Synergistic effects of titanium dioxide and cellulose on the properties of glass-ionomer cement

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In this study, we evaluate the effect of co-doping with TiO2 nanoparticles and sisal cellulose nanocrystals (CNCs) on the physical and biological properties of a conventional glass-ionomer cement (GIC). Test samples were characterized by scanning electron microscopy, and Fourier-transform infrared spectroscopy, and subjected to mechanical tests to evaluate the mechanical performances. Antimicrobial activity was evaluated against Candida albicans, and cytotoxicity experiments were conducted using L-929 cells. Unmodified GIC served as a control. Compared with the control group, the co-doped group demonstrated an increased compressive strength of 18.9%, an increased shear bond strength of 51%, the dissolution decreased by 18.3%, the volume wear rate was reduced by 5%. The antifungal effect against C. albicans was increased by 22%. In cytotoxicity experiments, the co-doped group had a slightly negative effect on the viability of L-929 cells.

Keywords: Glass-ionomer cement, Cellulose nanocrystals, TiO2 nanoparticles, Synergistic effect, Antimicrobial

INTRODUCTION

Glass-ionomer cement (GIC) was invented by Wilson and Kent in 19711-5, and has since become the most widely applied material in dental atraumatic restorative treatments (ARTs). ART is a minimal intervention dentistry technology recommend by WFO which removed carious lesions with simple hand instruments and used dentistry technology recommend by WFO which removed carious lesions with simple hand instruments and used GIC as a filling material for preventive filling. This is attributed to the very unique properties of GICs for clinical applications that irreplaceable, such as good adhesion to tooth structure, excellent anticariogenic properties, thermal compatibility with tooth hard tissue, and low cytotoxicity3). However, the numerous disadvantages of GICs, such as poor wear resistance, low mechanical strength, high porosity, and poor polishing properties, have restricted their use to low-stress-bearing sites3). Therefore, it is imperative to overcome these defects, which cannot meet the requirements of permanent filling materials. Oral restorations are required to maintain their integrity for as long as possible, while being subjected to strong compression, shear, and wear forces, variations in pH and temperature, and chemical attack. For example, it is well known that copious amounts of saliva are generated by thousands of chewing actions every day, making the humid oral environment extremely challenging for restorative materials. In addition, the human bite force can vary from 100 to 500 N depending on teeth involved and on the individual4,5). Moreover, even the formation of small cracks between restorative materials and tooth tissue may lead to the growth of bacteria, resulting in the development of secondary caries. As is well known, the generally good antimicrobial properties of GIC are mostly due to its release of fluoride6). However, investigation has shown that fluoride ions released from GICs cannot entirely inhibit bacterial growth and the formation of secondary caries7,8). Thus, different methods have been applied to improve the strength and antimicrobial properties of GIC.

One strategy proven to enhance mechanical performance is to modify the glass powder by adding materials such as metal powders9,10), bioactive glass11), and nano-bioceramics12). Another strategy that has demonstrated dramatically enhanced mechanical performance involves modifying polyacid by introducing activated chemical materials, such as amino acid derivatives13,14), N-vinylpyrrolidone15), and acrylate with other branched chains16), to the polyacid backbone. In addition, efforts have been made to enhance the antimicrobial properties of GICs. Hu et al.17) reported that the addition of 0.1 wt% epigallocatechin-3-gallate (EGCG) not only can improve the mechanical performance, but also enhances the antimicrobial properties of conventional GIC. Takahashi et al.18) demonstrated that a concentration of 1 wt% chlorhexidine (CHX) is optimal for enhancing the antimicrobial, mechanical, and bonding properties of GIC. However, other scholars have found that the addition of antimicrobial agents negatively affected the physical properties of GICs. Palmer et al.19) found that the compressive strength was decreased with the addition of CHX, while working and setting times were prolonged. In addition, researchers developed a novel quaternary
ammonium salt (QAS) or furanone-containing GIC with a long-acting antimicrobial function\textsuperscript{20,21}. In this case, the compressive strength of the GIC was significantly decreased, which was likely due to the negative effect of the charge and the hydrophobic chain of QAS on the strength of the cement.

