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Development, characterization and comparison of two strontium doped nano hydroxyapatite molecules for enamel repair/regeneration

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ABSTRACT

Objectives. Enamel damage resulting or arising from/associated with orthodontic treatment such as white spot lesions and surface deterioration after debonding brackets along with incipient carious lesions are considered problems not amenable for routine restorations due to its invasive nature. The present study was aimed at synthesizing and characterizing nHAp and 25 and 50 mol% strontium nHAp as a surface application modality for dental enamel remineralization/repair.

Methods. 25 and 50 mol% Sr nHAp was synthesized and characterized in comparison with custom made pure nHAp initially with the help of transmission and scanning electron microscopy as well as toxicological assessment. Further, comparative evaluation of these novel synthesized strontium substituted particles was assessed for its efficacy in repairing damaged enamel with the help of atomic force microscopy, scanning electron microscopy and micro indentation testing.

Results. There is increase in crystallinity and reduced particle size favoring dissolution and re-precipitation through small incipient carious lesions and soft white spot areas with 25% Sr-nHAp. Sr doped specimens showed more cell viability in comparison with pure nHAP make it less cytotoxic and hence a biologically friendly material which can be safely applied in patient's mouth. AFM images obtained from 25% and 50% Sr nHAp treated specimens clearly indicated increased roughness in surface topography and performed well with micro indentation test.

Significance. The novel synthesized Sr doped nHAp forms an improved treatment modality to tackle the long standing quest for solving the problem of enamel loss with incipient carious lesions and WSL from orthodontic procedures.

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1. Introduction

Orthodontic treatment leads to formation of White Spot lesions (WSL; areas with demineralized enamel) on the tooth surface, incidence rate of which range from 25% to 96%, when the oral hygiene habits are poor [1,2]. The components of the appliance and bonding materials often promote bacterial colonization in areas of poor oral hygiene, especially *Streptococcus mutans* and *Lactobacillus*, which indulge in acid production, pH change in oral cavity favoring diffusion of calcium and phosphorous ions out of enamel surface leading to demineralization process [3]. The mechanical and crystallographic studies on white spot lesions revealed around 10% loss in mineral content, making the area softer and prone to enamel caries. In severe cases, frank cavitations are seen which even require restorative intervention. It is reported that any tooth in the mouth can be affected by the process with most common ones being the anteriors comprising of maxillary lateral incisors and canines [4].

The second most common problem faced by orthodontic clinicians is the loss of surface enamel while debonding/removing orthodontic brackets. It is roughly estimated that debonding procedures will lead to loss of around 55.6 μm of surface enamel. The search for safe and efficient method of adhesive removal lead to introduction of wide array of materials such as use of hand scalers, tungsten carbide burs with low speed handpieces, sof-lex discs and special composite finishing systems with zirconia paste or slurry pumice. All the reported techniques produce different degree of polish but a fool-proof method is yet to introduce as some of these methods introduced abrasion anomalies with significant loss of enamel. Based on enamel surface index (ESI) and adhesive remnant index (ARI) scores, it has been reported that the composite burs create smoothest surface but have longer resin removal times, followed by tungsten-carbide burs and diamond burs [5,6]. Recent studies have shown association between enamel color change and resin tag depth suggesting that resins with shorter tag penetration produce less iatrogenic color change in enamel following treatment [7].

It is of relief that in some cases, the induced iatrogenic damage gets treated by the therapeutic action of the saliva, but in patients with dietary deficiencies or bad oral health, this damage becomes permanent and leads to teeth sensitivity and even caries. If salivary capacity is not enough for a spontaneous remineralization process, the corrective approaches can be initiated with fluoride treatment as the first line therapy [8]. A low dose daily approach with fluoridated tooth paste (1000–1500 ppm) or mouthrinse (0.05% sodium fluoride daily rinse) is a better approach than high dose fluoride (20,000–25,000 ppm) [9]. In more severe cases acid/pumice microabrasion, with the help of 18% hydrochloric acid in fine pumice, can be advocated [10]. In recent years, a great deal of research in this area is focused around application of calcium phosphate, their nanoparticles and hydroxyapatite, which has the same chemical composition as of tooth enamel ($Ca_{10}(PO_4)_6(OH)_2$). The synthetic process to prepare biomimetic nanostructured micro sized crystal clusters starts with the synthesis of biomimetic carbonate-hydroxyapatite nanocrystals (CHAs) having a nearly stoichiometric Ca/P molar ratio of about 1.6–1.7 in bulk and containing 4 ± 1 wt% of carbonate ions replacing prevalently phosphate groups. Also at nano scale, hydroxyapatite (nHAp) shows higher Ca^{2+} ion release rates and shows superior functional properties due to uniform grain size [11].

In comparison to the fluoride technology, nano hydroxyapatite (nHAp) use promises a long lasting solution to this problem without any side effects as seen in case of the former, such as gastrointestinal problems, chronic ailments and skeletal fluorosis [12,13]. Another benefit of nHAp is its ability to induce mineralization from within the teeth, which can be further enriched by natural therapy of the saliva, as opposed to fluoride which has been known to cause hyper mineralization of the surface layers and thus failing to strengthen the teeth from within [14]. Recently nHAp has been marketed in the form of tooth paste in view of its effects on repair of demineralizing lesions in enamel, tooth sensitivity and remineralization potential [15]. It is worth noting that the main drawbacks as far as nHAp is concerned are lack of strength, brittleness, high degree of crystallinity [16] and low solubility at neutral pH requiring an acidic pH to dissolve. It is reported that a wide range of ions, both cations and anions, may substitute into the structure of synthetic HAp, replacing calcium (Ca), phosphate, or hydroxyl ions [17]. The substitutions, such as carbonated hydroxyapatite and strontium substituted hydroxyapatite (above 10 mol% substitution), are more soluble than nHAp and brings in alterations in the degree of crystallinity and change some of the material properties, including phase stability, and reactivity.

Many researchers have tried replacing calcium (Ca^{2+}) ion with strontium ion (Sr^{2+}) in varied ratios to increase the acid reactivity of apatite, improvement in solubility and increased fluoride release successfully [18–21]. It is found that Sr-HAp (above 10 mol% substitution) is a more soluble material and its increased bioactivity, due to Sr^{2+} release, makes it more desirable *in vivo*.

In the present study an attempt has been made to synthesize nHAp with increased mol% substitution with strontium (25 and 50 mol% strontium incorporated nHAp) and characterize the product with respect to their application in dental enamel remineralization/repair. Basic physical properties of the above mentioned systems were analyzed and correlated to the possibilities for their final applications. Clinical utility of the synthesized samples were compared with the existing calcium based enamel re-mineralization product existing in the market.

2. Materials and methods

2.1. *Hydroxyapatite preparation*

Hydroxyapatite precursor powder was synthesized by wet chemical method involving precipitation of calcium phosphate from the aqueous solution of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, and ammonium dihydrogen orthophosphate (NH_4H_2PO4), in stoichiometric proportion (Ca/P = 1.67) maintaining the pH at 11 and temperature at 80 °C. The precipitate was aged for 24 h and washed in distilled water. The thick slurry was then spray-dried and calcined at