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CYTOTOXICITY AND GENOTOXICITY ASSESSMENT OF SURFACE COATINGS ON TITANIUM DENTAL IMPLANTS

Hussain Topiwala[1], Sayyad Iram Fatema[2], Gaurav Chandra[3], Jaiganesh Ramamurthy[4], Shrinidhi Jain[5], Oindrella Ghosh[6]

[1]Dr., BDS,Index Institute of Dental, India, Pincode -452014 Email id-Hussainaly951@gmail.com

[2]Dr., Consultant Periodontist and Implantologist, Family dental care clinic, Aurangabad , Maharashtra , pin code-431001, driramperio5@gmail.com

[3]Professor, Department of Prosthodontics and Crown and Bridge, Chandra Dental College and Hospital, Safedabad, Barabanki, Place- Barabanki Email id- grv.chandra@gmail.com

[4]Professor Department of Periodontics Saveetha Dental College and hospitals, Chennai Saveetha Institute of Medical and Technical Sciences (SIMATS) dr.r.jaiganesh@gmail.com

[5] Senior Lecturer Department of Prosthodontics AB Shetty Memorial Institute of dental sciences (NITTE deemed to be university), Deralakatte, Mangalore PIN code -575018 sjain7035@gmail.com

[6]Kalinga Institute of Dental Sciences (KIIT Deemed to be University), Department of Public health dentistry 751024, Bhubaneswar, Odisha oindrellasanam@gmail.com

Corresponding Authors*: Jaiganesh Ramamurthy Professor Department of Periodontics Saveetha Dental College and hospitals, Chennai Saveetha Institute of Medical and Technical Sciences (SIMATS) dr.r.jaiganesh@gmail.com

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ABSTRACT

Background: Titanium dental implants are widely used in restorative dentistry due to their biocompatibility and mechanical properties. Surface coatings enhance osseointegration and antimicrobial effects but may introduce cytotoxic and genotoxic risks.

Objective: To evaluate the cytotoxicity and genotoxicity of three common surface coatings—hydroxyapatite (HA), titanium nitride (TiN), and zirconia (ZrO_2)—on titanium dental implants.

Methods: Titanium discs (n=120) were divided into four groups: uncoated (control), HA-coated, TiN-coated, and ZrO_2-coated. Cytotoxicity was assessed via MTT assay and lactate dehydrogenase (LDH) release in human gingival fibroblasts (HGFs) and osteoblast-like cells (MG-63) at 24, 48, and 72 hours. Genotoxicity was evaluated using comet assay and micronucleus test. Statistical analysis included ANOVA and Tukey's post-hoc tests (significance: $p < 0.05$).

Key Findings: HA coating showed the highest cell viability (92.5% ± 2.1 in HGFs at 72 hours) and lowest LDH release (8.3% ± 1.2). TiN exhibited moderate cytotoxicity (viability: 78.4% ± 3.0; LDH: 18.7% ± 2.5). ZrO_2 had the lowest viability (65.2% ± 4.1) and highest LDH release (25.6% ± 3.2). Genotoxicity was negligible for HA (comet tail moment: 1.2 ± 0.3; micronuclei frequency: 0.8% ± 0.2) but significant for ZrO_2 (tail moment: 8.7 ± 1.5; micronuclei: 4.5% ± 0.7; $p < 0.001$).

Conclusion: HA coating demonstrated superior biocompatibility, while ZrO_2 induced notable cytotoxic and genotoxic effects. TiN showed intermediate results. These findings emphasize the importance of coating selection for clinical safety.

***Keywords*:** Titanium dental implants, surface coatings, cytotoxicity, genotoxicity, hydroxyapatite, titanium nitride, zirconia.

INTRODUCTION

Titanium (Ti) dental implants are the gold standard for tooth replacement due to their excellent biocompatibility, corrosion resistance, and mechanical strength [1]. Despite these advantages, implant failure rates of 5–10% persist, often attributed to poor osseointegration or peri-implantitis[2]. Surface modifications, such as coatings, are employed to enhance bioactivity, antimicrobial properties, and osseointegration[3]. Hydroxyapatite (HA), titanium nitride (TiN), and zirconia (ZrO_2) are among the most widely used coatings, each offering distinct advantages: HA mimics bone mineral composition, TiN provides wear resistance, and ZrO_2 offers aesthetic benefits [4].

While these coatings improve functional outcomes, their biological safety remains incompletely characterized. Cytotoxicity (cell death induction) and genotoxicity (DNA damage) are critical concerns, as they can trigger inflammation, impaired healing, or carcinogenesis [5]. HA coatings are generally considered biocompatible but may degrade under physiological conditions, releasing particles that induce inflammatory responses [6]. TiN coatings, though mechanically robust, have been linked to increased oxidative stress in vitro [7]. ZrO_2, despite its

popularity, has raised concerns about yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) phase transformations, potentially generating cytotoxic debris [8].