In contrast to the above discussed approaches for enhancing the mechanical and antimicrobial properties of GICs, titanium dioxide (TiO\textsubscript{2}) nanoparticles have a great many promising characteristics as an inorganic additive to dental materials, such as chemical stability, and photocatalytic antimicrobial properties\textsuperscript{22,23}. Xia et al.\textsuperscript{24} added TiO\textsubscript{2} nanoparticles to dental resin as a reinforcing agent, resulting in an improved microhardness and flexural strength. Elsaka et al.\textsuperscript{25} found that the addition of a low concentration of TiO\textsubscript{2} nanoparticles to GIC could dramatically improve its mechanical and antimicrobial properties. The enhancement of mechanical properties can be explained by the fact that the nanoparticles filled gaps between micrometer-sized particles, making the microstructure more compact. Cellulose fiber is another interesting potential additive for GICs. Cellulose fiber is a renewable resource obtained from plants, and has several advantages as an additive, such as low cost, low density, high specific strength, and high elastic modulus\textsuperscript{26-27}. This material has been widely used as different types of scaffolds in tissue engineering owing to its toughness, minimal cell flattening, and high rigidity\textsuperscript{28}. Silva et al.\textsuperscript{29,30} demonstrated that nano-cellulose fibers could enhance the mechanical properties of GIC due to their high specific area as well as the strong hydrogen bonds in their web-like structure.

These alternative additives to GIC are of substantial interest. However, there were no identified reports on the influence of co-doped of TiO\textsubscript{2} nanoparticles and cellulose nanocrystals (CNCs) into GICs. Therefore, the purpose of the present work was to evaluate the mechanical properties, antimicrobial properties, and biocompatibility of GIC co-doped with TiO\textsubscript{2} nanoparticles and CNCs. For this purpose, TiO\textsubscript{2} nanoparticles were added into glass powders and sisal CNCs were mixed with the polyacid liquid, and the resulting mechanical performance and biological properties of the prepared composites were evaluated. The mechanical properties considered here include compressive strength, surface hardness, dissolution, and shear bond strength. In addition, the prepared composites were characterized by numerous analytical methods. Antimicrobial activity was evaluated against Candida albicans, and cytotoxicity experiments were evaluated using L-929 cells. Unmodified GIC served as a control.

**MATERIALS AND METHODS**

**Materials**

A two-component conventional GIC system (Shanghai Medical Devices, Shanghai, China) composed of a polyacrylic acid liquid component and glass powder component of SiO\textsubscript{2}–Al\textsubscript{2}O\textsubscript{3}–AlF\textsubscript{3}–CaF\textsubscript{2}–NaF–AlPO\textsubscript{4} was chosen as the experimental material. We prepared TiO\textsubscript{2} nanoparticles using the sol-gel method employed in our pilot experiments\textsuperscript{32}. We prepared a dispersion liquid of sisal CNC whiskers (Qihong Technology, Guilin, Guangxi, China) with a length-to-diameter ratio greater than 18.

**Specimen preparation**

Three types of GICs were prepared: a non-modified GIC, denoted as CG; a GIC with 2 wt% TiO\textsubscript{2} nanoparticles, denoted as T1; and a co-doped GIC that included 2 wt% TiO\textsubscript{2} nanoparticles and 1 wt% CNC, denoted as C1. All weight measurements were conducted using an analytical balance with a precision of 0.0001 g (INESA, Shanghai, China). The powder components of the specimens included the glass powder and TiO\textsubscript{2} nanoparticles (in the case of T1 and C1 specimens), which were weighed separately, and then mixed. TiO\textsubscript{2} nanoparticles were mixed with the glass powder at a specified percentage (2 wt%) in an agate mortar under controlled grinding process. Anhydrous ethanol was added to the mortar, and the mixture was manually ground 2 h until the ethanol completely volatilized to ensure uniform distribution of the nanoparticles in the composite powders. In the case of C1 specimens, the CNCs were added directly to the liquid component of the GIC. Here, the dispersion liquid of the sisal CNC whiskers was first dried under freezing conditions for approximately 3–4 days to obtain the CNCs. After being pulverized, the CNCs were added to the polyacrylic liquid component, and subjected to sonication for 2 min for complete homogenization. The powder component was added to the liquid component to form GIC specimens in a powder:liquid ratio of 2:1. The mixing method was based on the manufacturer’s recommendations. During the sample preparation process, the surfaces of the various stainless steel molds were sprayed with a release agent to facilitate easy demolding.

