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Original research

Quantitative determination and antibacterial properties of TiO_2 nanoparticle-doped glass ionomer cement: an in vitro study

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Purpose

The aim of the present study is to determine the amount of titanium ions released into the artificial salivary medium by modified glass ionomer cement (GIC) doped with 3% and 5% (w/w) titanium dioxide nanoparticles (TiO_2-NPs), and to evaluate their antibacterial properties.

Materials and Methods

120 cylindrical discs with a diameter of 4 mm and a height of 6 mm were made with 3% and 5% w/w modified GIC containing TiO_2 NPs, divided into two groups of 60, and immersed in a chemically synthesized salivary medium. The samples were quantified over four-time periods: 24 hours, two months, four months, and six months, using inductively coupled plasma mass spectroscopy (ICP-MS), antibacterial properties were evaluated by means of colony forming count (CFU) method.

Results

The amount of titanium ions released from the discs that received 3%(w/w) TiO_2 was highest in the first two months, with no significant release at successive intervals. Also, the second group, which included 5% (w/w) TiO_2, saw a considerable ion release at every interval, with the second month seeing the maximum release. The levels in the 5% (w/w) group were consistently higher when the two concentrations were compared at each of the four time points, indicating a considerable increase in titanium release and antibacterial property with a concentration increase from 3% to 5%.

Conclusion

3% and 5% (w/w) concentrations may be considered safe and exhibit significant antimicrobial effect, titanium ions were discharged at higher rates in 5% (w/w) modified GIC containing TiO2-NPs than in 3% (w/w) modified GIC containing TiO2-NPs.

Keywords: *Antibacterial, Glass ionomer cement, Ion release, saliva, Titanium dioxide.*

Introduction

A common complication in orthodontic patients is the demineralization of enamel surfaces during fixed orthodontic treatment. Plaque accumulation is aided by fixed orthodontic attachments, which provide retentive areas. Streptococcus mutans and Lactobacillus cause a rapid shift in plaque microflora, resulting in an acidogenic environment. These acidic byproducts in plaque are responsible for subsequent enamel demineralization and the formation of white spot lesions (1, 2).

For many years, the orthodontic bonding material glass ionomer cement (GIC) has been used to bond and band orthodontic equipment to tooth structures. By including glass fiber, metals, resin, and zirconia particles, GIC's mechanical qualities have been enhanced (3). Silver, zinc, titanium, and silica nanoparticles are employed to enhance the antibacterial characteristics of bonding agents (4). Dental materials with silver nanoparticles offer great antibacterial characteristics with-

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out altering mechanical properties. Still, they also have drawbacks, including decolorizing the resin matrix, which makes them seem unattractive and makes them poisonous (5,6). Most recently, titanium dioxide-based metal nanoparticles received a lot of interest because of their attractive look, photocatalytic performance, and low toxicity (7).

In a recent study, titanium dioxide nanoparticles (TiO_2-NPs) were added to conventional GIC powder in various concentrations (3%, 5%, and 7% w/w), and it was shown that the addition of 3% and 5% w/w TiO_2-NPs enhanced the mechanical characteristics compared to traditional GIC. On the other hand, 7% weight-weight TiO_2 NP addition caused a loss of mechanical characteristics. The decrease in bond strength could be due to agglomeration of particles, which creates defect sites and disrupts the curing process (8,9). The investigation concluded that a potential bonding agent is 3% and 5% w/w TiO_2-NPs added to a typical GIC powder. However, prior research concentrated mostly on nanoparticles' antibacterial effects and mechanical capabilities, with relatively few studies in the orthodontic literature reporting on the ion release from the modified composite resins (10,11).

The amount of metal ions released by orthodontic equipment and adhesives must thus be taken into consideration in light of the aforementioned. This atomic absorption spectroscopy can be measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled plasma mass spectrometry (ICP-MS) atomic absorption spectroscopy (AAS). ICP-MS can now accurately complete routine multi-element determinations in biological samples and other matrices because of its enhanced sensitivity and resilience (12, 13).

