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Synthesis, Characterization, and Antibacterial Property of Strontium Substituted Hydroxyapatite Nanoparticles

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KEYWORDS

Nanoparticles, Orthodontics, strontium, hydroapatite, white spot lesion.

ABSTRACT:

Nanotechnology has emerged as a transformative force in medical disciplines, presenting innovative opportunities for enhancing therapeutic outcomes. This study delves into the integration of strontium-substituted hydroxyapatite nanoparticles (SrHA) into orthodontic composites, exploring their antimicrobial properties. Strontium, recognized for its roles in promoting cell proliferation and inhibiting bone resorption, is introduced into hydroxyapatite—a critical bioactive material in dentistry. The synthesis employs a chemical precipitation method, resulting in Sr-enriched HA nanoparticles with precise stoichiometry. Characterization techniques, including X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy, provide comprehensive insights into the resulting orthodontic composite.

Antimicrobial testing is conducted on SrHA-infused composites at concentrations of 0.5% and 1.0%, targeting S. aureus, S. mutans, and E. coli. The disc agar diffusion test reveals significant antimicrobial efficacy, with inhibition zones measuring 26-30 mm. Statistical analysis confirms concentration-dependent effectiveness against the tested bacteria.

The study underscores the potential of SrHA-infused composites in preventing white spot lesions, emphasizing their antimicrobial effects against S. mutans. Scanning electron microscopy exposes morphological shifts, indicating strontium-induced changes in particle size and shape. Energy-dispersive X-ray spectroscopy confirms strontium substitution within the nanoparticles.

In conclusion, the incorporation of SrHA nanoparticles into orthodontic composites showcases promising antimicrobial properties, hinting at their potential for preventing bacterial colonization and plaque formation. This research provides valuable insights into the synthesis, characterization, and antimicrobial efficacy of SrHA-infused orthodontic composites, contributing significantly to the ongoing advancement of dental biomaterials.

Introduction

Employee The incorporation of nanotechnology into several medical disciplines has significantly transformed treatment approaches in recent times, presenting unparalleled prospects for augmenting therapeutic results (1). The field of dentistry places emphasis on the maintenance of oral health and the regeneration of oral tissues, necessitating the use of materials that not only facilitate restoration but also encourage the inherent healing mechanisms inside the oral cavity (2). Strontium, which is known for its function in promoting cell proliferation, inhibiting bone resorption, and altering gene expression in osteoblastic cells, possesses a significant amount of promise for use in dental applications (3).

From a thermodynamic perspective, hydroxyapatite (HA), denoted as $Ca_{10}(PO4)6(OH)2$, stands out as the most enduring crystalline phase among calcium phosphate salts in bodily fluids (4). Recognized as the principal mineral in teeth and various calcified tissues,

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synthetic hydroxyapatite boasts remarkable bioactive properties, including osteoconductivity and cytocompatibility (5). In the realm of varied HA structures, there is substantial interest in biomedical applications focused on HA nanoparticles with precise stoichiometry and purity. These nanoparticles exhibit an ultrafine structure and high surface reactivity akin to the mineral structure found in bones (6). As biological HA manifests as plate-like crystals with nanoscale thickness, synthetic HA nanoparticles that parallel the characteristics of natural hard tissue minerals are considered optimal bioceramics for bone substitution and regeneration (7). The reduction in particle size to the nano-level not only impedes demineralization but also enhances densification and sintering properties, thereby preventing issues like micro-cracks (8). The superior cell proliferation and differentiation observed in HA nanoparticles are attributed to their enhanced surface functional characteristics compared to micro-sized particles. The synthesis of non-stoichiometric HA involves the incorporation of various ionic substitutions into the crystal lattice (8). Reports confirm that introducing ions such as strontium (Sr) into HA enhances biomaterial characteristics, including crystallinity and dissolution rate under physiological conditions. This enhancement has garnered attention in the research community, highlighting the potential impact on bioactivity (9). Strontium recognized as one of the most significant cations in hard tissues, not only stimulates cell growth and prevents bone resorption but also addresses osteoporosis-related concerns. Strontium-substituted hydroxyapatite (SrHA) demonstrates osteoinduction alongside high solubility, leading to the rational conclusion that the incorporation of fluorine and strontium into HA could be desirable for advancing bioactivity (10). The aim of the study was to prepare orthodontic composite containing Sr-substituted hydroxyapatite nanoparticles and to study its antimicrobial properties. The resulting orthodontic composite containing nanoparticles underwent comprehensive characterization through X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).

