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Titanium Dioxide Nanocomposite Gel for Safe and Effective Tooth Whitening: An In Vitro Investigation

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Abstract

Background: Tooth whitening has gained widespread attention as a noninvasive and aesthetic enhancement procedures to restore the natural or desired tooth color.

Objectives: This study aims to evaluate the tooth-whitening efficacy and surface characteristics of choline citrate (CC) gel with varying concentrations of titanium dioxide nanoparticles (1%, 3%, and 5%), as compared to the stained control group, using tea-stained bovine enamel samples in an in vitro setting.

Materials and Methods: This experimental, in vitro study was conducted at the Department of Dental Materials, Khyber Medical University, over a period of six months, from 1-09-2023 to 1-03-2024.Choline citrate gel was prepared and titanium dioxide nanoparticle-based CC gel in 1%, 3% and 5% concentration were prepared and FTIR spectra were obtained for these gels. Enamel samples were prepared and stained with black tea. Samples were distributed into experimental and control groups. Tooth color assessment via Vitapan shade matching and Surface characteristics were assessed via Micro-Hardness, Optical profilometry, Scanning Electron Microscopy.

Result: The FTIR spectra for choline citrate gel showed the peaks of both choline hydroxide and citric acid. No significant difference amongst the groups for shade matching was reported. Moreover, Microhardness analysis of all samples revealed no significant difference ($p>0.05$). Scanning Electron Microscopy analysis for stained tooth show rough surface while the surfaces of TiO_2 gel treated samples became smooth with increase concentration of TiO_2. The profilometry images for stained, 1%, 3%, 5% TiO2 treated showed an average roughness of 16.334, 9.855, 5.926 and 3.103 µm respectively.

Conclusion: Titanium Dioxide nanoparticles addition with CC gel created as a tooth whitening gel improved the color of stained enamel samples and can be further evaluated for non-invasive aesthetic tooth whitening.

Keywords: *Titanium Dioxide, Choline Citrate, Scanning Electron Microscopy (SEM), Optical profilometry, Vitapan shade matching.*

Introduction

Tooth whitening has gained widespread attention as a noninvasive and aesthetic enhancement procedures to restore the natural or desired tooth color.[1] According to a study, about 40% to 50% of the population under 35 years old has undergone or considered tooth bleaching to attain better tooth color.[2] The tooth whitening market worldwide has grown at a rate of 5.0% annually since the year 2022.

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Primarily peroxide based materials like hydrogen peroxide and carbamide peroxide are used as tooth whitening materials in the form of in-office laser-assisted tools or at-home bleaching kits respectively.[3]

They effectively oxidize stains but come with limitations such as tooth sensitivity, enamel demineralization, and gingival irritation.[3] Non-peroxide alternatives like activated charcoal and PAP (phthalimidoperoxycaproic acid) offer milder effects but may lack long-term efficacy.[4] There occurs a need for new materials to enhance whitening efficiency while minimizing adverse effects. Emerging approaches include biocompatible remineralizing agents like hydroxyapatite, enzyme-based whitening, nanotechnology-driven solutions, and smart hydrogels for controlled release. Advancements in whitening formulations can improve safety and overall patient satisfaction.[5]

Choline citrate, a bioactive compound known for its buffering and desensitizing properties, offers a promising alternative when formulated with innovative nanomaterials such as titanium dioxide (TiO_2) nanoparticles. TiO_2 nanoparticles, widely recognized for their photocatalytic properties[1], are increasingly being integrated into dental materials to improve their functional outcomes, particularly in aesthetic dentistry. Its high refractive index scatters light, creating a whiter appearance, while its hydrophilic nature reduces stain adherence. Additionally, nano-TiO_2 improves surface coverage, ensuring a uniform whitening effect. Despite the potential of such nanocomposites, limited research has been conducted to evaluate their effect on enamel surface characteristics when used in a tooth whitening context[3].

In the present study, we have synthesized titania reinforce choline citrate (CC) gels and investigated its effectiveness for tooth-whitening of extrinsically stained teeth. In addition, the influence of the fabricated gel was investigated on enamel roughness, and structural integrity using various characterization techniques Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), optical profilometry, and microhardness analysis.

