

# Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

Riya Changediya<sup>1\*</sup>, Anand Sabane<sup>2</sup>, Amol Patil<sup>3</sup>

<sup>1\*</sup>Post-Graduate Student (MDS), Department of Orthodontics and Dentofacial Orthopedics, Bharati Vidyapeeth (Deemed to be University), Dental College and Hospital, Pune - 411043.

Email: [changediyariya@gmail.com](mailto:changediyariya@gmail.com) (Corresponding Author)

<sup>2</sup>Associate Professor, Department of Orthodontics and Dentofacial Orthopedics, Bharati Vidyapeeth (Deemed to be University), Dental College and Hospital, Pune - 411043.

Email: [anandsabane@gmail.com](mailto:anandsabane@gmail.com)

<sup>3</sup>Professor, Department of Orthodontics and Dentofacial Orthopedics, Bharati Vidyapeeth (Deemed to be University), Dental College and Hospital, Pune - 411043.

Email: [amol.s.patil@bharatividyaapeeth.edu](mailto:amol.s.patil@bharatividyaapeeth.edu)

## ABSTRACT

**Background:** Titanium mini-implants (TMIs) are important in orthodontics for temporary anchorage, but their biocompatibility may be influenced by mouthwashes used during treatment. This in vitro study assessed the effects of two mouthwashes: chlorhexidine (CHX), fluoride-containing Listerine mouthwash on the surface roughness, cytotoxicity, and metal ion release from TMIs.

**Materials and methods:** A total of 39 TMIs divided in three groups equally were immersed in these solutions for 21 days at 37°C. The samples were then immersed in Dulbecco's Modified Eagle's Medium for 7 days at 5°C. Cytotoxicity was evaluated using the MTT assay on human gingival fibroblasts, metal ion release (Ti, Al, V, Fe) was measured with microwave plasma-atomic emission spectrometry, and surface morphology was examined using scanning electron microscopy (SEM), with data analyzed via one-way ANOVA and Tukey's post hoc test.

**Results:** Results indicated that CHX exhibited minimal cytotoxicity (cell viability of 91%) and the lowest metal ion release. In contrast, fluoride mouthwash showed higher ion release and greater cytotoxicity (cell viability of 79%). SEM analysis revealed significant corrosion and surface pitting in the Listerine group compared to the CHX and control groups.

**Conclusion:** Chlorhexidine mouthwash proved to have superior biocompatibility and minimal corrosive effects on titanium mini-implants, while fluoride-containing mouthwashes significantly heightened corrosion and metal ion release.

# Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

**Keywords:** Mini-implants, Mouthwash, Cytotoxicity, Ion release, Surface roughness, Orthodontic anchorage

**How to cite this article:** Changediya R, Sabane A, Patil A. Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study. *Int J Drug Deliv Technol.* 2026;16(19s): 711-720. DOI: 10.25258/ijddt.16.19s.82

**Source of support:** Nil.

**Conflict of interest:** None

## Introduction

The goal of orthodontics is to enhance both function and appearance by identifying, preventing, and correcting abnormalities of the teeth and face.<sup>1</sup> Anchorage control is a crucial component of treatment effectiveness because it stops undesired tooth movement when force is applied. Since then, numerous intraoral and extra oral anchorage techniques have been developed, each with unique benefits and drawbacks.<sup>2</sup>

Over the past 20 years, one significant development in orthodontics has been the introduction of mini-implants, or MIs.<sup>3</sup> Because of their small size, affordability, ease of insertion and removal, and little reliance on patient cooperation, these temporary anchoring devices are frequently utilized.<sup>4</sup> MIs enable precise three-dimensional anchorage control, enhancing treatment efficiency. However, their success rate varies from 70% to 95% when compared to traditional endosseous implants, depending on a number of criteria, including age, bone density, screw design, implantation site, and material qualities.<sup>5,6</sup>

