highest enamel remineralization (mean $\pm$ SD; $n = 6$) of $21.8 \pm 3.7\%$, significantly higher than the $5.7 \pm 6.9\%$ for fluoride-releasing composite ($p < 0.05$). The enamel group without composite had further demineralization of $-26.1 \pm 16.2\%$. In conclusion, a novel NACP nanocomposite was effective in remineralizing enamel lesions *in vitro*. Its enamel remineralization was 4-fold that of a fluoride-releasing composite control. Combined with the good mechanical and acid-neutralization properties reported earlier, the new NACP nanocomposite is promising for remineralization of demineralized tooth structures.

KEY WORDS: dental nanocomposite, calcium phosphate nanoparticles, human enamel, lesion remineralization, contact microradiography, caries inhibition.

DOI: 10.1177/0022034512458288

Received March 27, 2012; Last revision June 28, 2012; Accepted July 24, 2012

© International & American Associations for Dental Research

Remineralization of Demineralized Enamel via Calcium Phosphate Nanocomposite

INTRODUCTION

In the USA, 166 million dental restorations were placed in 2005 (Beazoglou *et al.*, 2007). Composites are increasingly used because of their esthetics and direct-filling capability (Ruddell *et al.*, 2002; Xu *et al.*, 2006; Ferracane, 2006; Drummond, 2008; Samuel *et al.*, 2009). Advances in fillers and polymers have improved the restorations (Watts *et al.*, 2003; Imazato, 2009; Spencer *et al.*, 2010; Ferracane, 2011). Nonetheless, half of restorations fail within 10 years, replacing them consumes 50 to 70% of the dentist's time, and secondary caries is the main reason for replacement (Mjör *et al.*, 2000; Jokstad *et al.*, 2001; Sakaguchi 2005; Ferracane, 2011; Demarco *et al.*, 2012).

Composites containing calcium phosphate (CaP) particles are promising for combating caries. These composites have been shown to release Ca and P ions and remineralize tooth lesions *in vitro* (Dickens *et al.*, 2003; Langhorst *et al.*, 2009). However, traditional CaP composites used particle sizes of 1 to 55 μm and had low mechanical properties that were inadequate for bulk restoratives (Dickens *et al.*, 2003; Langhorst *et al.*, 2009). Recent studies have reported novel nanocomposites which contained CaP and CaF_2 nanoparticles with sizes of about 50 to 100 nm (Xu *et al.*, 2010a). Nanoparticles of amorphous calcium phosphate (NACP) were synthesized with a mean particle size of 116 nm (Xu *et al.*, 2011). Nanocomposites containing CaP nanoparticles are advantageous because of the small size and high surface area of the nanoparticles (Xu *et al.*, 2010a). A previous study showed that the NACP nanocomposite had mechanical properties 2-fold those of traditional CaP composites (Xu *et al.*, 2011). The NACP nanocomposite neutralized acid attacks, while commercial controls failed to neutralize the acid (Moreau *et al.*, 2011). In addition, composite containing CaP nanoparticles released substantially more ions than that with micrometer-sized particles at the same filler level (Xu *et al.*, 2007), and CaP nanocomposite possessed much higher strength, fracture toughness, and wear resistance than traditional CaP composites (Xu *et al.*, 2010a). However, tooth lesion remineralization *via* a NACP nanocomposite has not been reported. Therefore, the next step is to show that the new nanocomposite, while possessing improved physical/mechanical properties, can indeed also remineralize tooth lesions.

The objective of this study was to investigate human enamel lesion remineralization *in vitro* via a NACP nanocomposite. Extracted molars were used, and enamel demineralization was created to be microradiographically similar to subsurface lesions *in vivo*. The NACP nanocomposite was placed on the top of demineralized enamel, in comparison with a commercial fluoride-releasing composite control, and a second control group in which no composite was placed on enamel. It was hypothesized that: (1) the new NACP nanocomposite would successfully regenerate the mineral lost in enamel due to acid attack; (2) enamel remineralization *via* the NACP nanocomposite would be higher than

that *via* a commercial fluoride-releasing composite; and (3) both composites would produce more remineralization than enamel without the NACP or fluoride-releasing composite.

