Antibacterial properties of Cu-doped TiO₂ prepared by chemical and heat treatment of Ti metal

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ABSTRACT

In this study, we proposed Cu-doped TiO₂, prepared by the alkaline and heat treatment of titanium (Ti) metal, for use in dental applications because of its bone-bonding and antibacterial properties generated by the release of Cu ions and visible-light-responsive photocatalysis. We successfully prepared Cu-doped TiO₂ on a Ti chip surface with a Cu content of 7 at.%. The apatite-forming ability of the Cu-doped TiO₂ was evaluated using a simulated body fluid and it was found that apatite formation occurred. Hence, we concluded that Cu-doped TiO₂ exhibits bone-bonding properties. The antibacterial properties of Cu-doped TiO₂ for Escherichia coli were higher than those of non-doped TiO₂ under visible light irradiation. The enhanced antibacterial effect was mainly caused by the visible-light-responsive photocatalysis of Cu-doped TiO₂. We confirmed that the reactive oxygen species generated by the visible-light-responsive photocatalysis of Cu-doped TiO₂ were hydroxyl radicals formed by the reaction of hydroxide ions (OH⁻) and holes (h⁺). Our findings are useful for the development of novel bioactive TiO₂-based coatings and bulk materials with antibacterial properties by Cu doping.

1. Introduction

Titanium (Ti) and its alloys have been used as implants in orthopedic and dental fields because of their excellent biocompatibility. Various surface modification processes have been studied to improve their bone-bonding ability [1,2] because the osteoconductivity of untreated Ti and its alloys can be enhanced. Alkaline treatment using sodium hydroxide (NaOH) solution and subsequent heat treatment can also improve the bone-bonding ability of Ti and its alloys [3]. Alkaline-heated treated Ti and Ti alloys form an apatite layer on their surfaces after implantation. Consequently, they bond directly to the living bone in bone defects [4]. Titanium dioxide (TiO₂) and its related compounds formed by alkaline-heated treatment are key materials to express the apatite-forming ability of Ti and its alloys. Apatite formation on material surfaces can be reproduced using the simulated body fluid (SBF) proposed by Kokubo [5]. The SBF has ion concentrations similar to those of the human blood plasma [6]. An apatite-forming ability test using the SBF is a useful indicator of the bone-bonding properties of artificial materials, although the apatite-forming ability in the SBF does not always correlate to in vivo bone-bonding ability [7].

In the implantation of artificial materials, surgical site infection (SSI) is an important and severe problem [8]. When SSI occurs after the implantation of artificial materials, surgery is required, which imposes a heavy burden on the patient. Therefore, during surgery, measures are required to control the incidence of SSI. It is desirable that the artificial material itself exhibits an antibacterial effect, and hence, various biomaterials combined with antibacterial elements have been developed, including Ag, Cu, and Zn [9–11]. In addition, the effect of photocatalysis on implant materials has been studied in recent years [12], and alkali-heated-treated Ti with antibacterial properties has been studied [13,14] as well.

To induce antibacterial properties and photocatalysis, Cu doping is an attractive approach because Cu is an antibacterial element. Moreover, Cu-doped TiO₂ can be synthesized by a micro arc oxidation [15,16] and a sol-gel method [17,18]. Such materials prepared by the latter method exhibit antibacterial properties under visible-light-irradiation conditions due to the reactive oxygen species formed by visible-light-responsive photocatalysis [17,18]. In our previous research, we established a technique to dope metal ions, including Cu ions, into the TiO₂ layer formed on Ti metal by modifying the alkaline-heated treatment method [19]. If a Cu-doped TiO₂ layer formed by the modified alkaline-heated treatment exhibits excellent antibacterial properties, then the material will...
be useful for dental applications, especially abutments. However, the material properties, especially the antibacterial properties, of Cu-doped TiO₂ layers formed by the modified alkaline-heat treatment remain unclear. Herein, we report that Cu-doped TiO₂ prepared by the modified alkaline-heat treatment exhibits improved antibacterial properties due to the release of doped Cu and visible-light-responsive photocatalysis. Additionally, to investigate the origin of antibacterial properties caused by visible-light-responsive photocatalysis, the reactive oxygen species generated from the sample under visible light irradiation were identified.