Material and Methods

This study was conducted from 1-09-2023 to 1-03-2024 after approval from Institutional Research Ethical Board of Khyber Medical university with reference NO: **KMU/IBMS/IRB/4th meeting/2021/8060-N** complying with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 197)

All the materials used in this study were of analytical grade and obtained from Sigma Aldrich, St. Louis, MO, USA.The CC was synthesized by treating 14.5 mL of choline hydroxide (44%) with 9.6 g of citric acid in one neck flask connected with a reflux setup at 70 °C and stirred at 300 rpm for 6 h. The solution was rotary evaporated to prepare clear, colorless CC gel.The optimized choline-based ionic liquid as subjected to adding of TiO_2, in different ratios (1%, 3%, 5% w/w) to CC. The synthesizedCC gels were subjected to FTIR (Thermo Nicolet 6700, Thermo Fisher Scientific, and Waltham, MA, USA). The range for collection of spectra was 4000–400 $cm^{-1}$and resolution of 4 cm^{-1}.

Collection of Tooth Samples: The tooth samples used for the study included permanent and sound bovine teeth obtained from Livestock, Pakistan, which fulfill the inclusion criteria of intact buccal surface without wear, caries, or fracture, and non-hypoplastic or non-fluorosis. The standard operating procedure of the Food Safety and Inspection Service (FSIS) was used to determine the age of animal[1]. The collected teeth were disinfected with sodium hypochlorite, washed with deionized water and stored in deionized water in the refrigerator at 4 °C until further experimentation.

Cutting Enamel Blocks: Tooth samples were prepared following ISO 28399:2020 (Products for External Tooth Bleaching) [1]. The remnants of soft tissue on the tooth surface were removed, washed with deionized water and stored in 0.1% thymol. Figure 1 shows tooth sample preparation. The crown portion was separated and cut into two equal halves inciso-cervical, separating facial/ buccal and palatal/lingual portions. The obtained portions we relabeled and grounded to 4mm X 4mm X 2mm enamel blocks under a continuous supply of waterwith Linear Precision Saw (ISOMET 4000, US). Cutting of tooth samples was done with a device blade of 0.5 mm thickness and cross-head speed of 175 mm/minute and a total of 70 enamel blocks were prepared. A Plus-shaped mark was made at the back of all the tooth samples with straight fissure bur to recognize the buccal aspect of the tooth.

Mounting and Polishing of Enamel Blocks: The samples were mounted in an impression compound (Kemdent, Swindon, UK). They were polished with 800 and 1200 grit silicon carbide sandpapers, followed by 3, 2, 1, and 0.5 microns Al_2O_3 on an automatic lapping and polishing machine (EQ-UNIPOL-1502, Qingdao, China) at 127 rpm speed. The samples were then washed, sonicated in deionized water for 30 s for removal of residual abrasives without affecting the structure.

The staining solution was prepared by boiling black tea (Tapal Tea Pvt. Ltd., Karachi, Pakistan) in water. The samples were stained by placing in staining solution in a water bath (at 37 °C for 24 h). After that, the samples were removed and rinsed with deionized water before analysis.

The artificially stained tooth samples were then randomly distributed into experimental and control groups (Table 1). They were immersed in 5 mL of respective gels for 48 h. The whole assembly was kept in an incubator (Memmert GmbH, Büchenbach, Germany) at 37 °C.

Table-1 Distribution of groups for study (n = 5 for each group)

Groups	Description
Experimental Groups	
1	1% TiO_2 CC gel treated tooth
2	3% TiO_2 CC gel treated tooth
3	5% TiO_2 CC gel treated tooth
Control group	
C	Stained tooth

ISO 28399:2020 specifications were for followed for shade evaluation of tooth samples.[1] Vitapan classical shade guide with 16 shade guide teeth in a color matching booth (GTI ColorMatcher, GTI Graphic Technology, Newburgh, NY, USA) using D65 daylight (6500 K) was used for evaluation of bleaching action of amongst the groups. According to the manufacturer's instructions, the shade guide teeth were arranged according to value from the lightest to the darkest on a white background. The best visually matched shade guide tooth with the target sample was selected, and the shade number was recorded. [1]

Vickers Hardness Numbers (VHN) for the samples was obtained via Vickers microhardness tester (Wolpert, 401

