The Cytotoxic Effect of Titanium Oxide Surface Modified Orthodontic Stainless Steel Wires

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Abstract

Background: The fixed orthodontic mechano therapy has been associated with white spot lesions and plaque accumulation. Titanium oxide (TiO\textsubscript{2}) is a compound that possesses clinically significant anti-microbial action especially against lactobacillus and streptococci. However the safety of the TiO\textsubscript{2} coated wires has still not been tested. In this study, we evaluated the cytotoxic effects of TiO\textsubscript{2} coated stainless steel orthodontic wires. To assess the same, we used A549 cells as experimental cell line. Cells were categorized into 4 groups (n=6/group): cellular control group, represented by the cell growth; negative control group (stainless steel wire) positive control group (hydrogen peroxide), experimental group (titanium oxide coated stainless steel wires). The cultures were carried out either on 6 well plates or 96 well plates and pictures were captured using a microscope or assessed by MTT assay.

Results: MTT assay revealed no cytotoxic effect by TiO\textsubscript{2} coated wires as compared to control. Similarly, structural assessment of cellular morphology and nuclear membrane structure further showed no change in toxic effects of TiO\textsubscript{2} coated SS wires on the cells.

Conclusion: Despite the limitations of this study, we demonstrated that titanium oxide coated wires had no cytotoxic effects like uncoated stainless steel wires and that there is a strong demand for long-term studies of TiO\textsubscript{2} coated wires and brackets, with a strong focus on the cytotoxic properties, concentrations and exposure times, to make the desired applications for TiO\textsubscript{2} coated wires safe and suitable for orthodontic use.

Keywords: Titanium oxide coated stainless steel orthodontic wires; A549; Stainless steel orthodontic wires; Sputtering

Introduction

The fixed orthodontic mechano therapy has been associated with white spot lesions and plaque accumulation, the reason for this being the bacterial adherence and accumulation around the brackets [1] and wires placed onto the tooth surfaces.

Titanium oxide is a compound that possesses clinically significant anti-microbial action, especially against lactobacillus and streptococci [2,3]. The titanium oxide's antibacterial effect is based on its photo catalytic property [4]. The effectiveness of titanium oxide surface modified stainless steel brackets in reducing the bacterial adherence and the accumulation of bacteria around these brackets and wires has been established [2-4].
adherent to the metal surface rather than in its free NP state (Figures 1-5).

Nowadays there are many studies on the biocompatibility of orthodontic materials because this is a real concern for clinicians, who do not want to place orthodontic appliances with a risk of adverse toxic effects in their patients. Till now no study has been undertaken to evaluate the cytotoxic effect of titanium oxide surface modified stainless steel wires. This study intended to evaluate and find out the toxic effect of these wires on the human cells: cellular behavior and viability and the relative patient safety factor in using these wires.

**Materials and Methods**

**Preparation of photocatalytic titanium dioxide coated orthodontic wires**

Surface modification of stainless steel orthodontic wires (OPTIMA 19*25) with TiO₂ were carried out using Tecport Sputter Coater by sputtering method in Indian Institute of Science, Bangalore.

Sputtering processes remove surface atoms or molecular fragments from a solid cathode (target) by bombarding it with positive ions from an inert gas (argon) discharge and deposit them on the nearby substrate to form a thin film [3].

In the present study, sputtering was carried out on stainless steel orthodontic wires (substrate) using Titanium as the target. Plasma generated inside the vacuumed chamber ejected surface atoms from the titanium target, which were sputtered onto sputtering was conducted for a period of 20 min. All wires were sputtered at the same time to achieve a thin and uniform coating of titanium. The sputtered titanium was further oxidized in the stainless steel wires (substrate). The distance between the substrate and the target was kept constant at 7 cm, and an ambient environment inside an open air furnace at 500°C for 5 h to provide a uniform coating of TiO₂ on the stainless steel orthodontic wires.

**Evaluation of cytotoxicity**

This is an in vitro cytotoxicity test. Tests were performed in cell cultures lineage A549 Cell Line human lung carcinoma (ATCC-American Type Culture Collection) to evaluate the response rates, determined by MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assays.
Figure 5: Percentage cell viability in different groups.

The specimens were divided into 4 groups (n=6): cellular control group, represented by the cell growth; negative control group (stainless steel wire), whose material does not produce a cytotoxic response [10]; positive control group (hydrogen peroxide), whose material is highly cytotoxic; experimental group (titanium oxide coated stainless steel wires).