Materials and methods:

Preparation of SR Substituted Hydroxyapatite Nanoparticle:

The Sr- enriched HA were synthesized through a chemical precipitation method using specific chemicals: Calcium nitrate tetrahydrate (Ca(N)3)2.4H20, MERCK), strontium nitrate (Sr(NO3), MERCK), and diammonium hydrogen phosphate ((NH42HPO4, MERCK). Each of these chemical components was individually dissolved in deionized water. The synthesis process involved adding the strontium solution to the calcium solution initially, followed by the introduction of the phosphate solution into the combined calcium and strontium solutions. To maintain the appropriate conditions, the pH level was adjusted to 10 using ammonia solution (NH4)H, MERCK). The Sr/(Ca+Sr) molar ratio was set at 25 or 4% and the (Ca+Sr)/P molar ratio was held constant at 1.67. The resulting HA solution was vigorously mixed for a period of 24 hours and allowed to precipitate for an additional 24 hours and then allowed to precipitate for an additional 24 hours at room temperature. Separation of the supernatant and HA precipitates was achieved directly through vacuum filtration, without any washing steps. The filtered HA slurry was subsequently subjected to a spray drying process to produce powder. To achieve a homogeneous concentration, the orthodontic adhesive was blended with 3 grams of orthodontic composite (Transbond XT, 3M Unitek, USA) using a mixer spatula and glass slab in a semi-dark setting. The spray-dried HA powder was added with orthodontic adhesive - Transbond XT Light Cure Adhesive Paste composite (3M Unitek, Monrovia, CA, USA). This orthodontic adhesive infused with Sr substituted hydroxyapatite nanoparticle was subjected to various analytical techniques, including scanning electron microscopy (Tescan Vega II SEM) *(Figure 3).* for morphological analysis, X-ray diffraction (Rigaku D/Max 2200, XRD) to identify phases, and FTIR analysis (Perkin Elmer) to explore molecular structures *(Figure 1).* FTIR and XRD analyses were performed on both the spray-dried and calcinated powders to investigate any potential alternations in nanoparticle infused orthodontic composite *(Figure 2).*

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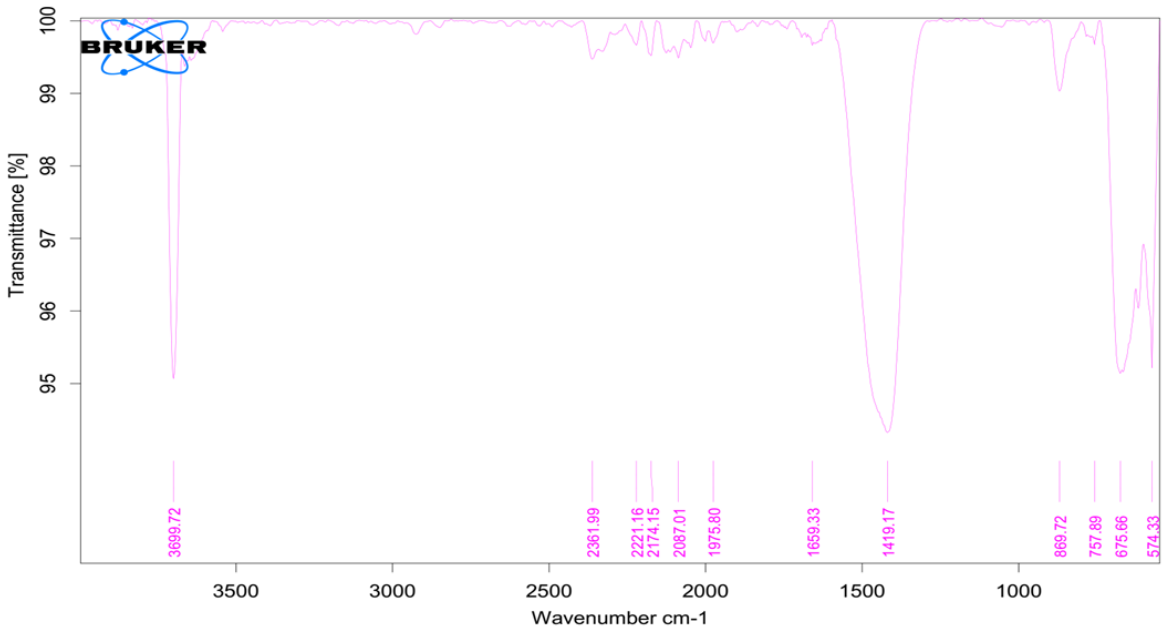

Figure 1: FTIR spectra for Sr- HAP.