**Specimen characterization**

1. **X-ray diffraction (XRD)**

XRD was conducted using a D/MAX-2400 diffractometer (Rigaku, Tokyo, Japan, \(\lambda=0.154181\) nm) with Cu \(K\alpha\) radiation to identify the crystalline structure of the as-prepared TiO\textsubscript{2} nanoparticles. Measurements were conducted at room temperature using a voltage of 40 kV and a current setting of 150 mA over wide angles of 20° from 20° to 80° with a step size of 0.02°.

2. **Scanning electron microscopy (SEM)**

SEM was conducted using a JSM-6701F microscope (JEOL, Tokyo, Japan) to characterize the surface morphology of the specimens. Qualitative element analysis of specific specimen areas was performed by energy-dispersive X-ray spectroscopy (EDS).

3. **Fourier-transform infrared spectroscopy (FTIR)**

FTIR spectra of the samples were obtained from 32 scans in the interval between 500 and 4,000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) using a NEXUS 670 FTIR spectrometer (Nicolet, Madison, WI, USA).
Mechanical testing

1. Compressive strength
Ten cylindrical specimens of each specimen type (i.e., CG, T1, and C1) were prepared in a stainless-steel mold (8 mm diameter and 6 mm high). A pressure of 2 MPa was applied with a dwell time of 5 min at room temperature for the preparation of each specimen until the cement was completely set. The specimens were then removed from the mold and ground with silicon carbide (SiC) metallographic abrasive papers (SHANGSHA, Shanghai, China) in the sequence of 600#, 800#, and 1200#. All specimens were immersed in distilled water at 37°C for 24 h prior to compression testing. We retreated and precisely measured the samples using the vernier caliper before testing. Compressive strength was measured using a universal testing machine (WDW-200, MTS, Shenzhen, China) with a 20 KN load cell at a crosshead speed of 0.5 mm/min. The compressive strength was calculated according to

$$\text{compressive strength} = \frac{4F}{\pi d^2} \text{ (MPa)},$$

where $F$ is the load (in N) at fracture and $d$ is the original diameter of the test specimen (in mm).

2. Surface hardness
Three block-like specimens of dimensions 18×12×5.5 mm were prepared for each specimen type using an equivalent preparation method as was applied for specimens subjected to compression testing. All specimens were immersed in distilled water at 37°C for 7 days prior to testing. A Rockwell hardness tester (XHRD-150, HY, Laizhou, Shandong, China) was employed for hardness testing. A stainless-steel ball with a diameter of 6 mm was selected and a normal load of 55.8 N was applied during testing. Each specimen was subjected to three independent tests under equivalent conditions at different positions on the specimen separated by a distance not less than 3 mm.

3. Enamel shear bond strength
A total of 24 extracted molars were randomly allocated into three groups of eight molar specimens each. All molars were employed within 1 month of extraction, and verified to be defect free. The molars were cut into cylinders of 5 mm length with a low-speed hand piece, inlayed with a resin mosaic (ZXQ-50S, SHOIF, Shanghai, China), and a 4-mm diameter hole was drilled through the center of each cylinder. The inner surfaces of the drilled holes were etched for 30 s with 37% phosphoric acid (HERAEUS, Hanau, Germany), rinsed with deionized water for 15 s, and dried with an oil-free air spray. The holes were then filled with the different GIC mixtures. Excess material was removed and the cylinders were immersed in distilled water at 37°C for 1 day prior to conducting the tests. Shear bond strength measurements were performed using the universal testing machine employed for compressive strength measurements with a 20 KN load cell by selectively applying a downward force to the filled GIC surfaces at a crosshead speed of 1 mm/min. The maximum applied force under which a filled GIC cylinder was extruded from the resin cylinder was recorded. The schematic diagram was shown in Fig. 1. The enamel shear bond strength was calculated according to

$$\text{shear bond strength} = \frac{F}{\pi dh} \text{ (MPa)},$$

where $F$ is the load (in N) at fracture, $d$ is the original diameter of the test specimen (in mm), and $h$ is the height of the cylinder (in mm).