Evaluation of the amount of titanium dioxide ions released into the synthetic salivary medium from GIC powder doped with 3% and 5% (w/w) titanium dioxide nanoparticles was unprecedented in the current study. The null hypothesis of the study is that there is no significant difference in the rate of titanium release and antibacterial properties between TiO2 NPs impregnated in GIC at 3% and 5% concentrations.

Materials and Methods

Study design

This in vitro study was performed to evaluate the antibacterial activity and quantification of TiO2 ions in modified GIC.

Sample size calculation

Using the G Power 3.1 software, sample size analysis was carried out. The power of this investigation was 80% at a 5% level of significance and an error of = 0.05. 120 observations spread across four distinct time intervals were needed to achieve the predicted effect size of 0.9. Two groups were created, one with GIC and 3% TiO_2 NPs (Group A, n=60), and the other with GIC and 5% TiO_2 NPs (Group B, n=60) of discs. Additionally, each group was split into four separate n=15 subgroups for each of four distinct time periods (i.e., 24 hours, 2 months, 4 months, and 6 months) (6).

Materials used in the study

A commercially available conventional GIC (GC Fuji II, Pyrax Polymers, Roorkee, India) and Titanium dioxide nanoparticles (TiO_2-NPs Anatase, Dry Powder form, Average particle size: 20-30nm, Purity: 99.9%, Nano Research Lab, Jamshedpur, Jharkhand, India) were used in the study.

Equipments used in the study

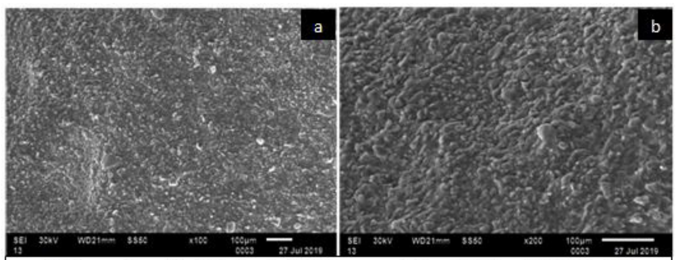
Vortex shaker, Generic, Elecoptomaufacturer, India (120-240 V, 50/60 Hz), speed range 0- 2000 rpm was used for mixing the GIC with TiO_2 nanoparticles. A scanning electron microscope (JEOL JSM-661 OLV, Tokyo, Japan) was used to assess the uniform distribution of nanoparticles in GIC at Advanced Analytical Laboratory, Andhra University, Visakhapatnam, Andhra Pradesh, India (Figure 1). Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Andhra University, Visakhapatnam was used for quantification of ions. Mueller Hinton agar plate, (HI-Media lab pvt.ltd., Mumbai, India) was used to evaluate the anti-bacterial activity for S. mutans.

Artificial saliva formulation

Artificial saliva composition included 0.381g NaCl, 0.213 g CaCl2.2H2O, 1.114 g KCl, 0.738g KH2PO4, and 2.2g mucin in 1000 ml distilled water and pH 7.

Preparation of 3 % and 5% GIC modification with TiO_2-NP's

To produce 3% modified glass ionomer powder, 9 mg of GIC powder and 0.3 mg of TiO2 NPs should be mixed in a high-speed vortex shaker at 1200 rpm. Similarly, to create 5% modified nano glass ionomer powder, 9 mg of GIC powder and 0.5 mg of titanium powder were mixed in a high-speed vortex shaker at 1200 rpm (14). Scanning electron microscopy (SEM) was also used to analyze the homogenous dispersion of nanoparticles within the glass ionomer pow-



SEM image of modified GIC doped with TiO_2 nanoparticles at 200X a) at 3%; b) at 5%

Figure 1. *SEM image of modified GIC doped with TiO_2 nanoparticles at 200x 3% and 5%.*

der at 200X magnification (Figure 1).