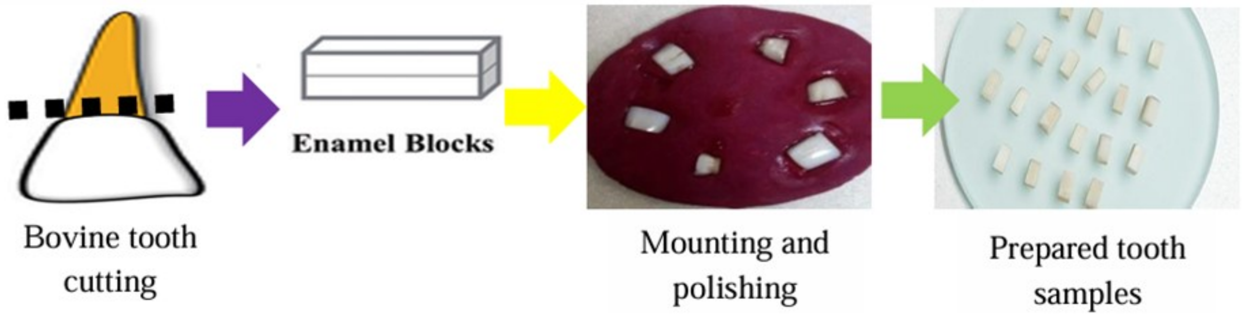


Figure 1 Tooth Sample Preparation

mvd eqpt 0002, Berg Engineering, Berlin, Germany). The readings were obtained with a load of 50 g for 15 s, with three readings on each sample.

All tooth samples were processed with dehydration and sputter coating with gold in a high-vacuum sputter coating machine (MED 5010, blazers Union Liechtenstein). The specimens were observed using the SEM (JSM-6490A, Jeol, Tokyo, Japan) at a voltage of 10 kV, magnifications 500× to 20,000×. For elemental analysis of tooth samples, a Semi-quantitative Energy Dispersive X-ray Spectroscopy (EDX; EDAX microanalysis system, Octane Plus Silicon Drift Detector, "TEAM Enhanced" v. 4.3) was used.

A 3D optical profilometer in non-contact mode (SPM-9500J3, Shimadzu Corp. Japan, Kyoto, Japan) was used for calculating the surface roughness of tooth samples. An optical scan was made with a probe at 1s clock speed and frequencies of 154 Hz and 175 Hz, probe tips 3–6 µm, heights 15–40 nm of the end radius, and a 20 × 20 µm scan area.

The results were statistically analyzed by SPSS version 22 software (IBM Chicago, IL, USA). Frequencies and percentages were presented for categorical variables, while means and standard deviation were used for quantitative variables. If the results followed a normality curve, then the one-way Analysis of Variance (ANOVA) and post hoc Tukey's test was used for comparison between groups. A *p*-value ≤ 0.05 was considered statistically significant.

Results

O-H and C-H aliphatic stretching vibration was observed at 3300 and 2950 cm-1 respectively as shown in Figure 2. CH3 deformation band at 1480 cm-1 and stretching vibration of the C–N bond was observed 1360 cm-1. C–O stretching band observed at 1080 cm -1. Peaks around 949 cm-1 and 879 cm-1 related to C-H deformation of –CH2– group were observed[1]. The characteristic peaks of citric acid i.e. C=O stretching were observed at 1722 cm-1, C-OH stretching at 1110 cm-1 and CH2 rocking at 780 cm-1[2].

The choline citrate spectrum (figure 2, C) contains the characteristic peaks of choline hydroxide and citric acid which confirmed its synthesis.

The Vitapan shade guide results were statistically analyzed via Fisher's exact test. The unstained tooth samples had A1 shade while stained ones had A4 shade. A bar chart in Figure 3 gives the frequency percentage of AI shade across four groups. The highest frequency percentage of A1 shade was noted in the EIII group, 83.3%, and the lowest by the CA group, 0. Meanwhile, in the EI and EII groups, the percentage was 50% and 33.3%, respectively.