2. Materials and methods

2.1. Chemicals

A Ti chip (dimensions 10 mm × 10 mm × 1 mm, 99.9%) was purchased from Kojundo Chemical Lab. Co., Ltd., Saitama, Japan. Sodium hydroxide (NaOH, 97%), copper nitrate trihydrate (Cu(NO₃)₂·3H₂O, 99.9%), phosphate buffered saline (PBS), sulfuric acid, ammonium iron (II) sulfate hexahydrate, xylene orange, tetrasodium salt, sorbitol, and hydrogen peroxide (H₂O₂) were purchased from Nacalai Tesque, Inc., Kyoto, Japan. A nutrient agar medium and a nutrient liquid medium were purchased from Becton Dickinson and Co., CA, USA. A Soybean-Casein Digest Broth with Lecithin & Polysorbate 80 "DAIGO" (SCDL) medium was purchased from Nihon Pharmaceutical Co., Ltd., Tokyo, Japan. 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) was purchased from Labotech Co., Kyoto, Japan, and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) was purchased from Sigma Aldrich, St. Louis, MO, USA. All other chemicals were purchased from Nacalai Tesque, Inc., Kyoto, Japan.

2.2. Sample preparation

A Ti chip was polished using a diamond pad (No. 400, Maruto Instrument Co., Ltd., Tokyo, Japan). The polished Ti chip was ultrasonically washed once with acetone (CH₃COCH₃, 99%) and twice with ultrapure water for 10 min. The washed chip was dried at room temperature (approximately 25°C) and atmospheric pressure. Subsequently, an aqueous NaOH solution was prepared by dissolving 1.031 g of NaOH into 5 cm³ of ultrapure water. The washed chip was immersed into the NaOH aqueous solution in a round-bottom polytetrafluoroethylene test tube. The test tube was shaken at 120 strokes/min for 24 h at 60°C using a shaking bath. After completion of the NaOH treatment, the Ti chip was removed from the test tube and washed with ultrapure water to obtain the alkaline-treated Ti chip.

Subsequently, 0.121 g of Cu(NO₃)₂·3H₂O was dissolved in 5 cm³ of ultrapure water. The copper nitrate aqueous solution was diluted 100-fold to obtain approximately 1 mol/m³ of copper nitrate solution. A 6 cm³ aliquot of diluted copper nitrate solution was transferred to a polytetrafluoroethylene, round-bottom test tube. The alkaline-treated Ti chip was then immersed and shaken at 120 strokes/min for 48 h at 80°C. After the treatment, the chip was removed and washed with ultrapure water. The Ti chip treated with the copper nitrate solution was heat treated at 600°C for 1 h using a muffle furnace (MSFS-1218, Yamada Denki Co., Ltd., Tokyo, Japan). The obtained samples treated with copper nitrate solution were named AL-Cu-HT. A sample obtained by alkaline treatment and further heat treatment at 600°C for 1 h was used as the control sample, and was named AL-HT.

2.3. Characterization

2.3.1. Surface structure analysis

The surface morphology of the sample was observed using a scanning electron microscope (SEM; VE8800, Keyence Corp., Osaka, Japan). The crystalline phase of the surface layer formed by the solution and heat treatments was characterized by thin film X-ray diffraction (TXRD; RINT2200VL, Rigaku Corporation, Tokyo, Japan) using Cu Ka radiation. In addition, the composition of the surface layer was evaluated using an X-ray photoelectron spectrometer (XPS; AXIS Ultra DLD, Kratos Analytical Ltd., Manchester, UK). The X-ray source involved a monochromatic Al Ka radiation (1486.7 eV), at 15 kV and 10 mA. The binding energy was calibrated using the C1s photoelectron peak at 284.8 eV as a reference.