4. Wear resistance
Ten disc-shaped specimens with a diameter of 45 mm and a thickness of 8 mm were formed for each GIC type. After setting, the discs were immersed in distilled water at 37°C for approximately 24 h, and polished with metallographic SiC abrasive papers in the sequence of 600#, 800#, and 1200#. The specimens were then washed with deionized water and subjected to wear resistance testing using an LSR-2M reciprocating friction tester (Zhongke, Lanzhou, Gansu, China) under a 7.5 N normal load with a sliding speed of 0.05 m/s for 30 min and a reciprocating distance of 10 mm. During testing, the specimens were all fixed on the test bench, which was filled with artificial saliva to simulate the human oral environment. A new metal grinding ball with dimension of φ=4 mm was employed for each test to ensure the accuracy of the results. The schematic diagram was shown in Fig. 2. Three replicated wear tests were conducted for each specimen, and the average

Fig. 1 (a) The schematic diagram of the shear bond strength test, (b) calculation of the bonding area.

Fig. 2 The specimen was subjected to the wear test (schematic diagram).
of the three wear-rate values obtained were recorded. The volume wear rate was calculated according to

$$\omega=\frac{\Delta V}{L \cdot N} \left(\text{mm}^2 \cdot \text{N}^{-1} \cdot \text{m}^{-1}\right),$$

where $\Delta V$ refers to the measured volume of material removed during testing (mm$^3$), $L$ is the total sliding distance (m), and $N$ is the actual load on the specimen (N). The friction was measured by the sensor and the friction coefficient was calculated by the software according to the formula:

$$\mu=\frac{F}{N},$$

where $F$ is the friction (in N), $N$ is the normal load (in N).

5. Dissolution

The sample preparation procedure employed for solubility tests was equivalent to that employed for compression tests, and the dimension of the sample is 8 mm in diameter and 6 mm in height. After being polished, the samples were placed in an oven at a temperature of 37°C for 12 h, and the initial mass ($m_i$) of each tested sample was measured using the analytical balance discussed above. The samples were then immersed in artificial saliva under a constant temperature of 37°C for 30 days. The artificial saliva was replaced every day, and the samples were washed with deionized water to eliminate any dissolved matter. After 30 days, the samples were again dried for 12 h at 37°C, and the final mass ($m_f$) of each tested sample was measured. The amount of material dissolved over the 30 days period was regarded as the dissolution rate according to formula:

$$\text{dissolution rate}=\frac{(m_i-m_f)}{m_i} \times 100\%.$$

6. Antimicrobial properties

The evaluation of the antimicrobial properties of the different GIC types employed C. albicans (ATCC 10231), which is a common oral pathogen. Testing was conducted in accordance with China National Standard GB/T 30706-2014 and the colony counting method. Disc-shaped GIC specimens with a diameter of 40 mm and a thickness of 3 mm were formed for testing, where three identical samples were prepared for each GIC type. In addition to the CG, T1, and C1 materials, a block of polyethylene (PE) was also tested at the same time for blank control.

Prior to testing, the surface of each sample was wiped with alcohol to ensure that it was sterile and completely dry. The initial concentrations of C. albicans were controlled at 10$^6$ CFU/mL. During the experiments, each sample was placed in a sterile petri dish, and a 0.2-mL microbial suspension was dropped onto the surface of the sample and covered with a PE film to insure even dispersal. All samples were placed in an incubator at a temperature of 37°C and a relative humidity of no less than 85%, and subjected to UV light (UV-A) (SANKYO, Shenzhen, China) exposure for 2 h. Afterwards, the surface of each sample was rinsed using 10-mL phosphate-buffered saline solution (PBS; HyClone, Logan, UT, USA) that contained a 1 wt% surfactant (Tween 80; Solarbio, Beijing, China). The eluate was diluted tenfold, and 100 µL was applied to a solid plate of Sabouraud medium (Solarbio) to test the antifungal property with respect to C. albicans. Afterwards, the strains were incubated for 24 h at 37°C, and the experiments were repeated three times. The antimicrobial rate was calculated using the following formula:

$$R=\frac{(C-C_0)}{C_0} \times 100\%,$$

where $C$ is the CFU of the control group and $C_0$ is that of the experimental group.