MATERIALS & METHODS

A spray-drying technique was used to synthesize NACP as previously described (Xu *et al.*, 2011), which yielded NACPs with a mean size of 116 nm (Xu *et al.*, 2011). The resin consisted of ethoxylated bisphenol A dimethacrylate (EBPADMA = 62.8%; all % values are mass fractions), triethylene glycol dimethacrylate (TEGDMA = 23.2%), 2-hydroxyethyl methacrylate (HEMA = 10.4%), and methacryloyloxyethyl phthalate (MEP = 2.6%), photo-activated with 0.2% camphorquinone and 0.8% ethyl 4-*N,N*-dimethylaminobenzoate (Langhorst *et al.*, 2009). The NACP nanocomposite had 40% NACP and 20% glass particles. Barium-boroaluminosilicate glass particles (median diameter = 1.4 μ m, Caulk/Dentsply, Milford, DE, USA) were silanized with 4% 3-methacryloxypropyltrimethoxysilane and 2% *n*-propylamine. A commercial fluoride-releasing nanocomposite (Heliomolar, Ivoclar, Mississauga, ON, Canada) was used as control. Heliomolar was filled with 66.7% of silica and ytterbium-trifluoride nanoparticles of 40 to 200 nm.

Caries-free human molars were collected from the School of Dentistry clinics according to a protocol approved by the University of Maryland. Teeth were disinfected in a 0.005% promodyne solution for 4 hrs and stored at 4°C in distilled water until use. Each tooth was cut with a diamond blade (Buehler, Lake Bluff, IL, USA) into sections of approximately 200 μ m in thickness, then polished to a thickness of 110 to 130 μ m (Schmuck and Carey, 2010). For alignment of the “Before” and “After” microradiographic images, transmission electron microscopy (TEM) grids were adhered to the enamel sections. The sections were embedded in epoxy. The epoxy block holding the tooth section was polished until the enamel surface was exposed.

We created caries-like subsurface enamel lesions by immersing each tooth section-epoxy block, with the polished enamel surface exposed, in 2 mL of an acidic solution for 72 hrs at 37°C. This acidic solution consisted of 8.7 mmol/L CaCl₂, 8.7 mmol/L KH₂PO₄, 0.05 ppm F from NaF, and 75 mmol/L acetic acid (pH = 4.0). This solution had been demonstrated, in a previous study, of being capable of producing subsurface enamel demineralization in 3 days (Langhorst *et al.*, 2009). This demineralized state of enamel sections was designated as the “Before” state, because it preceded the placement of a composite to cover the enamel for cyclic demineralization/remineralization treatment. The “After” state was after the 30-day cyclic demineralization/remineralization treatment, as described below.

Each enamel section was sandwiched between parafilm and glass slides (Fig. 1A) (Langhorst *et al.*, 2009). The demineralized enamel edge was located approximately 1.5 mm below the edge of glass slides. Composite paste was placed on demineralized enamel to a thickness of 1.5 mm. Two composites were tested: NACP nanocomposite and Heliomolar. The third group had no composite on enamel (no-composite control). Six samples were tested for each group (n = 6). Each sample with composite was photo-polymerized for 60 sec (Triad-2000, Dentsply, York, PA, USA). The irradiance of Triad-2000 was previously measured at

the specimen position, by means of a radiometer, to be 35 mW/cm² (Stansbury and Dickens, 2001). The depth-of-cure of NACP nanocomposite and Heliomolar was measured (Jung *et al.*, 2001). Each paste was placed in a Teflon mold (diameter = 3.5 mm, thickness = 5 mm), clamped on one side to a glass slide, and to opaque Teflon on the other side. The glass slide was irradiated *via* Triad-2000 for 1 min. Specimens were demolded, and uncured paste was removed with a razor blade. The cured specimen thickness was measured, and the thicknesses of 4 specimens (n = 4) were averaged to be the depth-of-cure (Jung *et al.*, 2001).