Recent research has shown that corrosion and the release of titanium (Ti) ions are two contributing causes to peri-implant inflammation, which is a

common cause of MI failure.<sup>7,8</sup> Due to exposure to saliva, pH fluctuations, and chemical agents, metallic orthodontic components, such as brackets, wires, and mini-implants, can corrode in the oral environment.<sup>8</sup> The titanium alloys used to make the majority of orthodontic mini-implants comprise aluminum (Al) and vanadium (V), which improve mechanical strength but may also make them more susceptible to corrosion.<sup>9</sup>

Fluoride-containing mouthwashes are commonly used to prevent white spot lesions; nevertheless, fluoride ions and low pH can cause metal ion release from Ti alloys, resulting in corrosion and discoloration.<sup>9</sup> Similarly, chlorhexidine (CHX) mouthwash, while effective in lowering peri-implant inflammation, can change implant surface integrity and trigger ion leakage with continuous use.<sup>10</sup> These metal ions can cause oxidative stress, cell damage, and inflammatory responses.<sup>10,11</sup>

Given the importance of biocompatibility and corrosion resistance to the long-term effectiveness of orthodontic mini-implants, it is critical to assess their cytotoxic and corrosive behavior under various mouthwash exposures. The purpose of this study is to analyse and compare the effect of commonly used mouthwashes on titanium

# Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

temporary anchoring devices in terms of surface roughness, cytotoxicity, and metal ion release.

## MATERIALS AND METHODS

This in-vitro experimental study evaluated the effect of commonly used mouthwashes on titanium temporary anchorage devices (TADs) in terms of surface roughness, cytotoxicity, and metal ion release, with Institutional Ethics Committee approval.

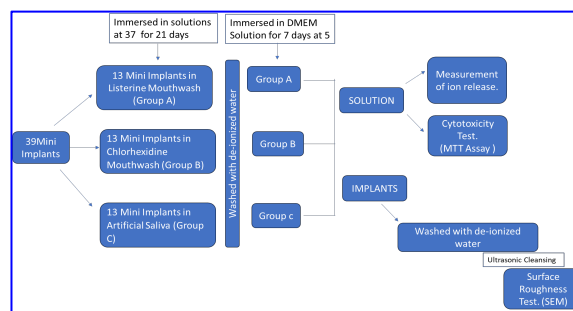
Thirty-nine titanium mini-implants ( $1.5 \times 6$  mm; SK Surgical, Pune) were divided into three groups ( $n = 13$ ):

-Group A: Chlorhexidine mouthwash (ICPA Health Products Ltd)

-Group B: Fluoride-containing Listerine (Johnson & Johnson)

- Group C: Artificial saliva (Wet Mouth; ICPA Health Products Ltd, control)

Cytotoxicity testing was performed at Maratha Mandal's Central Research Laboratory, Belgaum; metal ion release at the National Chemical Laboratory; and surface analysis (SEM) at the Central Instrumentation Facility, Savitribai Phule Pune University. Materials included human gingival fibroblast strains (HEK-293, HT-29, HepG2, K562, A549, KB Mouth; NCCS Pune), MTT reagent, DMEM medium (Gibco, Thermo Fisher Scientific), Microwave Plasma Atomic Emission Spectrometer (Agilent 4200 MP-AES) and Scanning Electron Microscope (FEI Nova Nano SEM 450).



**Fig 1: Flowchart explaining the methodology of the study**

## Preparation of Eluates (Figure 2)

Thirty-nine titanium Mini screws were divided into three groups equally ( $n = 13$ ). Group A: Chlorhexidine mouthwash; Group B: Listerine with fluoride; and Group C: Artificial saliva (control). The screws were immersed in 385  $\mu$ L of their respective solution (1 mL per 0.2 g ratio per DIN EN ISO 10271) and incubated at 37°C for 21 days. Control solutions that were without MI's were also incubated. Samples were washed with deionized water, ultrasonically cleaned (40 kHz, 15 min), and immersed in 10 mL of DMEM (5°C, 7 days) for metal ion measurement and cytotoxicity testing.