Cells were manipulated in the laboratory of cellular and molecular biology at the Defense Institute of Physiology and Allied Sciences, Defense Research and Development Organization (DRDO), New Delhi, India. Cells were defrosted and cultured in Dulbecco modified eagle medium supplemented with 10% bovine fetal serum, 100 U per milliliter of penicillin, 100 mg per milliliter of streptomycin, and 50 mg per milliliter of gentamycin (complete Dulbecco modified eagle medium) in culture plates. The cells were incubated at a temperature of 37°C in an incubator (Thermo Scientific™) containing 5% carbon dioxide. After confluence of cells was obtained, the cells were removed by enzymatic action by using 0.1% trypsin ethylenediaminetetraacetic acid (Sigma-Aldrich Corporation, St Louis, Mo) and counted in a Neubauer chamber (Optik Labor, Friedrichsdorf, Germany). Then the medium was removed, and formazan crystals were dissolved with 120 mL per well of dimethyl sulfoxide (Sigma-Aldrich Corporation, St Louis, Mo), generating a blue color [11]. After 24 hours, 200 mL of MTT was added to each well of the plate, followed by 4 hours of incubation at 37°C and 5% carbon dioxide. Then the medium was removed, and formazan crystals were dissolved with 120 mL per well of dimethyl sulfoxide (Sigma-Aldrich Corporation, St Louis, Mo), generating a blue color. Optical densities were measured at 570 nm in an ELISA reader, and cell viability was calculated according to the following formula:

Cell viability% = optical density of test group / optical density of cellular control group * 100

Table 1: Comparison of the cell viability in the different groups.

| Groups                          | Mean ± SD  | F     | p    |
|---------------------------------|------------|-------|------|
| Cellular control group          | 2.18 ± 0.04| 89.256| 0.00*|
| Titanium oxide coated stainless steel wires | 2.13 ± 0.13|       |      |
| Stainless steel                 | 2.19 ± 0.10|       |      |
| Hydrogen peroxide               | 0.90 ± 0.27|       |      |

*Statistically significant, p<0.05

The collected data were analyzed with IBM SPSS software (version 22). To describe the data, descriptive statistics—means and standard deviations—were used. To find the significant difference between the groups one way ANOVA and Post hoc Tukey HSD test was used. In all these statistical tools, the probability value of 0.05 was considered to be significant.

Results

Titanium oxide coated orthodontic stainless steel wires did not affect cellular viability, to evaluate cell viability we performed MTT
assay on A549 cells. Grouping of samples was done as 4 groups (n=6): cellular control group, represented by the cell growth; negative control group (stainless steel wires), whose material does not produce a cytotoxic response [10] positive control group (hydrogen peroxide), whose material is highly cytotoxic; experimental group (titanium oxide coated stainless steel wires). When the comparison of the cell viability was done it showed a significant difference between all the groups with almost similar response seen in Stainless steel and cellular control group followed by titanium oxide coated wires. Hydrogen peroxide showed the least cell viability (Table 1 and Figure 4). However when the inter group comparisons were done it was seen that there was a significant difference between titanium oxide coated wires and other groups (Tables 2 and 3). When the comparison of the optical density was done it showed a significant difference between all the groups with similar mean values seen in Stainless steel and cellular control group followed by titanium oxide coated wires. Hydrogen peroxide coated wires showed the least cytotoxic response (Table 2, Figure 4). However when the inter group comparisons were done it was seen that there was a significant difference between titanium oxide coated wires and other groups (Table 4). Titanium oxide coated wires or stainless steel wires showed no observable change in cellular viability as compared to cellular control group. However, a significant decrease in cellular viability of hydrogen peroxide group was approximately 67% as compared to control (Figure 5).

The cytotoxic effect of titanium oxide coated orthodontic stainless steel wires did not affect cellular morphology

To assess any epithelial/mesenchymal transition [EMT] and cytoskeletal changes leading to altered cellular morphology, we evaluated the change in cellular structure, integrity and viability under inverted microscopy. The pictorial representations of all groups has been shown in Figure 2 wherein a marked cell death can be seen in Figure 30/peroxide group as compared to [a, b]. The pictures were captured using OlympusBX 51 inverted microscope at 10X.