2.3.2. Evaluation of apatite-forming ability

The apatite-forming ability of the samples was evaluated using an SBF. The SBF was prepared as follows: 700 cm³ of ultrapure water was poured into a polyethylene container. The temperature of ultrapure water was maintained at 36.5 ± 1.5°C using a water bath. The reagents shown in Table 1 were added in order from No. 1 to No. 8. The pH of the solution was measured using a pH electrode (GST-5731 C, DKK-TOA Corp., Tokyo, Japan) set in a portable pH meter (HM-21P, DKK-

| No. | Reagent       | Amount     |
|-----|---------------|------------|
| 1   | NaCl          | 7.996 g    |
| 2   | NaHCO₃        | 0.350 g    |
| 3   | KCl           | 0.224 g    |
| 4   | K₂HPO₄ · 3H₂O | 0.228 g    |
| 5   | MgCl₂ · 6H₂O  | 0.305 g    |
| 6   | 1.0 mol·dm⁻³ HCl | 40 cm³ |
| 7   | CaCl₂         | 0.278 g    |
| 8   | Na₂S₂O₃       | 0.071 g    |
| 9   | (CH₃OH)₂CNH₂  | 6.057 g    |
2.3.3. Cu ion release behavior

To investigate the Cu ion release behavior of the sample, 10 cm³ of PBS was placed in a polypropylene vessel. The AL-Cu-HT sample, where n = 3, was immersed in PBS at 36.5°C. The PBS was refreshed at appropriate periods. The accumulated-released amounts of Cu ion from the samples at 1, 3, 7, 14, and 28 days were calculated based on the Cu concentrations in the PBS, which was measured using an inductively coupled plasma atomic emission spectrometer (ICP-AES, ICP600, Thermo Fisher Scientific Co., Ltd., Kanagawa, Japan).

2.3.4. Antimicrobial activity evaluation

In the antibacterial test, a nutrient agar was used in petri dishes (Falcon® plastic dish for general bacteria, Corning Inc., New York, USA) in 15 cm³ aliquots. Physiological saline was prepared by dissolving 8.5 g of NaCl into 1000 cm³ of ultrapure water, which was used after sterilization at 121°C for 20 min using a high-pressure steam sterilizer.

_Escherichia coli_ (E. coli, JCM5491) was used as the test bacterial strain, which was used after application to the nutrient agar medium and culturing at 37°C for 24 h. The bacterial mass of the cultured _E. coli_ was taken with a platinum loop and dispersed in physiological saline to prepare a stock bacterial suspension (approximately 10⁸ CFU/cm³). This stock suspension was diluted with a nutrient liquid medium to obtain a test bacterial suspension (approximately 10⁷ CFU/cm³).

The bacterial test was carried out for each sample, where n = 4. A cell strainer (Corning Inc.) attached to a 6-well plate was used as a device for setting the sample. The sample was placed on the cell strainer with the sample surface facing upward, and 10 mm³ of the test bacterial suspension was dropped. Subsequently, the sample surface was covered with a plastic film (9 × 9 × 0.06 mm) for close contact. To reduce the effects of increasing temperature and drying during visible light irradiation on the bacteria,
a cooler was placed behind the 6-well plate, and 1.5 cm³ of pure water was added to the wells to prevent sample drying.

LED light (460 nm; SPA-10SW, Hayashi Clock Industry Co., Ltd., Tokyo, Japan) was used as the light source. The distance from the lower part of the lens to the sample surface was 10 cm, the irradiance was 250 W/m², and the irradiation period was 30 min. This irradiation period was set assuming visible light irradiation to the abutment of dental implants during dental treatments. A schematic illustration of the antimicrobial activity evaluation system is shown in Figure 1. As a control experiment, an antibacterial test without visible light irradiation was also conducted.