The demineralizing solution consisted of: 3.0 mmol/L CaCl₂, 1.8 mmol/L K₂HPO₄, 0.1 mol/L lactic acid, and 1% carboxymethylcellulose (pH = 4.0) (Langhorst *et al.*, 2009). The remineralizing solution consisted of 1.2 mmol/L CaCl₂, 0.72 mmol/L K₂HPO₄, 2.6 μ mol/L F, and 50 mmol/L HEPES buffer (pH = 7.0). The demineralization/remineralization solutions were used to simulate oral fluid conditions (Langhorst *et al.*, 2009). Each day, specimens were immersed in 20 mL of fresh demineralizing solution for 1 hr, and 20 mL of remineralizing solution for 23 hrs at 37°C, according to previous studies (Chow *et al.*, 1992; Skrtic *et al.*, 1996; Langhorst *et al.*, 2009). This was repeated for 30 days, which created the “After” state in enamel. The demineralization treatment *in vitro* was somewhat more aggressive than what is typical *in vivo*. The reason for the use of an aggressive acid attack was to complete the experiments in a reasonable period of time, while quickly accumulating the damaging effects of acid attacks during years of restoration service *in vivo* (Langhorst *et al.*, 2009). The 1-hour duration in the demineralization solution approximates the accumulated acid challenge times in a 24-hour period (Chow *et al.*, 1992; Langhorst *et al.*, 2009). It should be noted that individuals may have more or fewer acid challenges than those recorded in the 1-hour total time, depending on dietary habits and biofilm compositions.

Mineral profiles of each enamel section were measured *via* quantitative analysis of contact microradiographs (Chow *et al.*, 1992; Langhorst *et al.*, 2009). Microradiographs before and after cyclic demineralization/remineralization were produced on holographic film (Integraf, Kirkland, WA, USA) exposed for 30 min to Cu-K α radiation (Faxitron, Hewlett Packard, McMinnville, OR, USA). Images were captured with an Evolution-MP5.0 digital microscope camera (Media Cybernetics, Silver Spring, MD, USA) with 12-bit grayscale values, at an intensity resolution of 4,096 gray levels and a spatial resolution of 1.4 μ m/pixel (Schmuck and Carey, 2010). Digitized images were analyzed with ImageJ software (NIH, Bethesda, MD, USA). Mineral profiles were aligned *via* TEM grids, and normalized by the black background being set to 0% and sound enamel to 100% of mineral density. An example of a “before” enamel lesion is shown in Fig. 1B, and the mineral profile is shown in Fig. 1C. Mineral loss, ΔZ , was calculated as the area between the mineral profile and lines ab and bc (Chow *et al.*, 1992; Dickens *et al.*, 2003; Langhorst *et al.*, 2009). ΔZ was obtained for each “Before” and “After” mineral profile in the same area of the same lesion. Remineralization in the enamel lesion that occurred during cyclic demineralization/remineralization is calculated as:

$$\text{Remineralization } R = (\Delta Z_{\text{Before}} - \Delta Z_{\text{After}}) / \Delta Z_{\text{Before}}$$

Figure 1. Enamel demineralization/remineralization treatment. (A) Schematic of tooth section between 2 glass slides with composite to be used in demineralization/remineralization treatment. (B) Example of enamel lesion before the demineralization/remineralization treatment, illustrating the method and the various features on the image. (C) Mineral profile. Mineral loss ΔZ was calculated as the area between the mineral profile and lines ab and bc. The leading portion of the profile is sloped rather than vertical, because the front face of the thin enamel section is usually not perfectly square with the upper and lower surfaces of the section. It is nearly impossible to cut a section that is perfectly perpendicular to the original tooth surface. Hence, the leading portion of the profile in (C) can be visualized as the front edge of the tooth section. These features are similar to those of previous studies (Chow *et al.*, 1992; Dickens *et al.*, 2003; Langhorst *et al.*, 2009).

where ΔZ_{Before} is mineral loss in enamel section before cyclic demineralization/remineralization, and ΔZ_{After} is mineral loss in the same area of the lesion after cyclic demineralization/remineralization (Dickens *et al.*, 2003; Langhorst *et al.*, 2009).