7. Cytotoxicity testing and cell morphology observation

The three types of GIC were subjected to an evaluation of their in vitro cytotoxicity. A medium having no extract derived from the tested specimens was employed as a negative control (NC) and Dulbecco’s Modified Eagles Medium (DMEM; Hyclone) containing 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Milwaukee, WI, USA) was employed as a positive control (PC). The cytotoxicity of the samples was evaluated by examining both the viability and morphology of L-929 fibroblasts according to ISO Standard 10993.5:1999. The L-929 cell line (Cell Bank at the Chinese Academy of Sciences, Shanghai, China) was cultured in DMEM supplemented with 10% (volume/volume) fetal bovine serum (FBS; Sijiqing, Hangzhou, China) in a wet incubator with 5% CO$_2$ at 37°C. Block-like specimens with dimensions 20×10×4 mm were fabricated using the method described above for surface hardness measurements, and the specimens were thoroughly sterilized prior to testing. Extraction media of the materials were prepared using a DMEM serum free cell culture medium with a surface-area-to-volume ratio of 1 cm$^2$/mL according to the ISO standard (i.e., 0.5–6.0 cm$^2$/mL)$^{30}$, and then incubated in a humid environment with 5% CO$_2$ at 37°C for 3 days to prepare the eluates. Cells were incubated in 96-well cell plates with a 2×10$^5$ cells/(100 µL) cell culture medium in each well, followed by incubation for 24 h to allow attachment. The medium of each well was then replaced with 100 µL of extraction media for all GIC types. Afterwards, the culturing of the cells was continued in a humid incubator with 5% CO$_2$ at 37°C for 3, 5, and 7 days. Then, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT; Sigma-Aldrich) was added to the well. The specimens were incubated with MTT for 4 h before termination at 37°C in darkness, and 100 µL of a formazan solubilization solution (10% sodium dodecyl sulfate in 0.01 M HCl) was added to each well and cultured for another 24 h. The spectrophotometric absorbance was measured using a microplate reader (Bio-Tek, Winooski, VT, USA) at a wavelength of 490 nm. This experiment was independently repeated three times for each GIC type. Photos of cell morphology were obtained using an inverted optical microscope (Olympus, Tokyo, Japan).

8. Statistical analysis

Statistical Package for Social Sciences software 16.0 (SPSS; IBM, Armonk, NY, USA) was employed to analyze the experimental data. After testing for homogeneity of variance (the Leven statistical test), all data were analyzed using one-way analysis of variance (ANOVA) and Tukey’s test to determine evidence of significant
differences between the experimental GIC results and those of the control group, adopting a level of significance of 95% \((p=0.05)\).

**RESULTS**

**XRD and morphology analysis**

Figure 3 presents the XRD patterns of TiO\(_2\) sintered at 500°C. It can be seen from the XRD patterns that diffraction peaks exist at (101), (004), and (200) planes, indicating an anatase phase of TiO\(_2\).

Figure 4(a) shows a representative SEM micrograph of intertwined cellulose whiskers at a magnification of 30,000×. Figure 4(b) shows a representative SEM micrograph of TiO\(_2\) nanoparticles at a magnification of 30,000×. It can be seen that the TiO\(_2\) nanoparticles are regularly spherical in shape and uniform in size, with diameters of about 50 nm. Figure 4(c) shows an SEM micrograph of blended glass powder and TiO\(_2\) nanoparticles at a magnification of 500×. The larger particles are glass powders, while the smaller particles are TiO\(_2\) nanoparticles. Figures 4(d), (e), and (f) show SEM micrographs indicative of the surface morphology of the CG, T1, and C1 specimens, respectively. The modified specimens (T1 and C1) exhibit a higher degree of integrity, smoother surfaces, and fewer cracks than the unmodified CG specimen.

**Chemical structure analysis**

The FTIR spectra of the GIC shown in Fig. 5(a) includes the typical broad peak indicative of -OH groups at 3,430.9 cm\(^{-1}\), a band indicative of C=O is observed at 1,722.1 cm\(^{-1}\), and two bands indicative of C–O are observed at 1,167.6 and 1,078.0 cm\(^{-1}\). The FTIR spectrum of TiO\(_2\) nanoparticles shown in Fig. 5(b) includes peaks at 3,177.6 and 1,625.4 cm\(^{-1}\) due to -OH groups. The CNC spectra shown in Fig. 5(c) presents bands indicative of -OH groups at 3,423.1 cm\(^{-1}\) and of the –CH\(_2\) of methylene groups at 2,904.9 cm\(^{-1}\). Bands at 1,638.7, 1,318.2, 1,059.8, and 1,034.4 cm\(^{-1}\) are due to the C=O bonds of the carbonyl group. Bands at 1,161.8 cm\(^{-1}\) are attributed to the C-O-C groups of the saccharide structure of cellulose, and the band at 899.4 cm\(^{-1}\) corresponds to the bending vibration of the C–H groups. Figure 5(d) shows FTIR spectra of the CG, T1, and C1 specimens. An intense band indicative of -OH groups is observed for the C1 specimen at 1,639.1 cm\(^{-1}\) due to TiO\(_2\) nanoparticles.