**Fig 2: Preparation of eluates from Mini screws in different types of mouthwash.**

## Cell Culture and Cytotoxicity (MTT Assay) (Figure 3)

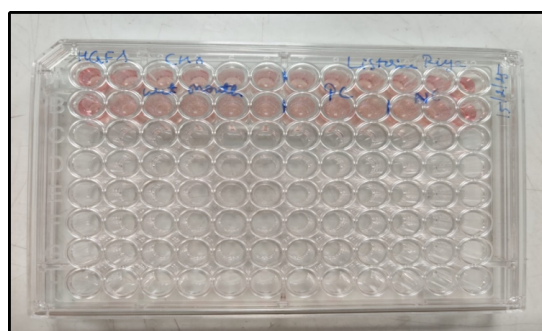
## Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

Human gingival fibroblasts (HGF) procured from NCCS Pune were grown in  $\alpha$ -MEM (pH 7.2) with 10% FBS, antibiotics, and amphotericin B at 37°C and 5% CO<sub>2</sub>. Cells ( $4 \times 10^4$ /well) were seeded onto 96-well plates and incubated for 48 hours. Next, mix 20  $\mu$ L of eluates or mouthwash with 100  $\mu$ L of medium and incubate for 24 hours. Controls: 2% CHX (positive); culture medium (negative). MTT solution (10  $\mu$ L, 5 mg/mL) was added and incubated for 3 hours. After dissolving formazan crystals in acidified isopropanol, absorbance was measured at 600 nm.

$$\text{Cell viability(\%)} = \frac{\text{Treated cells}}{\text{Negative control cells}} \times 100$$

Cytotoxicity was assessed (Utami et al., 2022)<sup>8</sup>

1. No cytotoxicity: cell viability more than 90%.
2. Slight cytotoxicity: cell viability 60%–90%.
3. Moderate cytotoxicity: cell viability 30%–59%.
4. Severe cytotoxicity: cell viability less than 30%



**Fig 3: Configuration of the 96-well flat-bottom plates. Eluates of Mini screws on the treatment solutions; (A) 0.2% Chlorhexidine gluconate mouthwash. (B) 0.2% Sodium fluoride mouthwash. 2% chlorhexidine gluconate as positive control (P) and complete medium**

**without treatment solution as negative control (N).**

### Metal Ion Analysis

Next, 15 mL plastic centrifuge tubes were cleaned by soaking in 10% nitric acid overnight and rinsed with deionized water. A total of 3 mL of culture media from each sub group was dispensed into a tube. Ti, Al, V and Fe ions were measured using Microwave Plasma atomic emission spectroscopy (MP-AES). Standard solutions (50, 100, and 200 mg/L) of each metal were prepared in DMEM for calibration. The mean of 3 values was calculated in ppm.

### Surface Analysis (SEM)

Surface morphology of miniscrew heads was examined before and after exposure using FEI Nova NanoSEM 450 at  $\times 5000$  magnification. Dried samples were mounted and analyzed for surface roughness and corrosion features.

### Statistical Analysis:

Data were entered in Microsoft Excel 2017 and analyzed using SPSS v26.0. Descriptive and inferential statistics were performed, and data normality was assessed. Intergroup comparisons were done using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at  $p < 0.05$  with a 95% confidence interval.

### Results:

The cytotoxicity, metal ion release, and surface roughness of titanium mini-implants were investigated. The MTT assay was used to determine cytotoxicity, and metal ion release (Ti, Al, V, Fe) was quantified by Microwave Plasma Atomic Emission Spectrometry (MP-AES) using

# Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

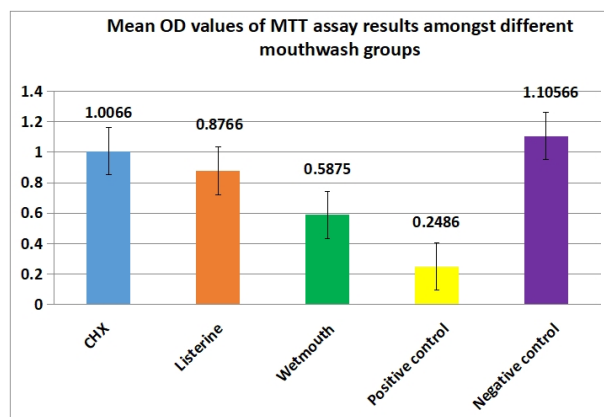
standard solutions (50, 100, and 200 mg/L) in DMEM. Surface roughness was qualitatively analyzed using a SEM (FEI Nova 450) at  $\times 5000$  magnification. Data were presented as mean  $\pm$  SD, and intergroup/intragroup comparisons were made using one-way ANOVA and Tukey's post hoc test ( $p < 0.05$ ).