Each enamel section generated 12 sets of “Before/After” curves at 12 different areas of the lesion, to provide the average value for that enamel lesion. Each set of “Before/After” curves generated one $\Delta Z_{\text{Before}} - \Delta Z_{\text{After}}$ value. There were 12 $\Delta Z_{\text{Before}} -$

Figure 2. Representative examples of microradiographs of enamel lesions. “Before” refers to the initial enamel demineralization created in the acidic solution. “After” means after the 30-day cyclic demineralization/remineralization regimen. (A) There was successful remineralization in enamel under the NACP nanocomposite after 30 days. (B) There was little remineralization in enamel with fluoride-releasing commercial composite control. (C) There was no remineralization in the control group without a composite. Arrows in (A) point to a twist in the TEM grid, indicating that the “Before” and “After” images were taken in the same area. Arrows in (B) point to the same wedge for “Before” and “After”. Arrows in (C) indicate the same specimen edge for “Before” and “After”, confirming that the “Before” and “After” images were taken in the same area.

ΔZ_{After} values for each enamel section, yielding the mean $\Delta Z_{\text{Before}} - \Delta Z_{\text{After}}$ value which in turn yielded the mean R value to represent

Figure 3. Representative mineral profiles of human enamel lesions before and after the 30-day cyclic demineralization/remineralization for: (A) no-composite control, (B) the NACP nanocomposite on demineralized enamel, and (C) the fluoride-releasing commercial composite on demineralized enamel. Enamel lesions without a composite showed further mineral loss. Enamel lesions with the NACP nanocomposite had a greater increase in mineral content, while those with fluoride composite had a smaller increase in mineral content.

that enamel section. Each group had 6 such R values ($n = 6$), which were used in one-way analysis of variance (ANOVA) to detect the significant effects of the groups. Tukey's multiple comparison was used at $p = 0.05$.

RESULTS

The depth-of-cure (mean $\pm$ SD; $n = 4$) was 3.2 ± 0.2 mm for the NACP nanocomposite, and 3.0 ± 0.2 mm for Heliomolar. They did not differ significantly from each other ($p > 0.1$).

Microradiographs showed typical enamel lesions before the cyclic demineralization/remineralization treatment (Fig. 2). There was significant remineralization in enamel under the NACP nanocomposite after demineralization/remineralization

Figure 4. Remineralization (mean $\pm$ SD; $n = 6$) of human enamel lesions in the 30-day cyclic demineralization/remineralization regimen: $R = (\Delta Z_{\text{Before}} - \Delta Z_{\text{After}}) / \Delta Z_{\text{Before}}$. Enamel lesions with the NACP nanocomposite had the highest remineralization, $21.8 \pm 3.7\%$; the fluoride-releasing commercial composite produced $5.7 \pm 6.9\%$ remineralization; enamel sections without a composite had $-26.1 \pm 16.2\%$, which means further demineralization during the 30-day cyclic demineralization/remineralization treatment. These 3 values are significantly different from each other ($p < 0.05$).

treatment. In contrast, enamel lesions under fluoride-releasing composite and for no-composite control had no noticeable remineralization (Figs. 2B, 2C).

Demineralized enamel without composite showed further mineral loss during cyclic demineralization/remineralization (Fig. 3A). In contrast, enamel with the NACP nanocomposite showed a significant increase in mineral content after cyclic demineralization/remineralization (Fig. 3B). There was a much smaller increase in mineral content after demineralization/remineralization for the fluoride-releasing group (Fig. 3C).

The remineralization values (Fig. 4) showed that enamel lesions with the NACP nanocomposite had the highest remineralization (mean $\pm$ SD; $n = 6$) of $21.8 \pm 3.7\%$, much higher than the $5.7 \pm 6.9\%$ for the fluoride-releasing composite. Enamel without composite had further demineralization of $-26.1 \pm 16.2\%$. These values are different from each other ($p < 0.05$).