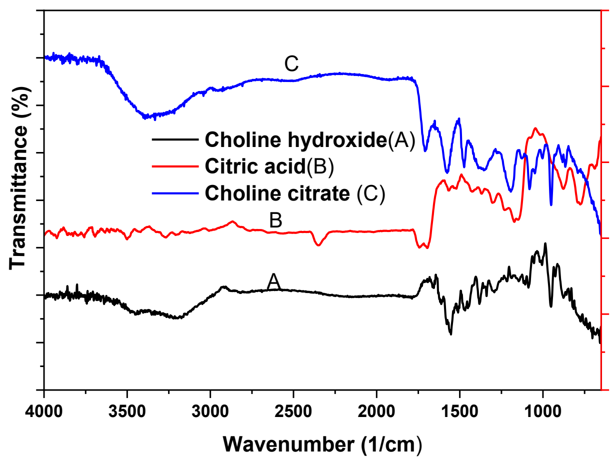


Figure 2. FTIR analysis of A: choline hydroxide, B: citric acid and C: Choline citrate ionic liquid

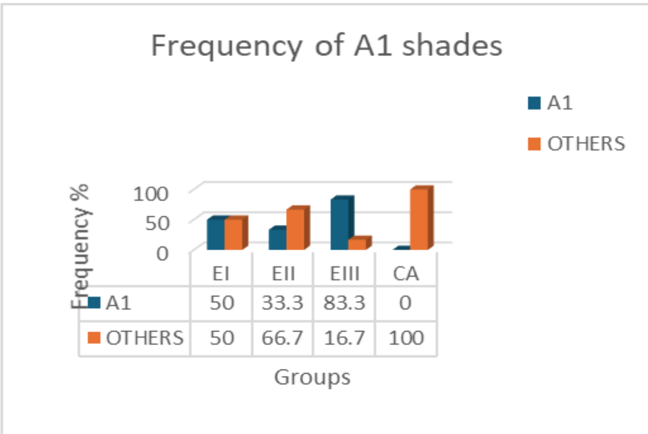
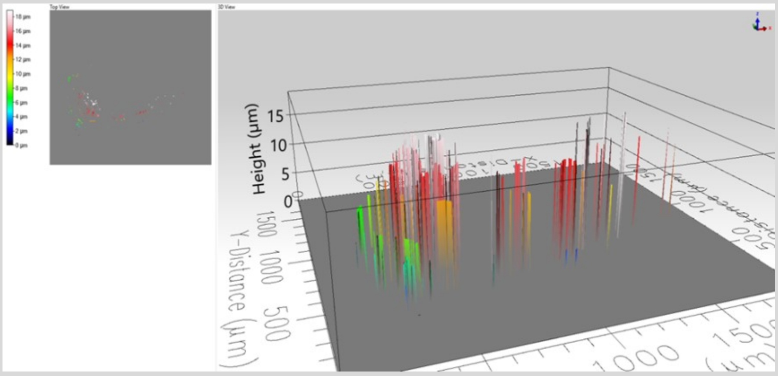
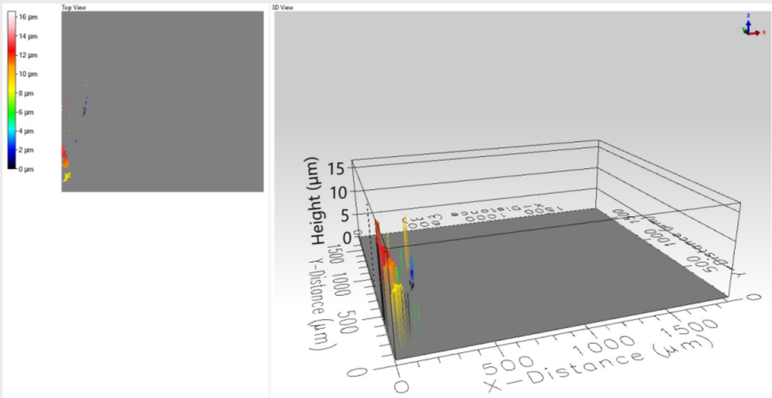
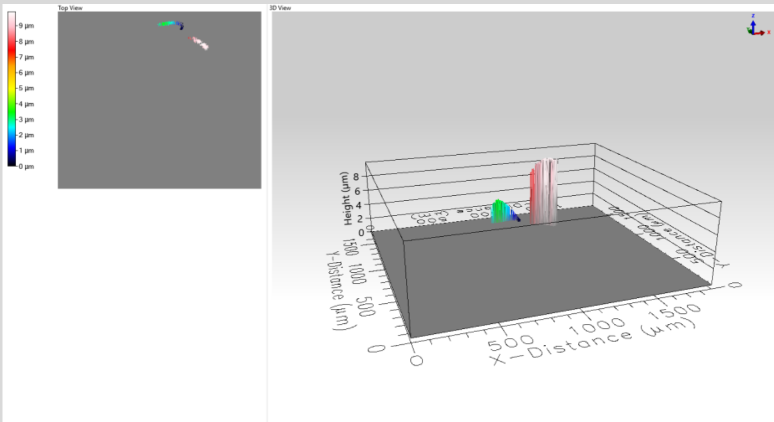
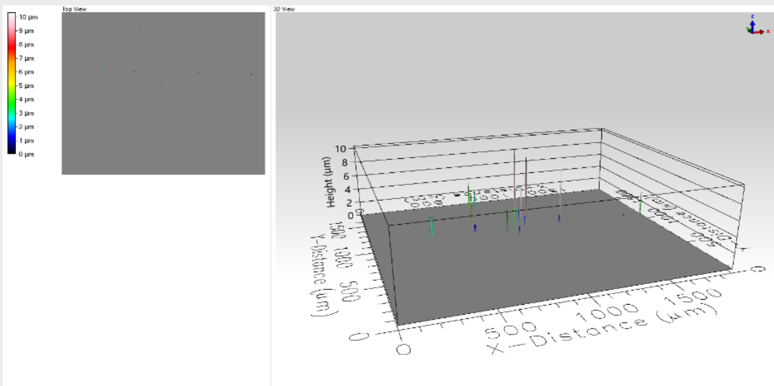


