Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 752-754 | ISSN:2251-6727

Evaluation of Surface Characteristics and Osteogenic Potential of Nano-Hydroxyapatite Coated Orthodontic Mini Screws: An In Vitro Study

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(Received: 16 July 2025 Revised: 20 August 2025 Accepted: 02 September 2025)

KEYWORDS

Nano-hydroxyapatite, Orthodontic anchorage, SEM (Scanning electron microscope), EDX (Energy dispersive X-ray), Cytotoxicity, MG-63, Mini screws, Osteointegration

ABSTRACT:

Introduction: Orthodontic mini screws, used as Temporary Anchorage Devices (TADs), are pivotal in modern orthodontics. Surface modifications like nano-hydroxyapatite (nHA) coatings may enhance their biological integration.

Objectives: To evaluate and compare surface morphology and osteogenic potential of uncoated versus nHA-coated mini screws.

Methods: Thirty-six titanium mini screws (1.5 mm × 6 mm) were split into coated and uncoated groups. Plasma spray coating with nHA was applied. Surface analysis was done via SEM and EDX. Osteogenic compatibility was assessed using MG-63 cell lines and MTT assay at 24 and 48 hours.

Results: SEM revealed nano-textured, uniform coatings. EDX confirmed Ca/P ratios matching hydroxyapatite. At 48 hours, coated screws showed significantly higher cell viability ($p = 0.042$), with no cytotoxicity.

Conclusions: nHA coatings improve mini screw bioactivity and osteoblast proliferation. Optimization of coating methods is necessary to avoid excess bulk and tip blunting.

1. Introduction:

Anchorage in orthodontics refers to the resistance to unwanted tooth movement and is vital for effective force application and treatment success. Conventional anchorage techniques like headgear or intraoral elastics frequently rely on patient compliance and anatomical limitations, often yielding suboptimal outcomes[1].

The introduction of Temporary Anchorage Devices (TADs) marked a paradigm shift in anchorage control. Orthodontic mini screws, typically fabricated from titanium, are small, biocompatible, and easy to place and remove. They are commonly inserted into alveolar bone and enable complex tooth movements without relying on other teeth[2].

Despite their clinical benefits, mini screws exhibit failure rates ranging from 10–30%. Common causes include inflammation, poor mechanical retention, and inadequate interaction with surrounding bone. These limitations have prompted researchers to explore surface modification strategies that enhance bone-implant integration, thereby increasing the success rates of TADs[2].

Hydroxyapatite (HA), a naturally occurring mineral in bone, has emerged as a promising biomaterial due to its bioactivity, osteoconductivity, and chemical similarity to human hard tissues. The nano-sized form of HA (nHA) is especially advantageous due to its high surface area and ability to mimic the nanostructure of bone, which promotes superior cellular adhesion and proliferation[3].

By coating titanium mini screws with nHA using plasma spray techniques, the aim is to create a bioactive surface that enhances osteoblastic response and facilitates better osseointegration. This study evaluates these modifications both structurally and biologically through in vitro assessment.

2. Objectives:

To evaluate and compare surface morphology and osteogenic potential of uncoated versus nHA-coated mini screws.

3. Material and Methods:

Study Design and Sample Preparation

An in vitro experimental study was conducted at SGT University's Department of Orthodontics and Dentofacial Orthopaedics. Institutional Ethical Committee clearance was obtained before initiating the study.

Thirty-six titanium Grade IV orthodontic mini screws, each 1.5 mm in diameter and 6.0 mm in length, were selected. Screws were divided into:

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- **Group A1:** Uncoated for SEM/EDX analysis (n = 3)
- **Group A2:** Coated with nHA for SEM/EDX analysis (n = 6)
- **Group B1:** Uncoated for MG-63 cell work (n = 9)
- **Group B2:** Coated with nHA for MG-63 cell work (n = 18)

FIGURE 1: UNCOATED SCREWS

FIGURE 2: COATED SCREWS

Coating Process

Nano-hydroxyapatite powder (20–80 nm, 99.9% purity) was procured from Nano Research Lab, Jamshedpur. Plasma spray coating was performed at EMPORIS Implants, Gujarat, using an F4 plasma spray gun with argon and hydrogen gas. The screw surfaces were roughened using WOLCUT abrasive P400 paper prior to coating. Multiple passes were made to ensure even coating.

Surface Analysis (SEM and EDX)

SEM (Zeiss Ultra Plus) was used at 500x and 1000x magnifications. Uncoated screws were examined for surface irregularities; coated screws were analyzed for coating uniformity and morphology. EDX was employed to confirm elemental composition and Ca/P ratios.

Cell Line and MTT Assay

MG-63 osteoblast-like human cells were used. Screws were sterilized and placed in 96-well plates with cells seeded at 1×10^4 per well. After 24 and 48 hours of incubation, MTT reagent was added. The formazan crystals formed were solubilized using DMSO and absorbance measured at 570 nm.