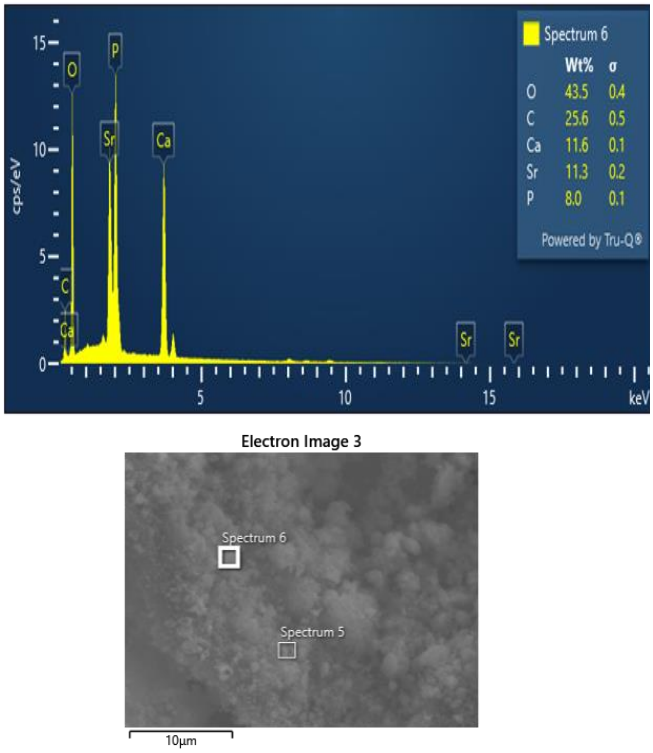

Figure 2: EDX spectrum of Sr-HAP

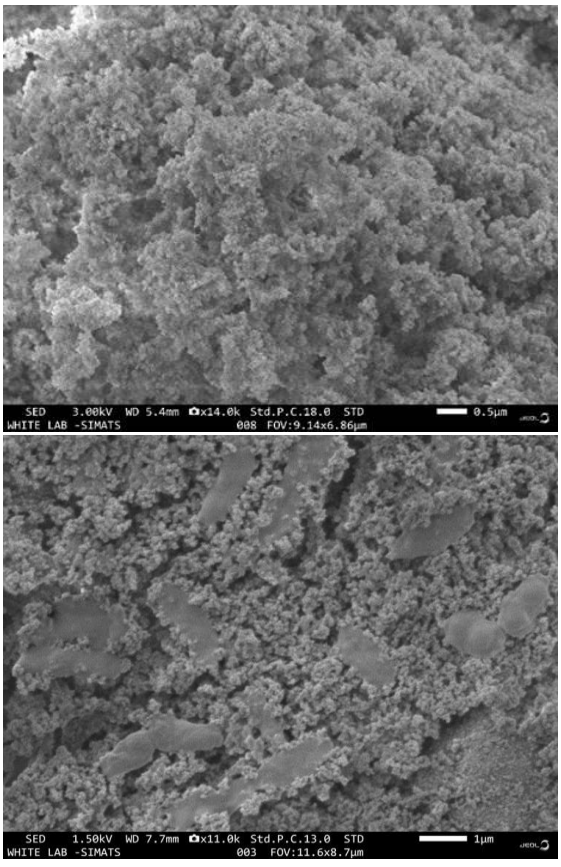

Figure 3: SEM micrographs of the biosynthesized Sr-infused hydroxypatite nanoparticles.