After either irradiation with visible light for 30 min or no irradiation for 30 min, the sample was collected together with the film, soaked in 2 cm³ of the SCDLP medium, and thoroughly stirred to wash out the bacteria. The washed-out medium was diluted 10-fold and 100-fold with the SCDLP medium, and 100 mm³ of each was seeded onto the nutrient agar medium. These media were cultured at 37°C for 48 h, the number of colonies was counted, and the viable cell count was calculated. The viable bacteria count between the AL-HT and AL-Cu-HT groups was compared by performing a one-way analysis of variance and conducting a multiple hypothesis test (Holm’s method).

2.3.5. Identification of reactive oxygen species induced by visible light irradiation

The hydroxyl radical (⋅OH) is one of the main reactive oxygen species generated by photocatalysis. There are two main routes for the generation of hydroxyl radicals due to photocatalysis:

Route 1. Reaction of a hydroxide ion (OH⁻) and a hole (h⁺), given by

\[ \text{OH}^− + \text{h}^+ \rightarrow \cdot \text{OH} \]

Route 2. Reaction assisted by H₂O₂ generated via the reaction of oxygen (O₂) and an electron (e⁻), as follows:

\[ \text{O}_2 + \text{e}^- \rightarrow \cdot \text{O}_2^- \]
\[ \cdot \text{O}_2^- + 2 \text{H}^+ + \text{e}^- \rightarrow \text{H}_2\text{O}_2 \]
\[ \text{H}_2\text{O}_2 + \text{e}^- \rightarrow \cdot \text{OH} + \text{OH}^- \]

Reactive oxygen species and free radicals are highly reactive, and it is difficult to measure them directly at around 25°C. Therefore, we measured these chemical species by electron spin resonance (ESR) using a spin trapping method. DMPO was used as a spin trapping agent. These adducts were measured using an ESR spectrometer (JES-FA-100, JEOL Ltd., Tokyo, Japan). The measurement conditions were as follows: microwave power, 4.0 mW; microwave frequency, 9428.954 MHz; magnetic width, 0.1 mT; field sweep width, ± 5 mT; field modulation, 100 kHz; modulation width, 0.1 mT; time constant, 0.03 s; and sweep time, 0.1 min. The samples were placed in a 24-well plate and 500 mm³ of DMPO solution (300 mol/m³) was added. The samples immersed in the DMPO solution were irradiated with visible light for 30 min under the same conditions as in the antibacterial property test using LED lighting. Subsequently, 200 mm³ of the DMPO solution, in which the sample was immersed, was removed and the reactive oxygen species were measured using the ESR spectrometer. We used TEMPOL to quantify the hydroxyl radicals. A control ESR spectrum was obtained from a solution without sample immersion and visible light irradiation.

The amount of H₂O₂, which is a reactive oxygen species, was measured using H₂O₂ colorimetry. Two types of solutions were used for this purpose. Solution 1 was prepared by mixing 6 cm³ of 100 mol/m³ sulfuric acid and dissolving 11.8 mg of ammonium iron (II) sulfate hexahydrate into 30 cm³ of pure water. Solution 2 was prepared by dissolving 9.1 mg of xylene-nil orange tetrasodium salt and 2.186 g of sorbitol into 30 cm³ of pure water. A calibration curve was prepared using solutions 1 and 2, and 8.821 mol/dm³ of H₂O₂ solution.

The sample was placed in a 24-well plate and immersed in 500 mm³ of pure water. After irradiation with visible light for 30 min under the same conditions as in the antibacterial property test using the LED lighting, 400 mm³ of the pure water, in which the sample was immersed, was removed and poured into a glass tube. Subsequently, 200 mm³ of solution 1 and 200 mm³ of solution 2 were added into the glass tube and mixed well. The glass tube was then maintained at approximately 25°C for 45 min. The absorbance of the mixture solution at a wavelength of 560 nm was then measured using an ultraviolet-visible spectrophotometer (GeneQuant 1300, Biochrom, Ltd., Cambridge, UK).