DISCUSSION

Traditional CaP composites had relatively low mechanical properties that were “inadequate to make these composites acceptable as bulk restoratives” (Skrtic *et al.*, 2000). It was suggested that CaP composites “could serve as a restoration-supporting lining materials” (Dickens *et al.*, 2003), and that ACP composites could be “useful as pit and fissure sealants” (Skrtic *et al.*, 2000). Currently available posterior/hybrid composites can be

used in stress-bearing restorations, but they do not release Ca and P ions; conversely, composites that release Ca and P ions cannot be used in stress-bearing restorations. Therefore, there is a need to develop new composites with a combination of load-bearing properties and CaP ion release and remineralization capability. Nanotechnology presents a promising route to the development of a new generation of composites with a combination of load-bearing and remineralization capabilities. Recent studies focused on the first half of this picture and synthesized new nanocomposites that possessed greatly improved strength, fracture toughness, and wear resistance compared with those of traditional CaP composites (Xu *et al.*, 2007, 2010a,b). The present study focused on the second half of this picture, remineralization capability, and showed that human enamel lesions were indeed remineralized *in vitro*, and that the lost mineral was regenerated *via* the NACP nanocomposite.

Caries is a dietary carbohydrate-modified bacterial infectious disease (Featherstone, 2004; ten Cate, 2006). Acidogenic bacteria ferment carbohydrates and produce organic acids, with biofilm pH dropping into the cariogenic region (Deng and ten Cate, 2004). To simulate *in vivo* pH cycles, previous studies tested cyclic demineralization/remineralization *in vitro* (Chow *et al.*, 1992; Langhorst *et al.*, 2009), with demineralization solution at pH 4, and remineralization solution at pH 7. Three points should be noted. First, pH 4 for the demineralization solution has been used in many studies as an accelerated model (*e.g.*, Chow *et al.*, 1992; Skrtic *et al.*, 1996; Langhorst *et al.*, 2009). While pH 4 is near the lower end of plaque pH, it is not unrealistic. For example, a previous study showed that *S. mutans* plaque pH reached 3.7 ± 0.3 (Kashket *et al.*, 1989). Another study showed that oral biofilms with lactic acid could reach pH 4 (Wijeyeweera *et al.*, 1989). Second, the 1-hour immersion in pH 4 demineralization solution thermodynamically favors demineralization; remineralization of enamel occurs during the 23-hour immersion in the remineralization solution at pH 7. Therefore, the fact that the NACP nanocomposite could release more ions at pH 4 (Xu *et al.*, 2011) indicates that it could reduce enamel demineralization; it does not mean that it caused remineralization at pH 4. Third, the present study had a positive control (fluoride-releasing composite) tested under the same accelerated demineralization/remineralization regimen. Therefore, the continued release of ions from the nanocomposite contributed to enamel remineralization, consistent with the fact that the control without CaP ions achieved little remineralization. However, the acid neutralization properties of the nanocomposite (Moreau *et al.*, 2011) likely also played a role in protection from demineralization. During the demineralization steps at pH 4, a localized higher pH near the enamel-nanocomposite interface due to acid neutralization would significantly reduce enamel mineral loss. While CaP ions and acid neutralization are both beneficial for remineralization and caries inhibition, further study is needed to separate the effect of CaP ions from the effect of local pH due to acid neutralization.

Calcium phosphate ions can help prevent enamel demineralization. For example, dentifrice with calcium was more effective than SiO₂ in reducing enamel demineralization (Cury *et al.*, 2003). Calcium in mouthrinses or chewing-gums remineralized enamel lesions (Reynolds *et al.*, 2003). Dentin remineralization was achieved *via* solutions containing calcium and phosphate