Figure 3. Frequency% of AI shade across groups

The mean microhardness values (VHN) with standard deviations of tooth samples for four groups are enlisted in Table 3. The comparison amongst the groups revealed no significant difference ($p > 0.05$).

The SEM images at 1000 X for stained tooth (A), 1% TiO2 gel treated tooth (B) 3% TiO2 gel treated tooth (C) and 5% TiO2 gel treated tooth (D) are shown in Figure 3. The stained tooth showed a coating of organic matrix that has been stained with black tea. The surface of the treated tooth became more clear as the percentages of TiO_2 increased from 1 to 5% in

Table 4 Roughness average and top and 3-D view of tooth samples

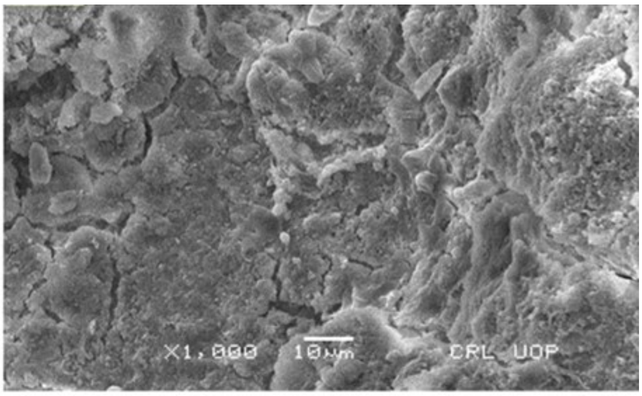
Sample	Roughness average	Top view and 3-D view
Stained tooth	16.334 µm	
1% TiO2 gel treated tooth	9.855 µm	
3% TiO2 gel treated tooth	5.926 µm	
5% TiO2 gel treated tooth	3.103 µm	

choline citrate gel. The smoothness of the surface confirmed the removal of the stain from the tooth.

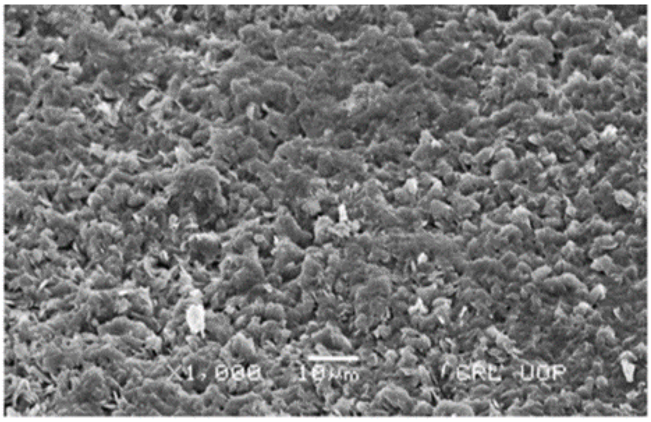
The top view, 3D topographical images and values of roughness average (Ra) of the tooth samples were taken. Ra values are enlisted in Table 4. The comparison of Ra values in the tested groups revealed no significant difference ($p > 0.05$).