Disc preparation of 3 % and 5% modified GIC with TiO_2-NP's

According to the manufacturer's instructions, glass ionomer cement was blended in a 1:1 powder-to-liquid ratio, and the modified GIC mix was then shaped into discs that were 4 mm in diameter and 6 mm high. Samples were submerged in 10 ml of artificial saliva at a temperature of 37^0

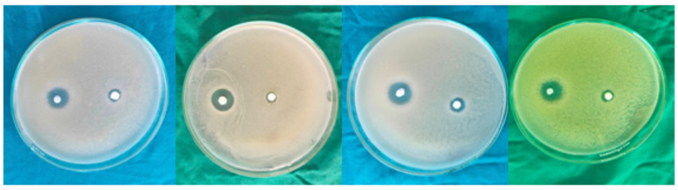
C for four intervals of time after that. Utilizing inductively coupled plasma mass spectrometry, TiO_2 was quantified (ICP-MS)

Quantification of TiO_2 nano particles

Samples with 3% w/w and 5% w/w concentrations of modified GIC discs were isolated from the artificial saliva at 24 hours, 2 months, 4 months, and 6 months. Transfer the salivary medium into separate tubes. The samples were centrifuged at 3200rpm for 15 minutes at -5^{0}C using a centrifuge (REMI C23 PLUS) to achieve impurity-free samples. Samples are diluted with distilled water from 10000 ppm (3% wt/wt) to 50000 ppm (5% wt/wt) to 100 ppb and 50 ppb, respectively, after centrifugation. The PPB range for ICP-MS quantification is limited 0-100. To remove organic materials and eliminate interference from other elements by the detector, the generated 100 PPB and 50 PPB through the dilution process were stabilizedwith supra pure HCL in ICP-MS.

Assessment of TiO_2antibacterial activity

A sterile inoculated loop was used to spread the material onto a blood agar plate with aseptic techniques to prevent growth contamination. To assess the antibacterial activity, the blood agar plates were streaked and then incubated for 24 hours at 37 degrees Celsius in an incubator. After 48 hours, colony-forming units (CFUs) of |S. mutans were counted in both the groups. (Figure 2).



Colony forming units (CFU's) at 24 hours, 2 months, 4 months and 6 months of 3% (right side) and 5% (left side) modified GIC doped with TiO_2

Figure 2. *Antibacterial activity of GIC doped with TiO_2 nanoparticles on colony forming units (CFU's).*

Statistical analysis

Statistical Package for Social Sciences SPSS(ver.26.0,IBM, Armonk, NY, USA) was used to analyze the data and repeated measures of analysis of variance (ANOVA) for multiple groups and independent sample T-test were used for pairwise comparisons. The confidence interval was set to 95% and p values less than 0.05 were considered significant.

Results

A total of 120 discs with GIC were impregnated with 3% (w/w) and 5% (w/w) titanium dioxide nanoparticles, and each sample was placed in a 10 ml artificial salivary medium. The time frames were 24 hours, 2 months, 4 months, and 6 months, and the ion release from the modified GIC discs was calibrated using ICP-MS, and antibacterial properties were evaluated using a colony counting machine. The mean titanium dioxide release from 3% w/w TiO_2 nanoparticles containing GIC (Group -A) was highest between the first and second intervals (0.33 ±0.54), which was statistically significant (p=0.05*). Nonetheless, there was no statistically significant release between the third and fourth intervals. (p=0.28) (Table 1). At the second interval (0.59 ± 0.02, p=0.001), the mean titanium dioxide release from 5% w/w nanoparticles containing GIC (Group-B) was significantly higher. However, at the third and fourth intervals (p=0.001*), the mean scores decreased significantly (Table 2). At all periods, the mean titanium dioxide release in Group B was significantly higher than in Group A. The second interval (p=0.001*) seemed to have the highest mean difference (0.59) (Table 3). The antibacterial test showed a statistically significant difference in CFU/ml of S. mutans in the experimental group between the four periods. There was a decrease in CFUs from first to second interval slight increase in colony counts from second to third interval and further increase from third to fourth interval. Pair-wise intergroup comparison amongst four intervals showed statistically significant p < 0.05 (Table 4) (Figure 3).