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Antimicrobial Properties of Sr Substituted Hydroxyapatite Nanoparticle

Antimicrobial testing was performed on three different groups. A control group (Transbond XT), Sr infused nano-composites with a low concentration of 0.5, and a high concentration of 1.0 wt%. The disc agar diffusion test was performed to investigate the antimicrobial capabilities of Sr infused. The adhesive was tested for its effectiveness against S. aureus, S. mutans, and E. coli. A suspension, matching a 0.5 McFarland turbidity (equivalent to 1.5x108 CFU/ml), was prepared from freshly cultured standard S. Aureus, S. Mutans and E.Coli after 24 hours of growth in brain-heart infusion (BHI) medium *(Figure 4)*. To create composite discs, plastic molds measuring 6 mm in diameter and 1 mm thickness were utilized. The procedure involved placing molds on a glass surface and applying adhesive into each mold. Afterward, the adhesives were exposed to a 20-second curing process (10 seconds from each side) using a light-cure device. This resulted in production of 10 discs, each measuring 6 mm in diameter and 1 mm in thickness for adhesive group with variable concentrations of nanopartilces. To ensure complete curing, the discs were subjected to an additional 10 seconds of curing cycle. Following this a portion of the prepared bacterial suspension was evenly spread on Mueller Hinton medium plates (Merck, Germany) containing 5% sheep blood, using swabs. All culture plates were placed in CO2 incubator at 37 C for a 24-hour incubation period. The diameter of the bacterial growth inhibition zones surrounding the disc was measured in millimeters.

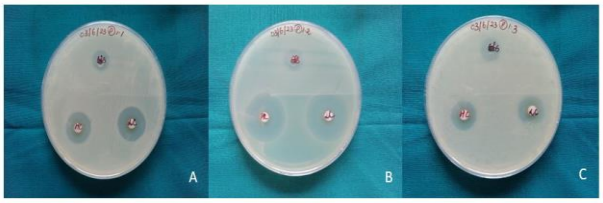

Figure 4: Antimicrobial test showing the efficacy of Sr-HAP against S. aureus, S. mutans, and E. coli.

To compare and examine the size of the inhibition zone, Kruskal-Wallis and Mann-Whitney tests were employed. For all the statistical analyses conducted, a significant level of 0.05 was set.

RESULTS:

The results obtained indicate the incorporation of Sr-HAP nanoparticles into the adhesives at 0.5% and 1% by weight. The antimicrobial assessment revealed the largest diameter of the zone inhibiting S. Aureus growth, with an average of 26 mm at low concentration and 27 mm at high concentration, for S.Mutans with an average of 26 mm at low concentration and 28 mm at high concentration, and for E.Coli with an average of 28 mm at low concentration and 30 mm at high concentration *(Table 1)*.

BACTERIA NAME	N	CONTROL	LOW CONCENTRATION	HIGH CONCENTRATION
S. AUREUS	10	24±0.12	26±0.32	27±0.38
S. MUTANS	10	24±0.23	26±0.29	28±0.31
E.COLI	10	20±0.34	30±0.42	30±0.56

Table 1: Comparison of different bacteria under low and high concentrations of Sr-HAP infused orthodontic adhesive.

Discussion

The incorporation of nanoparticles in adhesives could play a significant role in preventing the formation of white spot lesions (WSL). Research has shown that restorative composites containing 1% by weight of quaternary ammonium polyethylene imine can effectively prevent the growth of Streptocococcus mutans (S.Mutans), a knows contributor to WSL

developments. Additionally, composites containing Sr nanoparticles exhibited superior antimicrobial effects against S. mutans when compared to the control groups. Sr substituted HAP have found to possess potent antimicrobial properties, effectively inhibiting bacterial colonization and plaque formation (11). Notable Sr has demonstrated antimicrobial activity against a wide range of pathogenic bacteria. Given its cost-effectivess and greater chemical and physical stability, the antimicrobial capabilities of Sr substituted HAP nanoparticles and their impact have elevated the prevention of WSLs in orthodontic treatment (12).

Scanning electron microscopy (SEM) revealed a shift in the morphology of nanoparticles due to the introduction of strontium. It exhibited Sr replacing hydroxyapatite particles which are highly agglomerated. These appear porous and unordered morphology of hydroxyapatite pattern. While pure hydroxyapatite exhibits spherical shape (13). The average particle size observed were 31±4 nm, 43±3 nm and 56±9 nm (length x width) for Sr-HAP, 5% Sr-substituted HA and 10% Sr-substituted HA, respectively. This change in shape and size indicated that strontium substitution led to an increased aspect ratio of the particles, causing them to elongate along the c-axis direction (14–17). Furthermore, the energy-dispersive X-ray spectroscopy (EDX) spectra confirmed the presence of strontium substitution led to an increased aspect ratio of the particles, causing them to elongate along the c-axis direction (18). Furthermore, the energy dispersive X-ray spectroscopy (EDX) spectra, confirmed the presence of strontium substitution alongside other elements such as calcium and phosphate within the nanoparticles (19–23).

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