Table 2: Mean microhardness values of tooth samples

Groups	Mean VHN(N/ mm²)	SD
Experimental group		
EI	188.92	42.59
EII	195.77	52.58
EIII	196.92	54.59
Control group		
CA	185.77	50.58



A: Stained tooth

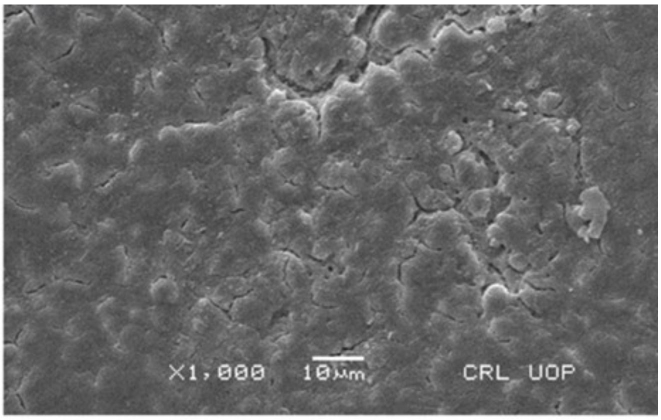


B: 1% TiO2 gel treated tooth

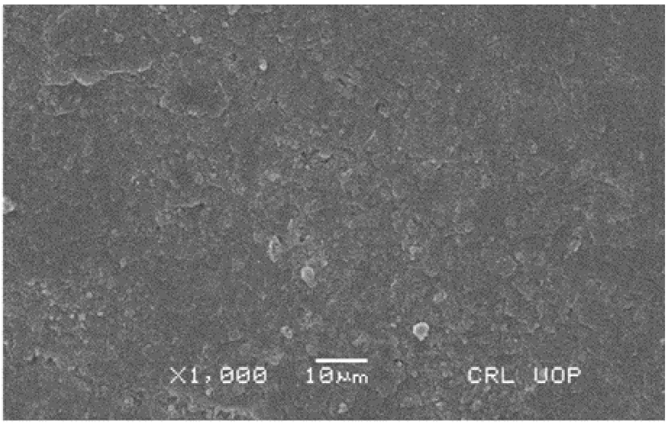
Discussion

The present study reported the synthesis of choline citrate nanocomposite tooth-whitening gels containing variable concertation titania nanoparticles (1%, 3%, and 5%). The nanocomposite tooth-whitening gels were assessed for their effectiveness against extrinsic stains. While the enamel surfaces treated with the gel were characterized as mechanical and chemical properties ensuring no deleterious effects caused by the tooth-whitening gel.

The process of whitening teeth is considered a cos-



C: 3% TiO2 gel treated tooth



D: 5 % TiO2 gel treated tooth

Figure 4. SEM micrographs of stained tooth and 1%, 3% and 5% TiO2 gel treated tooth

metic dental procedure. It is recommended for widespread or localized dental stains brought on by fluorosis, smoking, age, and food stains.[3] Some dental operations, such as orthodontic therapy, could also need teeth whitening after they're finished.[4]

TiO_2 is a fantastic photo synergist specialist because of its oxidizing characteristics across a broad pH range [5-7] and accelerates the chemical processes of bleaching gels by the impact of light sources.[5] Due to its biocompatibility, titanium dioxide has been employed to create a variety of orthopedic and dental implants. Additionally, the FDA advised using roughly 1 wt.% of TiO_2 as a coloring additive in a variety of food items.

TiO_2 was first used in teeth whitening because of many pilot studies that were carried out in this area. Numerous investigations using spectroscopy on TiO_2 have shown the existence of O-H, C-H, and C-N groups at wavelengths of 3200 cm-1, 2800 cm-1, and 1100 cm-1, respectively[10]. All these peaks were seen in 1, 3, and 5% TiO_2 gel's FTIR spectra (fig2 a, b, c). These groups' existence demonstrates that two synthetic gels were based on choline hydroxide. Choline hydroxide and citric acid are components of CC gel. Therefore, in addition to O-H, C-H, and C-N groups at 3200cm-, 2800cm-1, and 1100cm-1, respectively, the FTIR spectra of this gel also show the presence of an extra carboxylic acid, namely the COOH group at 1300cm-1 (Fig. 2). [11] The four groups therefore confirm that the CC gel is based on choline hydroxide and citric acid.