The results of this study are presented under three categories of titanium mini-implants (TMIs) following immersion in different mouthwash solutions.

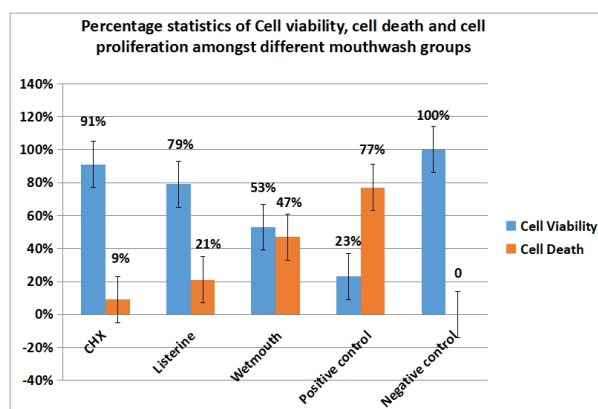
1. Cytotoxicity
2. Metal ion release
3. Surface roughness

## 1. Cytotoxicity Assessment

Cytotoxicity was evaluated using the MTT assay, and the optical density (OD) values were recorded. The mean OD values were highest in the chlorhexidine (CHX) group, followed by Listerine and Wet Mouth. A statistically significant difference was observed between the groups ( $p < 0.05$ ). The mean cell viability of Chlorhexidine group is 1.0066, Listerine group is 0.8766 and wet mouth is 0.5875. Compared to both the mouthwashes cell viability is more in Chlorhexidine group.



**Graph 1 - Mean OD values of MTT assay results amongst different mouthwash groups**



**Graph 2 - Percentage statistics of Cell viability, cell death, and cell proliferation amongst different mouthwash groups**

**Table 1. Percentage of cell viability and cell death**

Group	Cell Viability (%)	Cell Death (%)
CHX	91	9
Listerine	79	21
Wet Mouth	53	47
Positive Control	23	77

## Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

Group	Cell Viability (%)	Cell Death (%)
Negative Control	100	0

ANOVA revealed significant intergroup differences ( $p < 0.001$ ) among the mouthwash groups Post hoc Tukey's test indicated statistically significant differences between all groups ( $p < 0.05$ ).

### 2. Metal Ion Release

Metal ion concentrations (Al, V, Fe, and Ti) released into different solutions were measured using MP-AES. Listerine resulted in the highest ion release across all metals, followed by artificial saliva. The least ion release was seen with CHX.

**Table 2.** Mean metal ion concentrations (ppm) in each group

Group	Al (ppm)	V (ppm)	Fe (ppm)	Ti (ppm)
CHX	0.10	0.13	0.03	0.02
Listerine	0.42	0.19	0.31	0.05
Artificial Saliva	0.15	0.24	0.28	0.01

ANOVA confirmed significant differences in metal ion release for all metals ( $p < 0.05$ ) across the groups.

Tukey's post hoc test (Table 3) revealed:

- Significant differences in Al between CHX vs. Listerine and Listerine vs. Saliva.
- All groups differed significantly in V and Ti ion release.

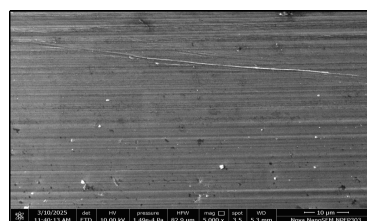
- Fe showed significant differences between CHX vs. other groups; Listerine vs. Saliva was not significant.