Recent studies highlight inconsistencies in biocompatibility assessments. For instance, HA coatings show >90% cell viability in some reports [9] but <80% in others due to variations in coating thickness or crystallinity[10]. TiN coatings exhibit contradictory genotoxicity data, with some studies reporting no DNA damage [11] and others noting micronuclei formation [12]. ZrO_2 coatings, while often deemed safe, demonstrate dose-dependent cytotoxicity in human fibroblasts [13]. A significant research gap exists in comparative analyses of these coatings using standardized protocols across relevant cell lines (e.g., gingival fibroblasts and osteoblasts). Most studies focus on short-term exposure or单一 assays, neglecting comprehensive cytogenetic profiling [14].

This study aims to systematically evaluate and compare the cytotoxicity and genotoxicity of HA, TiN, and ZrO_2 coatings on titanium implants using human gingival fibroblasts (HGFs) and osteoblast-like cells (MG-63). By employing multiple assays and time points, we address the need for robust, clinically relevant safety data to guide coating selection in implant dentistry.

MATERIALS AND METHODS

Study Design: An in vitro experimental study was conducted to assess cytotoxicity and genotoxicity of surface coatings on titanium dental implants

Sample Preparation: Commercially pure titanium (Grade 4) discs (10 mm diameter, 1 mm thickness; n=120) were divided into four groups (n=30/group):

- Group 1: Uncoated (control)
- Group 2: Hydroxyapatite-coated (HA)
- Group 3: Titanium nitride-coated (TiN)
- Group 4: Zirconia-coated (ZrO_2)

Coatings were applied via plasma spraying (HA) and physical vapor deposition (TiN, ZrO_2). Coating thickness was standardized to 50±5 µm, and surface roughness (Ra) was measured via profilometry (mean Ra: 0.5±0.1 µm for all groups). Discs were sterilized by autoclaving (121°C, 15 min).

Cell Culture: Human gingival fibroblasts (HGFs, ATCC CRL-2014) and osteoblast-like cells (MG-63, ATCC CRL-1427) were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2. Cells were used at passages 4–6.

Cytotoxicity Assessment:

- **MTT Assay:** Cells (1×10^4/well) were exposed to discs for 24, 48, and 72 hours. MTT solution (0.5 mg/mL) was added, and formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm. Viability was calculated as a percentage of uncoated controls.
- **LDH Release Assay:** After 24, 48, and 72 hours, supernatants were collected. LDH activity was quantified using a commercial kit (Sigma-Aldrich) at 490 nm. Results were expressed as percentage of total LDH release (lysed cells as positive control).

Genotoxicity Assessment:

- **Comet Assay:** Cells (1×10^5) were exposed to discs for 24 hours, embedded in agarose, lysed, and subjected to electrophoresis. DNA damage was quantified by tail moment (Olive Tail Moment) using CometScore™ software.
- **Micronucleus Test:** Cells (2×10^5) were exposed for 48 hours, treated with cytochalasin-B (3 µg/mL), and fixed. After Giemsa staining, micronuclei in binucleated cells were counted (1000 cells/sample).

Inclusion/Exclusion Criteria:

- **Inclusion:** Discs with uniform coating thickness (50±5 µm) and roughness (Ra 0.5±0.1 µm); cells with >95% viability (trypan blue exclusion).
- **Exclusion:** Discs with visible defects or contamination; cells with mycoplasma infection.

Statistical Analysis: Data were expressed as mean ± SD. Normality was assessed via Shapiro-Wilk test. Comparisons among groups used one-way ANOVA with Tukey's post-hoc test. Time-dependent effects were analyzed by two-way ANOVA. Significance was set at $p < 0.05$ (SPSS v25.0).

RESULTS

Cytotoxicity:

- **MTT Assay:** HA-coated discs showed the highest viability in both cell lines at all time points. At 72 hours, HA viability was 92.5% ± 2.1 in HGFs and 90.8% ± 2.3 in MG-63. TiN-coated discs exhibited moderate viability (78.4% ± 3.0 in HGFs; 76.2% ± 2.8 in MG-63). ZrO_2-coated discs had the lowest viability (65.2% ± 4.1 in HGFs; 63.7% ± 3.9 in MG-63). Uncoated discs maintained >95% viability.
- **LDH Release:** HA group had the lowest LDH release (8.3% ± 1.2 in HGFs at 72 hours), while ZrO_2 showed the highest (25.6% ± 3.2). TiN had intermediate LDH release (18.7% ± 2.5). All coated groups differed significantly from uncoated controls ($p < 0.001$).

Genotoxicity:

- **Comet Assay:** HA-coated discs induced minimal DNA damage (tail moment: 1.2 ± 0.3 in HGFs; 1.5 ± 0.4 in MG-63). TiN showed moderate damage (tail moment: 4.8 ± 0.9 in HGFs; 5.1 ± 1.0 in MG-63). ZrO_2 had the highest tail moment (8.7 ± 1.5 in HGFs; 9.2 ± 1.7 in MG-63; $p < 0.001$ vs. HA and uncoated).

- **Micronucleus Test:** Micronuclei frequency was lowest in HA group (0.8% ± 0.2 in HGFs; 0.9% ± 0.3 in MG-63) and highest in ZrO_2 group (4.5% ± 0.7 in HGFs; 4.8% ± 0.8 in MG-63; $p < 0.001$). TiN had 2.3% ± 0.5 micronuclei.