**Evaluation of mechanical properties**

The average compression measurements of the CG, T1, and C1 groups are shown in Fig. 6(a). The average compressive strength of the CG group is thereby obtained as 94.4 MPa. For the T1 group, the average compressive strength (CS) was slightly increased to 101.8 MPa. The addition of TiO\(_2\) nanoparticles did not obviously improve the CS of the GIC composite \((p>0.05)\). However, the average CS of the C1 group increased to 112.7 MPa \((p<0.001)\).

Figure 6(b) illustrates the variation in the surface hardness for the different GIC composites. The results indicate that the surface hardness remains stable in the range of 100–106 HRL for the three different groups \((p>0.05)\).

Figure 6(c) illustrates the shear bond strengths of the different GIC composites. For the specimens embedded in extracted human teeth, the average
Fig. 5 FTIR spectra of (a) unblended glass ionomer cement, (b) TiO$_2$ nanoparticles, (c) sisal cellulose nanocrystals, (d) unblended glass ionomer cement (CG), a 2 wt% TiO$_2$ modified group (T1), and a composite group (C1).

Fig. 6 Graphs about comparisons of the mechanical properties of different groups. (a) compressive strength, (b) surface hardness, (c) shear bond strength, (d) friction and wear rate. CG is unmodified glass ionomer cement, T1 is GIC modified with 2 wt% TiO$_2$, and C1 is GIC blended with 2 wt% TiO$_2$ and 1 wt% CNC.
shear bond strengths of the three groups exhibited a prominent increasing trend, where the average shear bond strength of the CG group was 9.69 MPa, that of the T1 group was 13.23 MPa, and that of the C1 group was 14.61 MPa. While the statistical significance between the average shear bond strengths of the CG and C1 groups was appreciable \((p=0.003)\), the statistical significance between the T1 and C1 groups is not obvious \((p=0.427)\).

**Evaluation of tribological properties**
The tribological properties of the different GIC composites are shown in Fig. 6(d). It can be seen that the average volume wear rate of the composites proceeded in the order of CG>C1>T1, where the highest average wear rate of 20.1 mm\(^3\)•N\(^{-1}\)•m\(^{-1}\) was obtained for the CG group, and the lowest average wear rate of 10.8×10\(^{-5}\) mm\(^3\)•N\(^{-1}\)•m\(^{-1}\) was obtained from the T1 group. No significant differences were obtained for the friction coefficients of the three testing groups.

**Evaluation of dissolution**
Figure 7 shows the average dissolution of the different GIC composites. The average dissolution rates proceeded in the order of T1<C1<CG, where the average dissolution rate of the T1 group was decreased significantly relative to that of the CG group \((p=0.033)\), while no significant difference was observed between the average dissolution rate of the C1 group and that of the CG group \((p>0.05)\).

**Evaluation of antimicrobial properties**
Figure 8 illustrates the antimicrobial effects of PE and the different GIC groups on *C. albicans*. The Figure is divided into subfigures showing the effects of (a) PE, (b) CG, (c) T1, and (d) C1 groups.

Figure 9 illustrates the antimicrobial effects of *C. albicans* treated on the different samples after 2 h of UV-A light illumination. The antifungal activity of *C. albicans* (ATCC10231) was 92.3% on C1, 93.1% on T1, and 70% on CG. In accordance with China National Standard GB/T 30706-2014, an antimicrobial rate greater than 90% demonstrates that the material has an antimicrobial ability, and greater than 99% demonstrates excellent antimicrobial activity. Therefore, both modified GIC composites demonstrate significant antimicrobial properties.