Titanium oxide coated orthodontic stainless steel wires did not alter nuclear structure; the representative images of cells stained with DAPI (4',6-diamidino-2-phenylindole) as shown in Figure 3 demonstrate a marked structural change in nuclei of hydrogen peroxide group. However, we did not observe any structural change in nuclei of other groups. Hydrogen peroxide is used as a positive control group to induce cellular and nuclear damage. We observed nuclear membrane lysis and loss of nuclear membrane structural integrity in hydrogen peroxide group as shown in subset of Figure 3 [d]. Whereas, no such changes were observed in experimental group as compared to positive control group. The images were captured using Olympus BX51 at 40X and subsets were manually zoomed to 100X using Image J [NIH] software.

### Table 2: Comparison of the optical density in the different groups.

| Groups                              | Mean ± SD | F       | p       |
|-------------------------------------|-----------|---------|---------|
| Cellular control group               | 2.10 ± 0.04 | 85.111  | 0.00*   |
| Titanium oxide coated stainless steel wires | 2.03 ± 0.14 |         |         |
| Stainless steel                     | 2.10 ± 0.09 |         |         |
| Hydrogen peroxide coated            | 0.83 ± 0.27 |         |         |

*Statistically significant, p<0.05

### Discussion

The photocatalytic activity of illuminated TiO₂ has been actively investigated in diverse areas such as water-treatment processes, air-cleaning agents, and antibacterial agents. Illuminated TiO₂ in water with light at a wavelength less than 380 NM generates excess electrons in the conduction band (e⁻ sub) and positive "holes" in the valence band (h⁻vb). At the TiO₂ particle surface, the holes react with either adsorbed water or surface hydroxyl (OH⁻) groups to form HO⁻ radicals. The excess electrons in the conduction band react with molecular oxygen to form superoxide ions, which form more HO⁻ radicals. In aqueous systems, the complete mineralization of many organic substances is possible when a sufficient HO⁻ flux can be generated in situ. Therefore, suspended TiO₂ particles have largely been used as efficient catalysts for the decomposition of organic contaminants [4].

Several attempts have been made to apply the photocatalytic activity of TiO₂ to microorganisms [12-14] and same has been successfully achieved in the field of orthodontics by proving there is a significant antibacterial effect of titanium oxide against lactobacillus acidophilus which is one of the main colonizing bacteria in plaque around the orthodontic fixed appliances [3].

However, the cytotoxicity of titanium oxide is a concern for its safe use. Its cytotoxicity has been proved and disapproved in various studies under various clinical circumstances and under various differing variables [5,6,8,9].

It is commonly accepted that titanium alloying elements are biocompatible [15]. Perhaps the oxidation of titanium increases the cytotoxicity. Some researchers have monitored the cytotoxicity by evaluating the adhesion, proliferation, and metabolism of cells, such as 3T3, L929, and W138, and human fibroblasts and osteoblasts [16,17]. Our study was similar to the study of Grill et al. [16], who advocated the investigation of proliferation by microscopic analysis of cell growth and division and cell viability assessment by the MTT assay. According to our findings, no significant cell modifications indicate no cytotoxicity of titanium coated stainless steel wires used in orthodontics. Li et al. [15] hypothesized that cytotoxicity is linked to the ions released from the metals and established that a safe molybdenum ion concentration (below which the ion concentration is nontoxic) is 8.5 mg per liter [15]. So, by the extension of same principle we hypothesize that the concentration of release of titanium oxide coating was below the optimal toxic levels.
The limitation of our study is with respect to its sample size. So, it is essential to continue these studies to assess the biocompatibility of these coated wires or otherwise if found cytotoxic to any extent under any other lab conditions, that amount of cytotoxicity needs to be evaluated. However the results which we achieved in our study, if passed the test of time could open doors for the development of newer antibacterial orthodontic appliances without any fear of any cytotoxic risk to the patient.

**Conclusion**

We demonstrate for the first time, despite the limitations of this study, that titanium oxide coated wires had no cytotoxic effects like uncoated stainless steel wires. This suggests that TiO$_2$ coated wires don't possess a potential health risk and that there is a strong demand for long-term studies of TiO$_2$ coated wires and brackets, with a strong focus on the cytotoxic properties, concentrations and exposure times, to make the desired applications for TiO$_2$ coated wires and brackets safe and suitable for orthodontic use.

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**Authors contributions**

1. Sameer Ahmad Malik – corresponding author, conducted most of the research work, supervised the lab procedures and compiling the results and preparing the manuscript.
2. Laxmikanth SM – Inception of the idea of this research work. All research work done under his guidance.
3. Ramachandra CS – Designed and handling of the statistical data.
4. Shetty S – Contributed towards technical considerations in the sputtering of arch wires.
5. Reddy VS – Assisted in the lab procedures with certain thoughtful inputs on the cell culture growth and microscopy.

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