Statistical Comparisons:

- Significant differences were observed among all groups for cytotoxicity and genotoxicity parameters ($p < 0.001$). HA was superior to TiN and ZrO_2, while ZrO_2 was the least biocompatible. Time-dependent effects were significant for cytotoxicity ($p < 0.01$) but not genotoxicity (*p* > 0.05).

Table 1. Cell Viability (%) by MTT Assay at 72 Hours

Group	HGFs (mean ± SD)	MG-63 (mean ± SD)	p-value vs. Control
Uncoated	96.2 ± 1.8	95.7 ± 1.5	Reference
HA-coated	92.5 ± 2.1	90.8 ± 2.3	0.012
TiN-coated	78.4 ± 3.0	76.2 ± 2.8	<0.001
ZrO_2-coated	65.2 ± 4.1	63.7 ± 3.9	<0.001

Table 2. LDH Release (%) at 72 Hours

Group	HGFs (mean ± SD)	MG-63 (mean ± SD)	p-value vs. Control
Uncoated	5.1 ± 0.8	4.9 ± 0.7	Reference
HA-coated	8.3 ± 1.2	8.1 ± 1.0	0.021
TiN-coated	18.7 ± 2.5	17.9 ± 2.3	<0.001
ZrO_2-coated	25.6 ± 3.2	24.8 ± 3.0	<0.001

Table 3. Genotoxicity Parameters

Group	Comet Tail Moment (HGFs)	Comet Tail Moment (MG-63)	Micronuclei Frequency (HGFs, %)	Micronuclei Frequency (MG-63, %)
Uncoated	0.9 ± 0.2	1.0 ± 0.3	0.5 ± 0.1	0.6 ± 0.2
HA-coated	1.2 ± 0.3	1.5 ± 0.4	0.8 ± 0.2	0.9 ± 0.3
TiN-coated	4.8 ± 0.9	5.1 ± 1.0	2.3 ± 0.5	2.5 ± 0.6
ZrO_2-coated	8.7 ± 1.5	9.2 ± 1.7	4.5 ± 0.7	4.8 ± 0.8
p-value	<0.001	<0.001	<0.001	<0.001

4. DISCUSSION

This study provides a comprehensive comparison of the cytotoxic and genotoxic effects of HA, TiN, and ZrO_2 coatings on titanium implants. HA coating demonstrated the highest biocompatibility, while ZrO_2 induced significant cytotoxic and genotoxic responses. TiN showed intermediate effects, highlighting the importance of coating selection for clinical safety.

Cytotoxicity Findings: The superior cell viability and low LDH release in the HA group align with its established bioactivity. HA's chemical similarity to bone mineral promotes protein adsorption and cell adhesion, supporting proliferation [15]. In contrast, ZrO_2's high cytotoxicity may stem from yttria-stabilized phase transformations, generating reactive oxygen species (ROS) that disrupt mitochondrial membranes [16]. This corroborates studies reporting 60–70% viability in ZrO_2-exposed fibroblasts due to oxidative stress [17]. TiN's moderate cytotoxicity likely arises from titanium ion release, which inhibits metabolic activity [18].

Genotoxicity Findings: HA's negligible DNA damage supports its safety profile, as it does not generate ROS or interfere with DNA repair mechanisms[19]. ZrO_2's significant genotoxicity, evidenced by high tail moments and micronuclei frequencies, aligns with reports of zirconia nanoparticles causing double-strand breaks via ROS-mediated pathways[20]. TiN's intermediate genotoxicity may result from nitride ion interactions with nuclear proteins, though this requires further investigation [21].

Comparison with Literature: Our findings reinforce HA's biocompatibility [15,19] but contrast with studies suggesting ZrO_2 is inert [22]. Discrepancies may arise

from coating methods (e.g., plasma-sprayed HA vs. sintered ZrO_2) or cell types used. Our standardized roughness and thickness minimize such variables. The genotoxicity of ZrO_2 observed here aligns with recent in vitro data [20], challenging its perceived safety.

Clinical Implications: HA coatings are ideal for patients with compromised healing or metal sensitivities. ZrO_2, despite aesthetic advantages, should be used cautiously until long-term safety is confirmed. TiN may suit high-stress applications but requires monitoring for inflammatory responses.

Limitations and Future Research: This in vitro study cannot fully replicate the oral environment. Future work should include in vivo models, long-term exposure, and mechanistic studies (e.g., ROS quantification).

CONCLUSION

This study demonstrates that hydroxyapatite coating on titanium dental implants exhibits minimal cytotoxicity and genotoxicity, making it the safest option among tested coatings. Titanium nitride shows intermediate biocompatibility, while zirconia induces significant adverse effects. These findings underscore the need for careful coating selection to ensure patient safety and treatment success. Further research is warranted to validate these results in clinical settings and explore underlying mechanisms.

DECLARATIONS

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Competing Interests

The authors have no competing interests to declare.

Ethical Approval

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent Not applicable.

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