Statistical Analysis

Cell viability percentages were analyzed using unpaired t-tests, with significance set at $p < 0.05$.

4. Results:

SEM Observations

Uncoated screws displayed machining marks and relatively smooth surfaces, indicative of conventional milling. Coated screws revealed a uniform, nano-textured surface with clustered nHA particles. The coating thickness ranged from 200 to 500 nm. Needle-like crystalline structures and surface porosity were noted, which favour cell adhesion.

EDX Findings

Elemental mapping confirmed calcium and phosphorus presence, validating hydroxyapatite deposition. A Ca/P atomic ratio of approximately 1.67 was observed, aligning with the theoretical value for hydroxyapatite. Trace titanium suggested minimal substrate exposure, confirming good coating coverage.

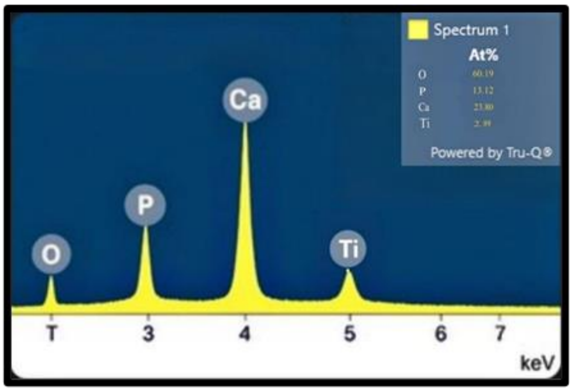

FIGURE 3: SPECTROGRAM

Spectrum 1			
Element	Signal Type	Wt%	Atomic %
O	EDS	60.11	60.19
P	EDS	20.85	13.12
Ca	EDS	34.83	23.80
Ti	EDS	1.12	2.89
Total		100.00	100.00

FIGURE 4: Showing amount of components of the coating

MTT Assay – 24 Hours

- **Uncoated:** Mean viability = 95.74%
- **Coated:** Mean viability = 92.90%
- **p-value:** 0.54 (not significant)

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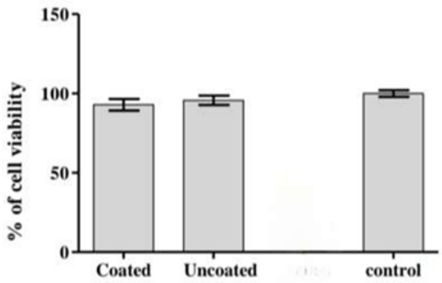

FIGURE 5: Cell count post 24hrs

MTT Assay – 48 Hours

- **Uncoated:** Mean viability = 97.27%
- **Coated:** Mean viability = 104.79%
- **p-value:** 0.042 (statistically significant)

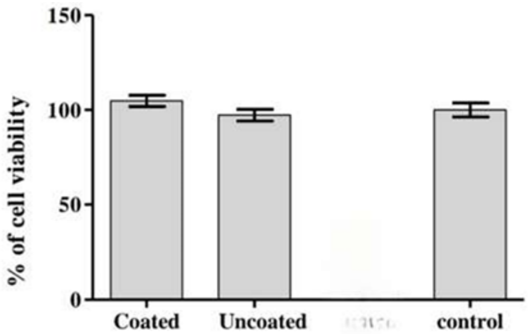

FIGURE 6: Cell count post 48hrs

Viability improved significantly over time in coated screws, indicating enhanced osteoblastic proliferation.

5. Discussion:

The successful implementation of orthodontic mini screws depends on both mechanical stability and biological compatibility. Surface modification is a key factor influencing osseointegration. This study focused on evaluating the effect of nHA coatings on mini screw surfaces and their subsequent interaction with osteoblast-like cells[3,4].

SEM results demonstrated the transformation of screw surface from a relatively smooth texture to a highly roughened and porous surface following nHA deposition. This modification increases surface energy and contact area, which enhances protein adsorption and osteoblastic attachment—critical factors for osseointegration[5].

EDX confirmed consistent elemental distribution of Ca and P with minimal titanium exposure, confirming full coverage of the screw body. The Ca/P ratio near 1.67 suggests high purity and crystallinity of hydroxyapatite, factors closely associated with bioactivity and bone-like integration[6,7].

Cytotoxicity assays using MG-63 cells revealed no toxicity for either group. However, enhanced cell proliferation in the coated group at 48 hours indicates a time-dependent positive biological response. These findings align with previous studies by Zhao et al. (2013) and Liu et al. (2014), which demonstrated enhanced cellular behaviour on nano-textured and HA-coated surfaces[8,9].

Limitations: Plasma spraying led to excessive coating on some screws, resulting in tip blunting. This could impede mechanical insertion and reduce primary stability. Future studies should optimize coating protocols to balance bioactivity and mechanical efficacy. Additionally, in vivo validation is required to confirm clinical translatability.

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