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Surface morphology and mechanical properties of extracted teeth after coating with nanohydroxyapatite and graphene oxide

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Abstract

Objective: This study evaluated surface morphology and compressive strength of teeth after application of a bioactive coating composed of nanohydroxyapatite (nHA), graphene oxide (GO), and calcium carbonate ($CaCO_3$).

Methods: Twelve human teeth were collected and divided into two groups (n=6). The test group was coated with the bioactive mixture of nHA, GO, and $CaCO_3$, while the control group remained uncoated. The coating was applied to cleaned tooth surfaces using the dip-coating method. The surface morphology of the coated teeth was examined after immersion in simulated body fluid (SBF) for 14 and 21 days using high-resolution scanning electron microscopy (HRSEM). The compressive strength and elastic modulus of teeth were measured with an Instron machine. An independent samples t-test was used to compare the values between groups, with significance set at $P<0.05$.

Results: Coated teeth showed a rough and dense surface after SBF immersion, suggesting enhanced surface interactions and an increased potential to support mineral nucleation. The average thickness of the bioactive coating was 14.4 ± 1.2 µm. Coated teeth showed significantly higher compressive strength than uncoated specimens ($P=0.01$). The elastic modulus of coated teeth (65.20 ± 12.56 GPa) was lower compared to uncoated teeth (69.50 ± 10.11 GPa), but this difference was not statistically significant ($P=0.53$).

Conclusions: The bioactive coating of nHA, GO, and $CaCO_3$ significantly increased the compressive strength of teeth while having no significant effect on their elastic modulus. Given its ability to enhance surface interactions and promote mineralization, this coating method shows promise for applications of tooth particles in ridge preservation and bone regeneration.

Keywords: Bone graft, Bone regeneration, Calcium phosphates, Graphene oxide, Hydroxyapatites, Tooth extraction

Introduction

Preserving the alveolar ridge after tooth extraction is crucial for maintaining bone volume and ensuring successful dental restorations. Following extraction, bone grafting materials are often used to preserve the ridge and repair alveolar defects, helping to maintain the bone structure needed for future implant placement (1). Although autogenous bone grafts are considered the gold standard, their use is limited by donor site morbidity and availability. As a result, alternative materials such as allografts and xenografts are commonly used, but these carry risks of immune reaction and disease transmission (2).

Recently, autogenous tooth-derived materials have gained interest as alternatives to allografts and xenografts due to their biocompatibility, osteoconductivity, and low risk of immune reaction (3). However, the structure and mineral composition of extracted teeth differ from bone, which limits their effectiveness for ridge preservation (4). This difference may influence the performance of tooth-derived materials in bone regeneration. To address this, various biomimetic materials (synthetic substances that mimic the properties of natural tissues) have been investigated to modify tooth surfaces and make them more similar in structure and composition to bone (5, 6). This method may enhance the use of tooth-derived materials in post-extraction sockets to support ridge preservation (7, 8).

Conventional coatings such as demineralized bone matrix (DBM), β-tricalcium phosphate (β-TCP), and

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collagen-based grafts have demonstrated osteoconductive properties but often lack sufficient mechanical strength. Recently, bioactive coatings that contain graphene oxide and nanohydroxyapatite have been developed (9). Such coatings may demonstrate potential to enhance apatite formation (10). It is assumed that the high surface area and functional groups present in these coatings can enable chemical bonding with calcium and phosphate ions, promote protein adsorption, and support cell attachment and osteogenic differentiation (11-13).

A potential concern with coated graft particles derived from tooth material is the reduction in their mechanical properties. The strength of the graft material is important for ridge preservation because the mechanical stability of the graft or biomaterial placed in the extraction socket directly influences the maintenance of alveolar bone volume and shape. Inadequate mechanical strength may lead to collapse or resorption of the grafted area, compromising the ridge's structural integrity needed for future dental restorations such as implants.

The present study aimed to investigate the surface morphology, compressive strength, and elastic modulus of extracted human teeth coated with a bioactive layer composed of nanohydroxyapatite (nHA), graphene oxide (GO), and calcium carbonate ($CaCO_3$).

Materials and Methods

Specimen Preparation

Extracted teeth used in this study were collected from routine dental extractions performed at Saveetha Dental College & Hospitals, Chennai, India. The study was conducted under institutional ethical approval (IHEC/SDC/FACULTY/23/PUB.HEALTH.DEN/108).