**Table 3.** Summary of significant pairwise comparisons ( $p < 0.05$ )

Metal	Significant Pairwise Comparisons
Al	CHX vs. Listerine, Listerine vs. Saliva
V	CHX vs. Listerine, CHX vs. Saliva, Listerine vs. Saliva
Fe	CHX vs. Listerine, CHX vs. Saliva
Ti	All pairwise comparisons significant

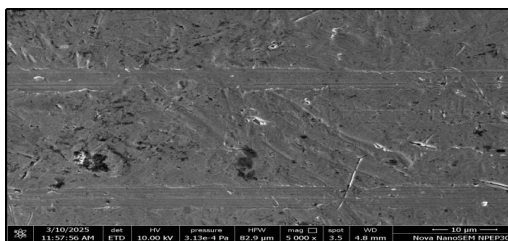
### 3. Surface Roughness (SEM Analysis)

Scanning Electron Microscopy (SEM) was performed to find any possible manufacturing flaws on the titanium mini-implants' (TMIs) surface before they were immersed in mouthwashes and artificial saliva. The SEM picture taken at 5000 $\times$  magnification, as seen in Figure 4, depicts the implant's initial surface shape and provides a baseline against which post-immersion alterations can be compared.

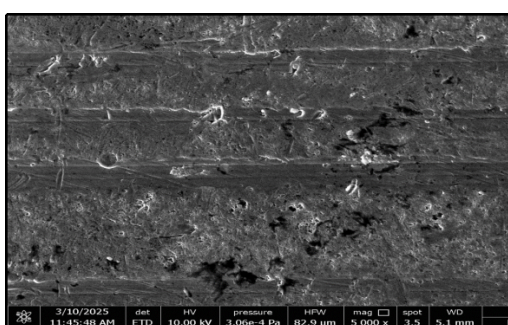


**Fig 4:** High-magnification SEM photomicrographs of titanium mini-implants without immersion ( $\times 5000$ ).

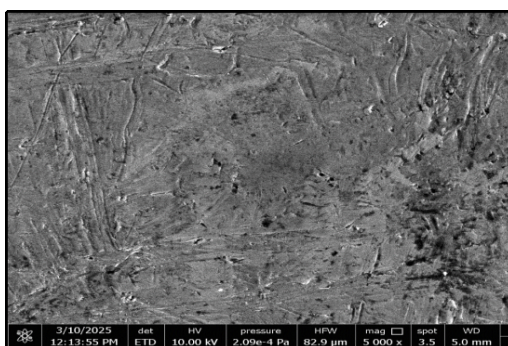
# Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study



• Fig 5: High-magnification SEM photomicrographs of titanium mini-implants with immersion in chlorhexidine mouthwash (×5000).



• Fig 6: High-magnification SEM photomicrographs of titanium mini-implants with immersion in Listerine mouthwash (×5000).



• Fig 7: High-magnification SEM photomicrographs of titanium mini-implants with immersion in Artificial Saliva (×5000).

Table 4. Summary of surface roughness changes after immersion

Group	Observations	Corrosion Severity
CHX	Slight roughness, minimal surface changes	Low
Listerine	Pits, cracks, dark spots, extensive corrosion	High
Artificial Saliva	Mild to moderate roughness and deposits	Moderate
Control	Uniform, no visible defects	None

SEM images supported quantitative findings from metal ion release, showing the **most surface degradation in the Listerine group.**

### Discussion:

Orthodontic treatment success depends on proper anchorage control, especially in complex cases where precise force delivery is required. Mini-implants (MIs), which have been used over the last two decades, have been a preferred way for achieving reliable three-dimensional anchorage due to their tiny size, ease of installation and removal, and reduced reliance on patient compliance.<sup>12,13,14</sup> Despite these benefits, MI success rates range from 70 to 95%, depending on bone density, closeness to adjacent roots, implant design, placement technique, and material composition.<sup>15</sup> Peri-implant inflammation has also been linked to implant loosening.<sup>16</sup>

## Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

For orthodontic patients with fixed appliances, maintaining oral hygiene can be difficult, which can result in gingival irritation, biofilm buildup, and enamel demineralization. Mouthwash formulations containing fluoride or chlorhexidine (CHX) are frequently suggested to reduce microbial colonisation.<sup>17, 18</sup> It is difficult to determine the exact contact duration between TMIs and mouthwash, but there is an assumption that in each 30–60 s, the mouthwash is present for 2–4 h. Orthodontic treatment is usually done in a long period, for about 1–2 years; therefore, 21 days is the arbitrary total duration assumption to immerse the TMIs into the mouthwashes<sup>9</sup>. Long-term exposure of TMIs to these solutions may impact metal ion release and corrosion, which may impact implant lifespan and cytotoxicity.<sup>19,20</sup>

CHX alone showed low cytotoxicity to gingival fibroblasts (<30%) according to the MTT experiment, whereas fluoride-containing solutions increased cytotoxicity, most likely because of higher metal ion release and dissolution of the TiO<sub>2</sub> protective layer. According to MP-AES measurement, fluoride solutions had the highest aluminum release (0.42 ppm), followed by artificial saliva (0.15 ppm) and CHX (0.10 ppm). All groups showed a minimal release of titanium ions, which is consistent with its low toxicity.<sup>23</sup> These results were supported by SEM examination, which revealed surface pitting and corrosion, especially in implants exposed to fluoride, whereas implants treated with CHX showed very minor surface alterations.<sup>24</sup> Similarly, Pavlic *et al* assessed corrosion of MIs in response to chlorhexidine and probiotic agents. They

reported that for patients undergoing orthodontic TMIs, CHX could be recommended for oral-hygiene maintenance.<sup>11</sup>

These findings are also consistent with earlier research showing that while CHX is somewhat safer for implant surfaces, fluoride ions can weaken titanium's protective oxide layer, create soluble titanium-fluoride complexes, and increase corrosion.<sup>25, 26</sup> According to clinical evidence, TMIs can be used with CHX-based mouthwashes without risk, although prolonged use of high-fluoride solutions may call for caution. The study is constrained by its in vitro methodology, though, as corrosion and ion release may be impacted differently by in vivo factors such as mechanical pressures, pH variations, and salivary flow. The significance of choosing the right oral hygiene products during orthodontic treatment is highlighted by the fact that CHX-based mouthwashes seem to preserve TMI biocompatibility, whilst mouthwashes containing fluoride may enhance cytotoxicity and corrosion. Some limitations of in vitro studies are that they are conducted under static conditions, which do not fully replicate the dynamic oral environment. Furthermore, more metal could release in real life because of the fluidity of saliva in the mouth and because oxide layers are removed by tooth brushing.

### **Clinical implication:**

Despite these limitations, chlorhexidine mouthwash appears safe for routine use with titanium mini-implants in orthodontic patients.

### **Conclusion**

## Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study

Chlorhexidine (CHX) mouthwash is gentle on titanium mini-implants, causing minimal corrosion and ion release while helping protect against infection. In contrast, fluoride-containing mouthwashes (NaF) promote corrosion and metal ion release. Surface analysis confirms that CHX preserves implant integrity better than NaF, making it the safer and more biocompatible choice for patients with mini-implants.