**Cytotoxicity evaluation**
Figure 10 shows cell metabolism following the application of the MTT tests assessing the cytotoxicity of five groups with or without the experimental materials for 3–7 days. It can be seen that the cell proliferation rate slightly
increased over the period from 3 to 7 days. The L-929 cell viability cultured with the extracts of T1 and C1 were all less than 75%, which verifies that they have slight toxicity.

Figure 11 presents optical microscopy images reflecting the morphologies of L-929 cells cultured in DMEM and extracts derived from (a) GC, (b) T1, (c) C1, and (d) the NC for 7 days. The number of cells in the cell cultures with T1 and C1 extracts was clearly less than that in the cell cultures with the CG extract and the NC. However, the cell morphologies were in all cases normal, and similar to the cell morphology observed for the NC culture.

**DISCUSSION**

In this experiment, when compared with unmodified group, the operation performance of the group T1 was well and the setting time was not significant prolonged. While in the group C1, the setting time was slightly decreased due to the addition of cellulose increased the viscosity of the polyacrylic acid liquid. A shorter setting time is advantageous clinically because it can prevent dissolution of the GIC from excessive water contamination in the early stages of hardening. Therefore, the experimental glass ionomers did not compromise the setting time, manipulation and working time in this experiment. We note from the SEM results that the number of surface cracks of the T1 and C1 group specimens clearly decreased. The reason for this is that the addition of a nanofiller can fill the cracks and pits of GIC, so that the material becomes more compact. No obvious network structures were observed in the C1 group, which could be related to the small amount of the added sisal cellulose.

Compared with the FTIR spectrum of the CG group, those of the T1 and C1 groups included a small peak at 1,639.1 cm\(^{-1}\) due to the addition of TiO\(_2\). A new peak representing the added CNC was not observed in the FTIR spectrum of the C1 group because the concentration of CNC was insufficient. Compressive strength means the ability of the material to resist the external force. If the compressive strength of the material is unsatisfactory, it will be hard to bear the chewing force when used in the oral, resulting in the disintegration and shedding of the material, so that the filling fails. Therefore, the clinical application of the material is meaningless. The surface of TiO\(_2\) has rich hydroxyl groups and it covalently bonded with GIC matrix. The addition of 2 wt% TiO\(_2\) nanoparticles was observed to slightly increase the CS of the composite relative to that of the unmodified GIC, which may be due to the small-size effect of nanoparticles. Here, the resulting experimental glass mixed with TiO\(_2\) filler had a wider range of particle-size distributions, and the TiO\(_2\) nanoparticles filling the spaces between GIC macromolecules may have served as a reinforcement agent, which may have imparted the somewhat superior mechanical properties. However, the increased CS of the C1 group was far more evident. The results obtained in the present study are similar to those of Silva, and are considered to be related to the network structure formed by cellulose. Self-association of the interacting hydrogen bonds between the CNCs can be expected to form a supporting architecture for transferring force into the cement matrix, and, hence, greatly improve the compressive strength.

The experimental surface hardness results presented here are in accordance with the work of Elsaka et al. The surface hardness of GIC is affected by various factors, such as exposure to water/saliva, the chemical components of the polyacid, and setting methods. No observable change was seen in the surface hardness of the three groups due to the small amount of additives could not affect the reaction process of the cement and thus reacted phase (polycrystalline) of the three GIC may have similar surface hardness each other. Wear is a crucial
factor in the evaluation of dental filling restorative materials. A material must be wear-resistant to be a clinically attractive dental restorative, particularly for posterior filling. The volume wear rate of the T1 group was considerably less than that of the CG group. This can be interpreted as a result of the appropriate amount of TiO$_2$ nanoparticles filling the empty spaces in the polymers, thus increasing the degree of cross-linking and cement compactness, and thus improving the wear resistance. Owing to the lack of acrylic acid in the C1 group, excess cellulose whiskers were retained in the polymer, and the CNCs could aggregate to a considerable degree, which would promote weakening and a higher mass loss$^{40}$.