3. Results and discussion

3.1. Surface structure

The surface structures of AL-HT and AL-Cu-HT are shown in Figure 2(a). Network-like structures were observed in both the samples. The network-like structure formation using alkaline-heat treatment by Ti was reported previously [20–22]. No significant differences in the surface structures were observed in these samples. The crystalline phases of the sample surfaces were characterized by TF-XRD. The TF-XRD patterns of the samples are shown in Figure 2(b). Rutile and metallic Ti (substrate), and a small amount of sodium titanate (Na₂Ti₅O₁₈), were detected in AL-HT. Meanwhile, rutile, anatase, and metallic Ti (substrate) were detected in AL-Cu-HT. Enhancement of the anatase formation by Cu doping has also been reported [19]. Therefore, reasonable experimental results were obtained by the TF-XRD measurement. The compositions of the sample
surfaces were analyzed by XPS, as per our previous report [19], which are summarized in Table 2. In both the samples, TiO₂ was the main component, and these results corresponded to the TF-XRD results (Figure 2 (b)). In the AL-HT surface, 15.0 at.% of Na was detected, while 7.0 at.% Cu was detected in the AL-Cu-HT surface. Negligible amounts of N, S, Cl, and Ca were detected in both samples. Since any crystalline Cu-related compounds, such as copper oxides, were not detected by XRD, the Cu ion was likely incorporated into the TiO₂ phases.

We confirmed the presence of Cu on the AL-Cu-HT surface by XPS analysis. Subsequently, we evaluated the release behavior of Cu ions from the sample in PBS. Figure 3(a) shows the release behavior of the Cu ions (accumulated-released amount of Cu vs. soaking period). The Cu ions were gradually released from the samples, and the accumulated-released amount of Cu in PBS reached approximately 40 μmol after 28 days of soaking. No significant initial release burst was observed. To clarify the release mechanism, Figure 3(b) shows the accumulated-released amount of Cu against the square root of

Table 2. Surface composition (atomic percentage). Numbers in parentheses correspond to composition prior to the removal of the C1s contribution.

| Sample  | O   | Ti  | Cu  | Na  | N   | S   | Cl  | Ca   | C    |
|---------|-----|-----|-----|-----|-----|-----|-----|------|------|
| AL-HT   | 58.7| 25.3| -   | 15.0| 0.4 | < 0.1| 0.5 | 0.1  | 0    |
|         | (48.6)| (21.0) | (12.4) | (0.3) | (0.1) | (0.4) | (0.1) | (17.2) |
| AL-Cu-HT| 65.2| 26.8| 7.0 | -   | 0.6 | 0.3 | < 0.1| 0.1  | 0    |
|         | (51.1)| (21.0) | (5.5) | (0.5) | (0.2) | (< 0.1) | (0.1) | (21.7) |

Figure 3. Cu ion release behavior from AL-Cu-HT in PBS. (a) Accumulated-released amount of Cu vs. soaking period and (b) accumulated-released amount of Cu vs. square root of soaking period. The release of Cu ions from AL-Cu-HT was likely caused by ion substitution of cations in PBS and Cu ions. Note that the standard deviation of each set of data was small (< 0.7 μmol), therefore the error bars are omitted.
the soaking period. A linear relationship was obtained between the accumulated-released amount of Cu and the square root of the soaking period. Therefore, based on previous reports [23,24], the release of Cu ions was likely caused by ion substitution of cations in PBS and Cu ions, which would have been included in the TiO₂ phases.

3.2. Apatite-forming ability

The apatite-forming ability of the samples was evaluated using an SBF. Figure 4(a) shows the SEM images of the samples after immersion in an SBF for 7 days. The surface of AL-HT was entirely covered by the precipitate. Precipitates with a diameter of approximately 10 μm

![](image1)

**Figure 4.** (a) SEM images and (b) TF-XRD patterns of AL-HT and AL-Cu-HT after immersion in the SBF for 7 days. The surface of AL-HT was fully covered by the precipitate, and precipitates approximately 10 μm in diameter were observed on the AL-Cu-HT surface after soaking in the SBF. The crystalline phase of the precipitate was hydroxyapatite.