(Hara *et al.*, 2008). A previous study showed that CaP ion release from the NACP nanocomposite was greatly increased when pH was decreased from 7 to 4 (Xu *et al.*, 2011). The NACP nanocomposite was responsive and increased the ion release at cariogenic pH4, when these ions were most needed to inhibit caries. Similarly, Heliomolar had a cumulative fluoride release at 84 days of 7 $\mu\text{g}/\text{cm}^2$ at pH 4, significantly higher than 2 $\mu\text{g}/\text{cm}^2$ at pH 7 (Xu *et al.*, 2010b). Heliomolar remineralized enamel lesions by 5.7% in the present study, while control enamel without a composite had further demineralization of -26%. During the time period of 30 days, the NACP nanocomposite had enamel remineralization that was 4-fold that of the fluoride-releasing control. The effective remineralization of this study, coupled with the acid neutralization and good mechanical properties reported earlier (Moreau *et al.*, 2011; Xu *et al.*, 2011), indicates that the NACP nanocomposite is promising for caries-inhibiting restorations. Further studies should incorporate antibacterial agents into the NACP nanocomposite, with biofilm inoculation, to investigate its dual remineralizing and antibacterial effects on caries inhibition.

In summary, a NACP nanocomposite was shown, for the first time, to effectively remineralize demineralized human enamel *in vitro*. Its remineralization was 4-fold that of a commercial fluoride-releasing composite in a 30-day cyclic demineralization/remineralization regimen. Combined with its previously reported strong mechanical and acid-neutralizing properties, the new NACP nanocomposite is promising for restorations that can remineralize demineralized tooth structures.

ACKNOWLEDGMENTS

We thank Drs. S. Takagi, G.E. Schumacher, A.A. Giuseppetti, B.D. Schmuck, C.M. Carey, K.M. Hoffman, and D. Skrtic for fruitful discussions. We are grateful to Esstech (Essington, PA, USA) and Ivoclar Vivadent (Amherst, NY, USA) for donating the materials. This study was supported by NIH R01DE17974 (HX) and the University of Maryland School of Dentistry. The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article. Certain commercial materials and equipment are identified to specify the experimental procedures. This does not imply recommendation or endorsement by NIST or the ADA.

REFERENCES

- Beazoglou T, Eklund S, Heffley D, Meiers J, Brown LJ, Bailit H (2007). Economic impact of regulating the use of amalgam restorations. *Public Health Rep* 122:657-663.
- Chow LC, Takagi S, Shih S (1992). Effect of a two-solution fluoride mouth-rinse on remineralization of enamel lesions *in vitro*. *J Dent Res* 71:443-447.
- Cury JA, Francisco SB, Simões GS, Cury AA, Tabchoury CP (2003). Effect of a calcium carbonate-based dentifrice on enamel demineralization *in situ*. *Caries Res* 37:194-199.
- Demarco FF, Correa MB, Cenci MS, Moraes RR, Opdam NJ (2012). Longevity of posterior composite restorations: not only a matter of materials. *Dent Mater* 28:87-101.
- Deng DM, ten Cate JM (2004). Demineralization of dentin by *Streptococcus mutans* biofilms grown in the constant depth film fermentor. *Caries Res* 38:54-61.