Twelve intact human teeth extracted for routine dental procedures were collected. The teeth were randomly assigned into two groups (n = 6): a control group with uncoated teeth and a test group with teeth coated with the bioactive nHA-GO-$CaCO_3$ composite. All specimens underwent the same cleaning, sterilization, and drying procedures before coating or testing.

Preparation of Nanohydroxyapatite

Nanohydroxyapatite (nHA) was synthesized using a hydrothermal method with adenosine 5′-triphosphate disodium salt ($ATPNa_2$) as the phosphate source. Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$, 44 g) was dissolved in 10 mL of deionized water to prepare solution A. Separately, $ATPNa_2$ (30 mg) was dissolved in 10 mL of deionized water to prepare solution B. Solution B was then added to solution A, and the pH was adjusted using a 30% sodium hydroxide (NaOH) solution. After stirring for 20 minutes, the mixture was autoclaved at 180 °C for 12 hours. The resulting white precipitate was collected, thoroughly washed with deionized water, and oven-dried at 70 °C.

Preparation of Graphene Oxide

Graphene oxide (GO) was synthesized from graphite flakes following a modified oxidative process. Graphite (1 g) was dispersed in 50 mL of concentrated sulfuric acid under stirring in an ice-water bath, maintaining the temperature below 10 °C. Potassium permanganate ($KMnO_4$, 3 g) was gradually added, and the suspension was stirred at room temperature for 25 minutes, followed by sonication for 5 minutes. This stirring-sonication cycle was repeated 12 times.

Subsequently, 200 mL of distilled water was added to terminate the reaction. The suspension was then ultrasonicated for 2 hours, and the pH was adjusted to approximately 6 using 1 M sodium hydroxide, followed by an additional 1 hour of sonication.

Finally, L-ascorbic acid (10 g dissolved in 100 mL of distilled water) was added slowly as a reducing agent. The resulting black precipitate was filtered, washed with 1 M hydrochloric acid and distilled water, and freeze-dried to obtain GO powder.

Coating Process

A suspension containing nHA, GO, and $CaCO_3$ was prepared in phosphate-buffered saline (PBS), with chitosan added to enhance adhesion. The mixture was ultrasonicated for 50 minutes and then stirred for 3 hours to ensure uniform dispersion. The teeth specimens were cleaned in distilled water using ultrasonication, sterilized in 70% ethanol, and air-dried. The coating was applied by the dip-coating method, in which the teeth were immersed in the prepared suspension for 1 minute and then vacuum-dried. This procedure was repeated three times to achieve the desired coating thickness. Finally, the coated teeth were dried in a vacuum oven at 60 °C for 24 hours.

Microscopic Evaluation

The surface morphology of the coated specimens was evaluated after immersion in simulated body fluid (SBF), which mimics the ionic composition of human plasma. The SBF solution was prepared by dissolving a prescribed mixture of inorganic salts in distilled water, and its pH was adjusted to approximately 7.4 using tris-hydrochloric acid (14). Each specimen was immersed in

SBF for 21 days at 37 °C, with a specimen surface area-to-solution volume ratio of 0.1 cm^2/mL. After incubation, the specimens were rinsed with distilled water and oven-dried. High-resolution scanning electron microscopy (HRSEM) was employed to examine the surface morphology and formation of the apatite layer. The thickness of the bioactive coating was also determined using HRSEM images.

Mechanical Testing

The compressive strength of the specimens was evaluated using a universal testing machine (Instron, Norwood, MA, USA). Each specimen was positioned vertically between the loading platens, and a compressive force was applied at a crosshead speed of 1 mm/min until failure occurred. The maximum load at fracture was recorded and used to calculate compressive strength.

In addition, the load-displacement data were obtained from the testing machine, and the elastic modulus of each specimen was determined from the linear region of the stress–strain curve. The mean values for both the control and test groups were calculated for statistical analysis.

Statistical Analysis

Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY, USA). The normal distribution of the data was confirmed by the Shapiro-Wilk test ($P>0.05$). The independent samples t-test was applied to compare the compressive strength and elastic modulus between the two groups. A p-value of less than 0.05 was considered statistically significant.