With respect to the shear bond strengths of the GICs with extracted human teeth, studies have shown that phosphoric acid etching can dramatically increase the enamel micro-tensile bond strengths ($\mu$TBSs) of conventional GICs and resin-modified GICs$^{40}$. Porous structures may be formed on the enamel surface after etching. When the adhesive acts on the enamel surface, not only does an ionic interaction form between the GIC and the hydroxyapatite of the tooth substrate, but a mechanical interlocking of the polymer into the dentin also occurs$^{40}$. Thus, the bonding strength is significantly improved. The likely explanation for the enhanced shear bonding strengths of the T1 and C1 groups with extracted human teeth is that adhesion with the tooth structure is the result of an ion-exchange layer being formed slowly between the cement and the tooth$^{43}$. The addition of TiO$_2$ nanoparticles and CNCs intensified this reaction, and, thus, the solar and ionic attraction between the carboxylate groups and the tooth substrate were enhanced.

Dissolution represents the amount of soluble material in the material, which can reflect the degree of polymerization within the material$^{45}$. Therefore, it is important to measure the dissolution GIC in order to evaluate its stability and durability in water. In this study, the addition of TiO$_2$ or CNCs did not compromise the dissolution of the GIC, proven that stability of the two modified groups was well.

Conventional GIC exhibits some degree of antimicrobial action, and two primary points of view have been proposed to explain this. The first point of view is based on the fact that bacteria growth requires a pH in the range 7.1–7.4. Yesilyurt et al.$^{46}$ and Deschepper et al.$^{45}$ proposed that an acid-base reaction occurs when glass powder and acrylic liquid are mixed together, forming an acidic polymer that inhibits the growth of bacteria by promoting a pH value below 7. The other, and more acceptable, point of view is that GICs release fluoride, which interferes with oral metabolism, destroys the catabolism process of salivary glycosylation, and ultimately affects the growth of bacteria$^{45,46}$. Nakajo et al.$^{47}$ also demonstrated that fluoride released from GIC is responsible to inhibit the acid production of caries-related oral streptococci.

The anatase phase of TiO$_2$ nanoparticles has a proven photocatalytic activity and high stability$^{48}$. Upon UV light irradiation, electrons are excited from the electronic valence band to the conduction band, producing hole and electron pairs, and oxygen ions. The oxygen is activated in water, resulting in free hydroxyl radicals that have a very high oxidation capacity, and degrade organic matter effectively. When bacteria adhere to the surface of anatase TiO$_2$, reactive oxygen ions and hydroxyl radicals can penetrate the walls of bacteria cells, cutting off their respiratory system and electronic transmission system, and effectively killing the bacteria$^{49,50}$. Another advantage of TiO$_2$ is that it degrades toxic substances released from bacteria when they are killed$^{51}$. Moreover, a favorable antimicrobial property is also provided by the high surface area–to–volume ratio of nanoparticles$^{52}$. Both the T1 and C1 groups demonstrated a strong antimicrobial effect against C. albicans, indicating that the addition of cellulose did not jeopardize the antimicrobial activity of the material.

The L-929 cell viabilities cultured with the extracts of T1 and C1 for 3–7 days were all less than 75%, which verifies the slight toxicity of the as-prepared samples. This may be related to the addition of TiO$_2$ nanoparticles. Liu et al.$^{50}$ considered that TiO$_2$ nanoparticles induced apoptosis of the PC12 cell (a cell line derived from rat adrenal medulla pheochromocytoma). Gurr et al.$^{54}$ demonstrated that anatase TiO$_2$ nanoparticles (10 and 20 nm) in the absence of photoactivation induced oxidative DNA damage, lipid peroxidation, and increased hydrogen peroxide and nitric oxide production in BEAS-2B cells (a human bronchial epithelial cell line). Another study$^{55}$ indicated that TiO$_2$ nanoparticles induced tumor-like phenotypes in human gastric epithelial cells. All of these studies suggested that TiO$_2$ disturbs cell metabolism and causes cell damage and apoptosis, which is consistent with the results of our experiments. Therefore, to avoid pulp irritation, the proposed GICs cannot be employed for deep caries in clinical applications.

**CONCLUSION**

The physical properties of the GIC group co-doped with 2 wt% TiO$_2$ nanoparticles and 1 wt% CNC were significantly improved. Specifically, the CS increased by 18.9%, the dissolution decreased by 18.3%, the volume wear rate was reduced by 5%, and the enamel shear bond strength increased to 151% when bonding with extracted teeth. Antimicrobial activities were 92.27% (C. albicans). And the co-doped group had a slightly negative effect on the viability of L-929 cells. Therefore, we believe that the co-doped modified GIC represents a promising restorative dental material for surface applications.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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