The FTIR spectra of the stained teeth and teeth treated with varying concentrations of titanium dioxide enriched CC gel (1%, 3%, and 5%) show clear differences in transmittance and functional group presence, reflecting changes in the surface chemistry of the enamel (ref figure 2). The spectrum of the stained tooth (Group A) displayed prominent peaks associated with organic components indicative of staining, such as carbonyl (C=O) and hydroxyl (O-H) functional groups.

After treatment with titanium dioxide CC gel, significant reductions in peaks associated with staining components were observed, particularly in the higher concentration groups (3% and 5% TiO_2 gel-treated teeth, Groups C and D, respectively). This suggests an effective breakdown or removal of organic chromophores from the enamel surface. Additionally, the presence of peaks corresponding to Ti-O and Ti-O-Ti bonds in the treated groups indicates successful deposition or adsorption of titanium dioxide on the enamel surface, which might contribute to shade improvement and surface protection.

The observed changes correlate with the visual shade results and roughness average findings (ref table 4). While higher concentrations of titanium dioxide gel effectively reduced staining, their influence on enamel surface chemistry suggests a potential enhancement in aesthetic properties without compromising structural integrity.

It can be inferred from figure3 that the distribution of A1 shade frequencies amongst the experimental groups (EI, EII, and EIII) and the control group (CA) demonstrates notable trends influenced by varying concentrations of titanium dioxide gel and the staining condition of the samples. Group EI (1% titanium dioxide CC gel) exhibited an equal distribution of A1 (50%) and other shades (50%). In group EII (3% titanium dioxide CC gel), the frequency of A1 shades decreased to 33.3%, while other shades increased to 66.7%. Group EIII (5% titanium dioxide CC gel) showed the highest frequency of A1 shades (83.3%), with only 16.7% categorized as other shades. Conversely, the control group (CA), treated with deionized water, exhibited no A1 shades (0%), with all samples classified under other shades (100%).

It is important to note that the samples initially stained with tea had an A4 shade, while unstained samples corresponded to an A1 shade. This indicates that the titanium dioxide enriched CC gel effectively restored the samples closer to the A1 shade, with higher concentrations showing greater efficacy. The absence of A1 shades in the control group highlights the inability of deionized water alone to remove stains or enhance shade restoration.

The microhardness analysis of tooth samples across experimental groups (EI, EII, and EIII) and the control group (CA) as depicted in table3 revealed comparable Vickers Hardness Numbers (VHNs). The mean VHN values for the experimental groups ranged from 188.92 ± 42.59 (EI) to 196.92 ± 54.59 (EIII), while the control group (CA) had a mean VHN of 195.77 ± 52.58. Pairwise comparisons among the groups showed no statistically significant differences ($p > 0.05$) in microhardness values.

These findings indicate that varying concentrations of titanium dioxide gel (1%, 3%, and 5%) did not negatively impact the microhardness of the tooth samples compared to the control group treated with deionized water. This suggests that the application of titanium dioxide gels maintains the structural integrity of tooth enamel without compromising its hardness.

The two main tests used to evaluate how bleaching affects morphology, elemental content, and surface properties of enamel are SEM and optical profilometry. The tooth sample treated with synthetic 1% TiO2 gel may be seen to have a typical look of enamel with mineralized deposits in the micrograph in figure (4) A-E. Due to their tiny size, the particle size analysis in figure (4d) implies that these mineralized deposits might be hydroxyapatite crystals on the surface of the enamel.

The tooth sample shows leaf-like deposits in the micrograph shown in figure (4) A–E might likely represent hydroxyapatite crystals. The micrographs in figure (4) show indications of etching that may have happened because of bleaching. The chemical etching pattern seen in figure (4) mimics type II, where the prism peripheries are dissolved, leaving an undamaged prism core in the center and a darkened periphery. [12]

The granular deposits in figures (4)A–E of may represent hydroxyapatite crystals. In research that included fourteen days of recurrent teeth whitening with hydrogen peroxide and carbamide peroxide, the same granular pattern of the enamel surface was seen.[13] The use of hydrogen peroxide and carbamide peroxide for teeth whitening produced signs of erosions, an increase in porosities, and shallow indentations in the SEM tests. [14,15] The tooth samples used in the present investigation that were treated with synthetic TiO_2 gel exhibit an etching pattern. It was possible to see surface degradation in this group (Fig 4).