Table 1. *Comparison of TiO_2 release from 3% w/w nanoparticles containing GIC*

Time Frame	N	Mean(PPB)	SD	P value
24 hours	15	0.069	0.006	**0.001***
2 months	15	0.333	0.544	
4 months	15	0.274	0.001	
6 months	15	0.187	0.199	

* statistically significant

Table 2. *Comparison of TiO_2 release from 5% w/w nanoparticles containing GIC*

Time Frame	N	Mean(PPB)	SD	P value
24 hours	15	0.157	0.013	0.001*
2 months	15	0.595	0.022	
4 months	15	0.469	0.016	
6 months	15	0.337	0.019	

Repeated measures ANOVA, * statistically significant, PPB: Parts Per Billion

Table 3. *Comparison of means between 3% w/w TiO_2 nanoparticles and 5% w/w TiO_2 nanoparticles containing GIC*

Time Frame	N	1% w/w TiO_2 nanoparticles	5% w/w TiO_2 nanoparticles	P value
24 hours	30	0.069	0.157	0.03*
2 months	30	0.333	0.595	0.001*
4 months	30	0.274	0.469	0.02*
6 months	30	0.187	0.337	0.03*

Independent sample T test, * statistically significant.

Table 4. *Paired comparison of colony counts for 3% w/w and 5% w/w modified GIC*

Time interval	Concentration	Mean±SD	SD Error	value
24 hours	3% w/w	7.42±0.73	0.16	0.001*
	5% w/w	10.71± 0.48	0.28	
2 months	3% w/w	3.23±0.64	0.14	
	5% w/w	4.83± 0.56	0.32	
4 months	3% w/w	4.56±0.69	0.19	
	5% w/w	6.79±0.61	0.38	
6 months	3% w/w	5.36±0.87	0.22	
	5% w/w	8.19±0.75	0.41	

*sign*significant difference.ificant difference.

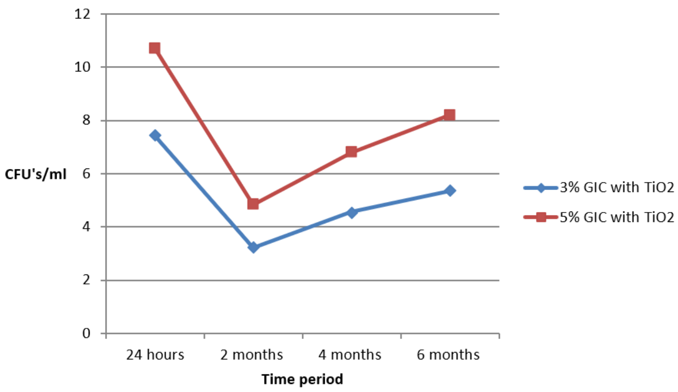


Figure 3. *Mean comparison of CFU's at four time intervals of 3% and 5% modified GIC with TiO_2.*

Discussion

The null hypothesis of the study was rejected, as there was a significant difference in the rate of titanium release and antibacterial properties between GIC impregnated with TiO2 NPs at 3% and 5% concentrations. During orthodontic treatment with fixed appliances, white spot lesions often develop close to the bands and brackets. Bacteria can adhere more effectively, survive longer, and cause more damage thanks to the scaffolding provided by the extracellular polymeric matrix in which they reside. Streptococcus mutans glucosyltransferases (GTFs) can facilitate biofilm development by producing extracellular glucans (15,16). Antimicrobial adhesives that reduce the expression of these GTF genes may help to prevent tooth decay. To prevent the interaction between these factors, it is essential to maintain good dental hygiene by physically removing plaque and applying fluoride or antimicrobial substances that enhance enamel and dentin resistance while limiting bacterial metabolism (17,18). Since patient participation is a challenge (19,20), many practitioners prefer techniques that rely on professional application rather than patient compliance.