Results

SEM Images of Coated Teeth

HRSEM images of the coated teeth were obtained after 14 days (Figure 1A) and 21 days (Figure 1B) of immersion in simulated body fluid (SBF). These images showed rough, dense surfaces rich in minerals, indicating the presence of apatite-like deposits. Cross-sectional SEM analysis showed that the average thickness of the bioactive coating was 14.4 ± 1.2 µm, with no notable variation across different surface regions, confirming uniform coverage.

Mechanical Testing

The uncoated extracted teeth exhibited a mean compressive strength of 370.42 ± 0.27 MPa, whereas the coated teeth showed a significantly higher value of 375.34 ± 0.56 MPa ($P = 0.01$).

The stress–strain curves for both groups are shown in Figure 2. Although the coated teeth had a slightly lower elastic modulus (65.20 ± 12.56 GPa) compared to the uncoated teeth (69.50 ± 10.11 GPa), this difference was not statistically significant ($P = 0.53$).

Discussion

This study evaluated surface morphology and mechanical properties of human extracted teeth covered by a bioactive coating composed of nanohydroxyapatite (nHA), graphene oxide (GO), and calcium carbonate ($CaCO_3$). The incorporation of GO into the bioactive coating was intended to improve mineral

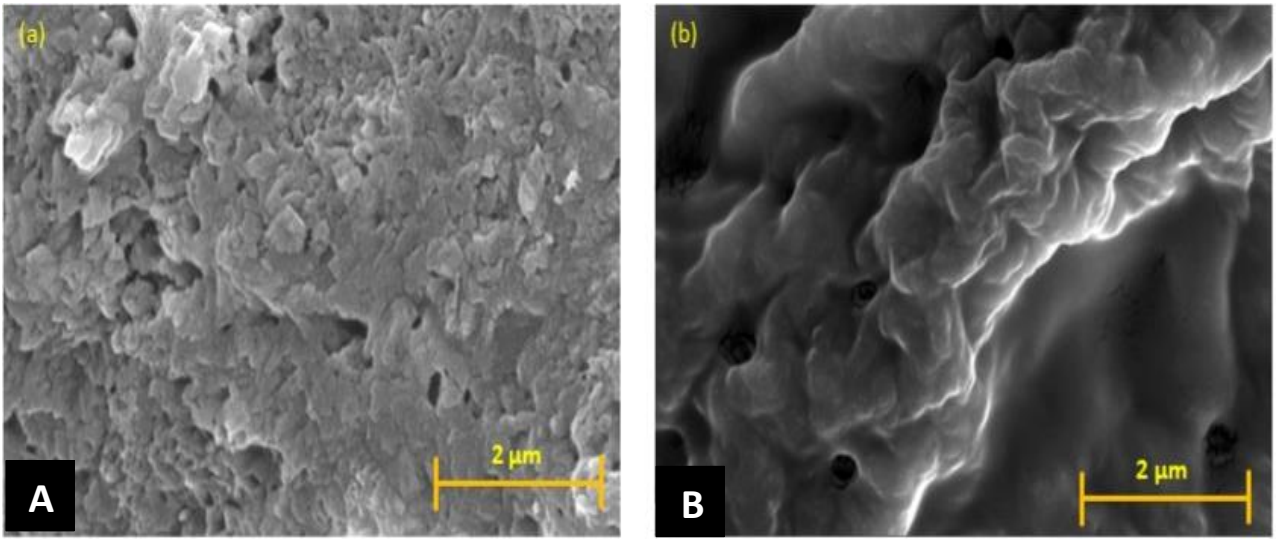

Figure 1. A) High resolution SEM image of the coated extracted tooth surface following 14 days of immersion in simulated body fluid, B) High resolution SEM image of the coated extracted tooth surface after 21 days of immersion in simulated body fluid

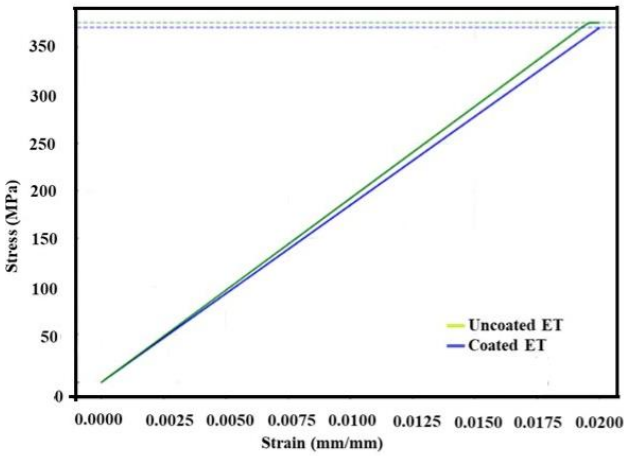

Figure 2. Stress-strain curve of coated and uncoated extracted teeth (ET)

deposition and coating stability. Previous studies have shown that GO substrates support osteoblast adhesion and mineral deposition, owing to their distinct surface chemistry and topographical features (15, 16).