### References:

1. Thomas M. Orthodontics in the 'Art' of Aesthetics. *Int J Orthod Milwaukee* 2015;26(4):23–28.
2. Alves CB et al. Evaluation of cytotoxicity and corrosion resistance of orthodontic mini-implants. *Dent Press J Orthod* 2016;21(5):39–46.
3. Mohammadi A, Moghaddam SF. Influence of perioperative chlorhexidine mouthwash regimen on immediate failure rate of orthodontic miniscrews. *Iran J Orthod* 2010;5(3):100–104.
4. Chang HP, Tseng YC. Miniscrew implant applications in contemporary orthodontics Kaohsiung. *J Med Sci* 2014;30(3):111–115.
5. Ananthanarayanan V, Padmanabhan S, Chitharanjan AB. Ion release from different orthodontic mini-implants: an in-vitro study. *Aust Orthod J* 2016;32(2):165–174.
6. Kharadi L. Applications of mini-implants in orthodontics. *Int J Appl Dent Sci* 2021;7(2):558–560.
7. Capecchi D. The principle of action and reaction according to Newton. *Encyclopedia* 2023;3(2):705–720.
8. Utami WS, Anggani HS, Purbiati M. Cytotoxicity effect of orthodontic miniscrew-implant in different types of mouthwash: an in vitro study. *J Orthod Sci* 2022;12(4):11–15.
9. Alavi S, Ahmadvand A. Ion release and corrosion of titanium mini-implant surface in response to mouthwashes. *Dent Res J* 2021;18(2):32.
10. Nayyar A, Sood A, Chaudhry S, Kaur H, Khanna C, Pawar P. Titanium Implant Corrosion and Peri-Implant Disease: Current Concepts and Future Directions. *Oral Sphere J. Dent. Health Sci.* 2026;2(1):47-51.
11. Pavlic A, Turco P, Contardo L, Spalj S. Do chlorhexidine and probiotic solutions provoke corrosion of orthodontic mini-implants? *Int J Oral Maxillofac Implants* 2019;33(3):1379–1388.
12. Gainsforth BL, Higley LB. Use of metal implants as a means of anchorage in orthodontics. *Am J Orthod Oral Surg*;1945; 31:418–426.
13. Brookes ZL, et al. Effects of chlorhexidine mouthwash on the oral microbiome. *J Dent Sci* 2021; 113:61–68.
14. Charoenpong H, Ritprajak P. Effect of metal ions released from orthodontic mini-implants on osteoclastogenesis. *Dent Med Probl* 2021;58:327–333.
15. Das A, Karande V, Tripathi A, Thomas LR, Pasha Z, Malhotra V, Kashwani R. Palatal bone thickness for mini-implant placement in different skeletal facial patterns: A CBCT approach. *Bioinformation.* 2025 Apr 30;21(4):635-641.
16. Caetano PL, et al. Corrosion resistance and surface characterization of miniscrews removed from orthodontic patients. *Maxillofac* 2019;60:1–7.

## **Effect of Commonly Used Mouthwashes on Titanium Temporary Anchorage Devices in Terms of Surface Roughness, Cytotoxicity and Metal Ion Release - An In Vitro Study**

17. Milošev I, et al. Fluoride ions and corrosion behavior of Ti alloys in artificial saliva. *Acta Chim Slov* 2013;60:543–555.
18. Danaei SM, et al. Ion release from orthodontic brackets in mouthwashes: an in vitro study. *Am J Orthod Dentofac Orthop* 2011;139:730–734.
19. Kang SH, et al. Fluoride-containing solutions on titanium orthodontic brackets. *Am J Orthod Dentofac Orthop* 2008;133:692–698.
20. Pavlic A, et al. Corrosion behavior of TMIs exposed to chlorhexidine. *Int J Med Res Health Sci* 2016;5:23–31.
21. Chellat H, et al. Cytotoxicity of chlorhexidine on fibroblast cell lines. *J Dent Res* 2022;101:789–797.
22. Quaranta A, et al. Corrosion of titanium alloy discs in 0.2% chlorhexidine mouthwash. *J Mater Sci Mater Med* 2010;21:2203–2211.
23. Bhola S, et al. Electrochemical study of chlorhexidine-induced corrosion on titanium. *J Appl Biomater Funct Mater* 2013;11:124–130.
24. Newbury DE, Ritchie NA. Elemental microanalysis using SEM/EDS. *J Mater Sci* 2012;47:1293–1306.
25. Abboodi HH, Al-Dabagh DJ. Assessment of metal ions released from orthodontic mini-implants in fluoridated mouthwashes. *Int J Med Res Health Sci* 2018;7:156–164.
26. Hwang CJ, et al. Effect of fluoride on corrosion resistance of titanium alloy. *Korean J Orthod* 2001;31:306–312.