Orthodontic bonding materials now have better mechanical and bactericidal properties due to nanotechnology, which involves changing matter atom by atom. They can be included in orthodontic adhesives/cements or coated onto the surfaces of orthodontic appliances to reduce microbial adhesion or enamel demineralization during orthodontic therapy (21). Titanium dioxide nanoparticles (TiO2 NPs) are the most commonly used nanoparticles in dentistry because of their low toxicity and antibacterial qualities (22). TiO2 nanoparticles break down organic molecules when exposed to non-lethal UV radiation by creating hydroxyl radicals and superoxide ions (23,24). The physical and chemical characteristics of standard orthodontic adhesives and equipment should not be affected when nanoparticles are introduced. However, safety must be ensured for a medically appropriate amount of time (21).

Although studies on metal ion release from orthodontic appliances into biological fluids ruled out the possibility of toxic concentrations, even nontoxic levels may cause biological changes in the oral mucosa, such as altered cellular functions, decreased DNA synthesis, and enzyme inhibition. Additionally, orthodontic adhesives must be biocompatible for clinical use in contact with gingival and oral tissues (25,26).

According to Baranowska *et al.* (13), TiO2 nanoparticles have been found to accumulate in the blood (1042 mg/kg bw/day), liver, heart (62.5, 125, and 250 mg/kg bw over 30 days), and spleen (0.324, 648, 972, 1296, 1944, 2592 mg/kg for 24 h, 48 h, 7, 14 days) (22). Some TiO2 nanoparticles have been shown to have inflammatory, oxidative, and genotoxic effects, and as a result, TiO2 has been designated as potentially carcinogenic to humans by the International Agency for Research on Cancer (IARC) (13).

After the materials had fully set, the amounts of ions released into the storage solution were monitored at predetermined intervals using inductively coupled plasma (ICP) analysis (12). The multi-element capacity of inductively coupled plasma mass spectrometry (ICP-MS) enables the simultaneous measurement of many elements in a single analysis, making it a preferable option for tracking element concentrations in solution with less sample preparation and a quicker analysis time (27). In this study, the quantity of titanium released from GIC with two distinct titanium dioxide concentrations (3% w/w and 5% w/w) was monitored over time. Both concentrations had significant antimicrobial activity and adequate shear bond strength, as shown in previous research. Therefore, the analysis was performed on the titanium released from these concentrations with proven qualities, considering both the allowable amounts of titanium and its antibacterial capabilities. Previous studies have demonstrated that the body may react negatively to titanium at levels between 10 and 50 mg/kg (11, 12, 28).

The quantity of titanium that leached out of adhesives at both dosages in the current investigation was substantially lower (about 0.0001-0.0003 mg/kg) than the amount required to cause the aforementioned negative effects discovered in prior studies (22,29). This was observed in both groups at all intervals. Although studies comparing the exact quantities of released titanium in saliva exist, the levels observed in our experiment using 3% and 5% TiO2 concentrations were safe and within allowed limits. The experimental group's antibacterial properties exhibited an increase in antibacterial activity from 24 hours to 2 months. However, there was a decrease in ion release, and the effect on antibacterial properties began to decline from the third and fourth intervals, i.e., 4 months and 6 months, respectively, after impregnation of nanoparticles in GIC. This is because the nanoparticles tend to clump together within the bonding agents. The findings are consistent with the research conducted by Nasalapur *et al.* (30).