- Dickens SH, Flaim GM, Takagi S (2003). Mechanical properties and biochemical activity of remineralizing resin-based Ca-PO₄ cements. *Dent Mater* 19:558-566.
- Drummond JL (2008). Degradation, fatigue, and failure of resin dental composite materials. *J Dent Res* 87:710-719.
- Featherstone JD (2004). The continuum of dental caries – evidence for a dynamic disease process. *J Dent Res* 83(Spec Iss C):C39-C42.
- Ferracane JL (2006). Hygroscopic and hydrolytic effects in dental polymer networks. *Dent Mater* 22:211-222.
- Ferracane JL (2011). Resin composite—state of the art. *Dent Mater* 27:29-38.
- Hara AT, Karlinsey RL, Zero DT (2008). Dentine remineralisation by simulated saliva formulations with different Ca and Pi contents. *Caries Res* 42:51-56.
- Imazato S (2009). Bioactive restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. *Dent Mater J* 28:11-19.
- Jokstad A, Bayne S, Blunck U, Tyas M, Wilson N (2001). Quality of dental restorations. FDI Commision Projects 2-95. *Int Dent J* 51:117-158.
- Jung H, Friedl KH, Hiller KA, Haller A, Schmalz G (2001). Curing efficiency of different polymerization methods through ceramic restorations. *Clin Oral Invest* 5:156-161.
- Kashket S, Yaskell T, Lopez LR (1989). Prevention of sucrose-induced demineralization of tooth enamel by chewing sorbitol gum. *J Dent Res* 68:460-462.
- Langhorst SE, O'Donnell JN, Skrtic D (2009). *In vitro* remineralization of enamel by polymeric amorphous calcium phosphate composite: quantitative microradiographic study. *Dent Mater* 25:884-891.
- 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.
- Moreau JL, Sun L, Chow LC, Xu HH (2011). Mechanical and acid neutralizing properties and inhibition of bacterial growth of amorphous calcium phosphate dental nanocomposite. *J Biomed Mater Res B Appl Biomater* 98:80-88.
- Reynolds EC, Cai F, Shen P, Walker GD (2003). Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res* 82:206-211.
- Ruddell DE, Maloney MM, Thompson JY (2002). Effect of novel filler particles on the mechanical and wear properties of dental composites. *Dent Mater* 18:72-80.
- Sakaguchi RL (2005). Review of the current status and challenges for dental posterior restorative composites: clinical, chemistry, and physical behavior considerations. *Dent Mater* 21:3-6.
- Samuel SP, Li S, Mukherjee I, Guo Y, Patel AC, Baran GR, *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.
- Schmuck BD, Carey CM (2010). Improved contact x-ray microradiographic method to measure mineral density of hard dental tissues. *J Res Natl Inst Stand Technol* 115:75-83.
- Skrtic D, Antonucci JM, Eanes ED, Eichmiller FC, Schumacher GE (2000). Physicochemical evaluation of bioactive polymeric composites based on hybrid amorphous calcium phosphates. *J Biomed Mater Res* 53:381-391.
- Skrtic D, Hailer AW, Takagi S, Antonucci JM, Eanes ED (1996). Quantitative assessment of the efficacy of amorphous calcium phosphate/methacrylate composites in remineralizing caries-like lesions artificially produced in bovine enamel. *J Dent Res* 75:1679-1686.
- Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, *et al.* (2010). Adhesive/dentin interface: the weak link in the composite restoration. *Ann Biomed Eng* 38:1989-2003.
- Stansbury JW, Dickens SH (2001). Network formation and compositional drift during photo-initiated copolymerization of dimethacrylate monomers. *Polymer* 42:6363-6369.
- ten Cate JM (2006). Biofilms, a new approach to the microbiology of dental plaque. *Odontology* 94:1-9.
- Watts DC, Marouf AS, Al-Hindi AM (2003). Photo-polymerization shrinkage-stress kinetics in resin-composites: methods development. *Dent Mater* 19:1-11.
- Wijeyeweera RL, Kleinberg I (1989). Acid-base pH curves in vitro with mixtures of pure cultures of human oral microorganisms. *Arch Oral Biol* 34:55-64.
- Xu HH, Weir MD, Sun L (2007). Dental nanocomposites with Ca-PO₄ release: effects of reinforcement, dicalcium phosphate particle size and silanization. *Dent Mater* 23:1482-1491.
- Xu HH, Weir MD, Sun L, Moreau JL, Takagi S, Chow LC, *et al.* (2010a). Strong nanocomposites with Ca, PO₄ and F release for caries inhibition. *J Dent Res* 89:19-28.
- Xu HH, Moreau JL, Sun L, Chow LC (2010b). Novel CaF₂ nanocomposite with high strength and F ion release. *J Dent Res* 89:739-745.
- Xu HH, Moreau JL, Sun L, Chow LC (2011). Nanocomposite containing amorphous calcium phosphate nanoparticles for caries inhibition. *Dent Mater* 27:762-769.
- Xu X, Ling L, Wang R, Burgess JO (2006). Formation and characterization of a novel fluoride-releasing dental composite. *Dent Mater* 22:1014-1023.