The citric acid component of the gel may have contributed to the etching that happened in the synthetic CC gel group, causing its pH to be decreased which resulted in bleaching activity, which eroded and etched the enamel's surface.[16] Identifying mineralized deposits and indentations in all five categories, even if they have various morphological morphologies, such as cube-shaped, leaf-like, and granular deposits, is of special importance.

3-D and 2-D optical profilometers are used to evaluate the surface profile of dental enamel subjectively and quantitatively. In the current study, profilometric photographs of the chosen area were used to analyze the surface profile qualitatively and a 3-D profilometer was used to figure out the average roughness of the tooth samples as depicted in table 4.

The texture of a surface under investigation is described by the roughness average, often known as surface roughness and typically represented as Ra.[17] It is measured by determining how much a surface under test deviates from its ideal shape. The surface becomes rougher the more it deviates from its ideal shape, and vice versa. Clinically, increased tooth surface roughness is characterized as coarseness that promotes bacterial adherence, plaque retention, and tooth discoloration.[17]The roughness average (Ra) analysis of tooth samples using optical profilometry across experimental groups (EI, EII, and EIII) and the control group (CA) demonstrated no statistically significant differences ($p > 0.05$) as revealed by one-way ANOVA. The roughness averages recorded for the experimental groups ranged from 3.103 µm (EI) to 16.334 µm (EIII), with the control group (CA) showing a Ra value of 5.926 µm.

The observed increase in roughness averages among experimental groups correlates with the increasing concentrations of titanium dioxide gel, with EI (1% gel) presenting the lowest roughness value and EIII (5% gel) the highest. Despite this variation, the lack of statistically significant differences indicates that the application of titanium dioxide gel does not result in meaningful changes in surface roughness when compared to the control group treated with deionized water.

According to research on peroxide-based bleaching chemicals, surface porosity and roughness have increased, significantly altering the surface topography.[18] In the present study, the graphical surface profile and surface roughness of the four groups are similar showing a minor change to surface enamel (table 4). According to research on peroxide-based bleaching chemicals, surface porosity and roughness have increased, significantly altering the topography of the surface as shown in profilometric pictures and roughness average. [18,19]

The findings align with the visual shade results, suggesting that higher titanium dioxide concentrations, while improving shade restoration (as seen in A1 shade frequencies), may slightly influence surface roughness but within a range that does not affect enamel integrity.

This study has several limitations that should be considered when interpreting the findings. First, as an in vitro investigation, the experimental conditions may not fully replicate the dynamic oral environment or the complex aging processes that occur in vivo. Additionally, the use of bovine enamel rather than human enamel may limit the generalizability of the results, given the potential differences in structural and compositional characteristics between the two. The study focused on extrinsic staining induced by tea, which may not accurately represent intrinsic staining issues commonly encountered in clinical settings. Furthermore, while the Vitapan shade guide was employed to assess color changes, this method carries an inherent potential for subjective error and variability. Future research should aim to overcome these limitations by incorporating in vivo studies, using human enamel specimens, and employing more objective color measurement techniques.

Conclusion

Within the limitations, this study highlights the potential of titanium dioxide-enriched CC gel as an effective and safer tooth whitening gel. The titanium dioxide enhances the bleaching effect while maintaining enamel microhardness and structural integrity. The higher concentrations of titanium dioxide (3% and 5%) showed greater efficacy in restoring a lighter shade of enamel. Moreover, SEM and optical profilometry revealed minimal surface alterations suggesting a gentler approach to enamel bleaching.

CONFLICT OF INTEREST: None

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Author Contributions

1. **Mobeen Ali**: Conceptualization of the study, experimental design, and laboratory work.
2. **Saad Liaqat**: Data collection, experimental procedures, and result documentation.
3. **Memuna Kausar Satti**: Study supervision, methodology refinement, and critical review of the manuscript.
4. **Muhammad Sohail Zafar**: Statistical analysis, data interpretation, and technical guidance.
5. **Nawshad Muhammad** : Literature review and assistance in manuscript drafting.
6. **Fatima Suhaib** : Data analysis support and preparation of figures and tables.
7. **Hasham Khan** – Final manuscript review and approval of the version to be published.