Conclusion

Based on the study's results, the release of ions was found to be higher at the second interval (second month) than the first, gradually decreasing at the third and fourth intervals (fourth and sixth months). Additionally, the release of titanium was higher in modified GIC with 5% weight TiO2-NPs compared to 3%. This in vitro study concluded that 1% and 5% concentrations of TiO2-NPs can be considered safe and exhibit strong antibacterial effects.

Türkçe özet: *Titanyum dioksit nanopartikül ilave edilmiş cam iyonomer çimentosunun in vitro olarak tükürük salınım miktarının nicel olarak belirlenmesi ve antibakteriyel özelliklerinin incelenmesi. Amaç: Bu çalışmanın amacı, %3 ve %5 (w/w) titanyum dioksit nanoparçacıkları (TiO2-NP) ile modifiye edilmiş cam iyonomer simanların (GIC) yapay tükürük ortamına saldıkları titanyum iyon miktarını belirlemek ve antibakteriyel özelliklerini değerlendirmektir. Gereç ve yöntem: 4 mm çapında ve 6 mm yüksekliğinde, TiO2 NPs içeren %3 ve %5 w/w modifiye GIC ile yapılmış 120 silindirik disk, 60'lık iki gruba ayrıldı ve kimyasal sentezlenmiş bir tükürük ortamında batırıldı. Örnekler, plazma kütle spektroskopisi (ICP-MS) kullanılarak 24 saat, iki ay, dört ay ve altı ay boyunca dört zaman aralığında ölçüldü. Antibakteriyel özellikler, koloni oluşturma sayısı (CFU) yöntemi kullanılarak değerlendirildi. Sonuç: %3 (w/w) TiO2 içeren disklerden salınan titanyum iyon miktarı, ilk iki ayda en yüksek düzeydeydi ve ardışık aralıklarda önemli bir salınım olmadı. Ayrıca, %5 (w/w) TiO2 içeren ikinci grupta, her zaman aralığında önemli bir iyon salınımı görüldü ve ikinci ay en yüksek salınımı gösterdi. Her dört zaman noktasında her iki konsantrasyon karşılaştırıldığında, %5 (w/w) gruptaki düzeyler, 3% ile karşılaştırıldığında tutarlı bir şekilde daha yüksekti, bu da konsantrasyon artışıyla titanyum salınımında ve antibakteriyel özelliklerde önemli bir artış olduğunu göstermektedir. Sonuç: %3 ve %5 (w/w) konsantrasyonlar güvenli olarak kabul edilebilir ve belirgin bir antimikrobiyal etki gösterir. TiO2-NP içeren %5 (w/w) modifiye GIC'de titanyum iyonları, %3 (w/w) modifiye GIC'den daha yüksek miktarda salınmıştır. Anahtar kelimeler: Antibakteriyel, Cam iyonomer siman, İyon salınımı, Tükürük, Titanyum dioksit.*

Ethics Committee Approval: The study was approved by the institutional ethical committee with reference number SSDC & RI/IRB/IEC/2019-2020/419/9/1.

Informed Consent: Not required.

Peer-review: Externally peer-reviewed.

Author contributions: TVDM, TSR, KSVR, PS, VSK, VM participated in designing the study. TVDM, TSR, PS, VSK, VM participated in generating the data for the study. TVDM, TSR, KSVR, VSK, VM participated in gathering the data for the study. TVDM, TSR, PS, VM participated in the analysis of the data. TVDM, KSVR, PS, VSK, VM wrote the majority of the original draft of the paper. TVDM, TSR, KSVR, PS, VM participated in writing the paper. TVDM, TSR, PS, VSK have had access to all of the raw data of the study. TVDM, TSR, KSVR, PS, VSK, VM have reviewed the pertinent raw data on which the results and conclusions of this study are based. TVDM, TSR, KSVR, PS, VSK, VM have approved the final version of this paper. TVDM, TSR, KSVR, VSK guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

Conflict of Interest: The authors had no conflict of interest to declare.

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