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Effect of calcium phosphate nanocomposite on *in vitro* remineralization of human dentin lesions

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ABSTRACT

Objective. Secondary caries is a primary reason for dental restoration failures. The objective of this study was to investigate the remineralization of human dentin lesions *in vitro via* restorations using nanocomposites containing nanoparticles of amorphous calcium phosphate (NACP) or NACP and tetracalcium phosphate (TTCP) for the first time.

Methods. NACP was synthesized by a spray-drying technique and incorporated into a resin consisting of ethoxylated bisphenol A dimethacrylate (EBPADMA) and pyromellitic glycerol dimethacrylate (PMGDM). After restoring the dentin lesions with nanocomposites as well as a non-releasing commercial composite control, the specimens were treated with cyclic demineralization (pH 4, 1 h per day) and remineralization (pH 7, 23 h per day) for 4 or 8 weeks. Calcium (Ca) and phosphate (P) ion releases from composites were measured. Dentin lesion remineralization was measured at 4 and 8 weeks by transverse microradiography (TMR).

Results. Lowering the pH increased ion release of NACP and NACP-TTCP composites. At 56 days, the released Ca concentration in mmol/L (mean ± SD; $n = 3$) was (13.39 ± 0.72) at pH 4, much higher than (1.19 ± 0.06) at pH 7 ($p < 0.05$). At 56 days, P ion concentration was (5.59 ± 0.28) at pH 4, much higher than (0.26 ± 0.01) at pH 7 ($p < 0.05$). Quantitative microradiography showed typical subsurface dentin lesions prior to the cyclic demineralization/remineralization treatment, and dentin remineralization *via* NACP and NACP-TTCP composites after 4 and 8 weeks of treatment. At 8 weeks, NACP nanocomposite achieved dentin lesion remineralization (mean ± SD; $n = 15$) of (48.2 ± 11.0)%, much higher than (5.0 ± 7.2)% for dentin in commercial composite group after the same cyclic demineralization/remineralization regimen ($p < 0.05$).

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Significance. Novel NACP-based nanocomposites were demonstrated to achieve dentin lesion remineralization for the first time. These results, coupled with acid-neutralization and good mechanical properties shown previously, indicate that the NACP-based nanocomposites are promising for restorations to inhibit caries and protect tooth structures.

1. Introduction

Approximately 166 million tooth cavity restorations were placed in 2005 in the Unites States [1], with at least half of the posterior direct restorations using composites [2]. Composites are increasingly used because of their excellent esthetics and direct-filling capability [3–7]. Advances in resin compositions, filler particles and the resin–filler interface have improved the composite properties [8–11]. However, the lifetime of composite restorations is limited by inferior properties such as polymerization shrinkage/stress formation, fracture, abrasion and wear resistance, and marginal leakage [2,12–15]. Marginal leakage can result in the formation of secondary caries, the main reason for composite restoration failures [16–20].

A promising approach to combat caries is to use composites containing calcium phosphate (CaP) particles. These composites have been shown to release calcium (Ca) and phosphate (P) ions and remineralize tooth lesions *in vitro* [21–23], *in situ* in the oral environment [24], and *in vivo* in human volunteers [25]. Mineral growth in tooth lesions can be stimulated by increasing the calcium and phosphate concentrations within the lesion to levels greater than those existing in oral fluids. Indeed, enamel subsurface lesions were remineralized by a CPP-ACP solution [26]. In this approach, CPP-ACP was included in a sugar-free chewing gum to control dental caries *via* active remineralization and salivary stimulation. Additionally, ACP was added to sealants to release supersaturating levels of calcium and phosphate ions, driving the solution thermodynamics toward formation of apatite [22]. A drawback with previous CaP composites for dental restorations was that these composites used traditional CaP particles and had low mechanical properties, which were inadequate for bulk restoratives [21,22].

Recent studies reported novel nanocomposites containing CaP and CaF_2 nanoparticles with sizes of about 50–100 nm [27]. Nanoparticles of amorphous calcium phosphate (NACP) with a size of 116 nm were synthesized *via* a spray-drying technique [28]. Nanocomposites containing NACP are advantageous because of the small size and high surface area of the nanoparticles [27]. A previous study showed that the NACP nanocomposite had mechanical properties 2-fold those of traditional CaP composites [28]. The NACP nanocomposite neutralized acid attacks, while commercial controls failed to neutralize the acid [29]. In addition, composites containing CaP nanoparticles released substantially more ions than that with micrometer-sized particles at the same filler level [30], and CaP nanocomposites possessed much higher strength, fracture toughness, and wear resistance than traditional CaP composites [27]. Recently, NACP nanocomposites were shown to remineralize lesions in human enamel in an *in vitro* model [23]. Additionally, NACP nanocomposite was shown to reduce caries in enamel in a human *in-situ* model [24]. Enamel remineralization was partially enabled by the presence of residual seed mineral crystals, which resulted in apatite mineral formation from the diffusion of calcium and phosphate ions into the carious lesion [31]. However, the previous studies focused on enamel without testing the effect of NACP nanocomposite on dentin [23,24].

Dentin contains 70% carbonated apatite, 20% organic matrix (mostly collagen) and 10% water [31]. When dentin lesions form, the mineral phase is damaged and may be destroyed. As the carious attack progresses, the collagen fibers are exposed and degraded, leading to a decrease in the mechanical properties of dentin [32]. In demineralized dentin, unlike enamel, there are fewer residual mineral seed crystals present, which may make it more difficult to remineralize dentin compared to enamel. Clinically, treatment of carious dentin lesions is dependent on the depth of the lesion. In shallow to moderate lesions, the carious material can be completely removed and restored with composite, amalgam or glass ionomer. In asymptomatic deep lesions, where there is a risk of pulp exposure but restoration of tooth function is possible, partial removal of the carious dentin may be considered the clinically conservative approach. The treatment can involve an attempt to remineralize the demineralized dentin by either indirect pulp treatment or stepwise caries removal. In indirect pulp treatment, most of the carious lesion is removed and the finished cavity preparation is lined with a remineralizing material (calcium hydroxide, resin-modified glass ionomer, *etc.*) and the final restoration is placed to provide a good seal. Stepwise caries removal is a 2-step process, requiring removal of less carious dentin, followed by an interim placement of glass ionomer cement to aid in remineralization. After several months, remineralization is assessed and, if successful, a permanent restoration is placed [25]. While CaP nanocomposites were shown to release more Ca and P ions and possess much better mechanical properties than traditional CaP composites [23,24,27–30], the remineralization of dentin caries *via* nanocomposite containing NACP has yet to be reported.

Accordingly, the objective of this study was to investigate the remineralization of dentin lesions in human teeth *in vitro via* nanocomposites containing NACP and NACP plus micron-sized tetracalcium phosphate (TTCP) particles. It was hypothesized that: (1) the cyclic demineralization/remineralization regimen would fail to remineralize dentin lesions when restored with the commercial composite; (2) the new NACP and NACP-TTCP nanocomposites would successfully regenerate the mineral lost in dentin; (3) dentin