HRSEM images of the coated samples in this study showed rough and dense surfaces, suggesting the presence of apatite-like deposits. These surface changes may also indicate the bioactivity of the coating and its increased potential to support mineral nucleation. Several studies reported that adding HA and GO created a rough surface texture, suggesting enhanced surface interactions (17-19).

The findings of this study showed that teeth treated with the bioactive coating exhibited higher compressive strength than uncoated teeth, whereas the elastic modulus of the two groups was comparable. The increased mechanical strength of the coated teeth suggests greater stability and better integration at the defect site. A stronger, bioactive tooth-derived material could better support the surrounding bone and soft tissues during healing, resist mechanical forces, and stimulate new bone formation. Moreover, this approach offers a sustainable and biocompatible alternative to current grafting methods, while being less invasive than autografts (20, 21). Our previous work also demonstrated that a coating containing reduced graphene oxide and calcium carbonate provided excellent tensile and flexural strength, as well as enhanced deposition of bone apatite crystals (4, 22).

The elastic modulus is calculated using the slope of the straight, linear part of the stress-strain curve. A higher elastic modulus indicates a stiffer material (it deforms less under the same applied load). For structural support and load-bearing applications, a higher elastic modulus is generally preferred because it means the material maintains its shape under stress. In the present study, coated teeth exhibited a slightly lower elastic modulus than uncoated samples, indicating greater flexibility. The difference in elastic modulus, however, was not statistically significant between the two groups, indicating that the addition of coating would not significantly reduce the stiffness of grafted materials.

The significantly higher compressive strength of coated teeth is consistent with previous studies. Pepla et al. (23) concluded that nanohydroxyapatite-based coatings enhance the mechanical properties of biomaterials used in dental applications. Several studies investigated HA/GO-based composites for bone regeneration, reporting improvements in bioactivity and mechanical strength (24-26). Daulbayev et al. (25) demonstrated enhanced osteogenic differentiation with GO/HA scaffolds, although the compressive strength values reported (280-310 MPa) were lower than those achieved in the present study (375 MPa).

The clinical relevance and superiority of the proposed bioactive coating lie in its ability to transform extracted teeth, a common biological waste, into a cost-effective and osteoconductive scaffold that supports bone regeneration. For clinical application, the extracted tooth material would need to be processed into a graft material. For this purpose, the cleaned tooth (often focusing on the dentin portion) should be crushed into small particles and coated with the bioactive layer, then placed into the socket to promote ridge preservation.

This study was conducted in vitro; therefore, the biological performance of the coated materials in vivo remains untested. In addition, the small sample size and absence of long-term degradation and mechanical stability evaluations may limit the generalizability of the results. Future studies should include in vivo experiments to examine the osteoconductivity, biocompatibility, and integration of the coated materials within bone tissue.

Conclusions

The bioactive coating of nHA, GO, and $CaCO_3$ significantly increased the compressive strength of teeth without affecting their elastic modulus. Coated teeth showed a rough and dense surface after SBF immersion, suggesting enhanced surface interactions and an increased potential to support mineral nucleation. Therefore, coated extracted teeth could serve as an autogenous graft material, potentially offering a biologically and mechanically suitable alternative to conventional grafts.

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Not Applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

R.S. designed the project, contributed to the methodology, and wrote the manuscript. R.P.K. contributed to the methodology, statistical analysis, and manuscript preparation. Both authors read and approved the final manuscript.

Ethical approval

Extracted teeth used in this study were collected from routine dental extractions performed at Saveetha Dental College & Hospitals, Chennai, India. The study was conducted under institutional ethical approval (IHEC/SDC/FACULTY/23/PUB.HEALTH.DEN/108).

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