![](image2)

**Figure 5.** Number of viable bacteria for AL-HT and AL-Cu-HT under conditions with and without visible light irradiation. Strong antibacterial properties of AL-Cu-HT under visible light irradiation caused by visible-light-responsive photocatalysis were observed.
were observed on the AL-Cu-HT surface after soaking in the SBF. The coverage ratio of AL-Cu-HT by the precipitate was clearly lower than that of AL-HT. The crystalline phase of the precipitate formed on the sample surface after immersion in the SBF was characterized by TF-XRD. The TF-XRD patterns of the samples after immersion in the SBF for 7 days are shown in Figure 4(b). In both samples, reflection peaks assigned to the TiO$_2$ phases and hydroxyapatite were detected. Hence, the precipitates formed by immersing the samples in the SBF were concluded to be hydroxyapatite. Although the apatite-forming ability of AL-Cu-HT was lower than that of AL-HT, apatite was actually precipitated on the surface of AL-Cu-HT after immersion in the SBF for 7 days, and hence AL-Cu-HT is expected to bond onto bone directly in an in vivo environment.

### 3.3. Antibacterial properties

Figure 5 shows the results of antibacterial tests using *E. coli*. When 10 mm$^3$ of the test bacterial suspension (approximately 10$^5$ CFU/cm$^3$) was applied, the number of *E. coli* cells on the sample surface immediately after the addition was estimated to be approximately 10$^5$ CFU. In AL-HT, the number of viable *E. coli* cells decreased upon visible light irradiation. The decrease in the viable *E. coli* was likely due to the increased temperature and drying in the culture system, based on a previous report [25]. In the case of no visible light irradiation, the number of viable bacteria on AL-HT and AL-Cu-HT was approximately the same. This result indicated that the Cu ions released from AL-Cu-HT did not contribute to its antibacterial properties in the short-term bacterial culture (30 min). As shown in Figure 3(a), Cu ions were gradually released from AL-Cu-HT. The accumulated-released amount of Cu on day 1 was ~5 µmol. Because 10 mm$^3$ of bacterial suspension was used in the antibacterial test, the Cu concentration of the bacterial suspension would be ~500 mol/m$^3$ if the amount of Cu ions released from the samples were the same. This Cu concentration is sufficient for antibacterial property expression, based on a previous report [9]. Therefore, in AL-Cu-HT, antibacterial properties in long-term bacterial culturing is expected owing to the sustained release of Cu. In the case of AL-Cu-HT, the number of viable *E. coli* dramatically decreased after visible light irradiation. This decrease could not be explained by the increased temperature and drying. The strong antibacterial properties of AL-Cu-HT under visible light irradiation were likely to have been caused by visible-light-responsive photocatalysis.

Subsequently, we investigated the chemical species, which are the source of the antibacterial properties that were generated from the AL-Cu-HT under visible light irradiation. Figure 6(a) shows the ESR spectra of the samples. The spin adduct, DMPO-OH, was assigned using the hyperfine coupling constants (HFCCs). The analysis details for identification of the radical species are shown in Figure 6(b), using the AL-Cu-HT sample as an example. The HFCCs of all of the samples were $a_H = a_N = 1.49$ mT, which coincided with those of the DMPO-OH adduct reported in a previous paper [26]. Therefore, it can be concluded that signals derived from DMPO-OH were observed in all of the samples and hydroxyl radicals were generated by visible light irradiation. The concentrations of the hydroxyl radicals in the control sample, AL-HT, and AL-Cu-HT were calculated to be 1.4, 1.5, and 2.5 mmol/m$^3$, respectively. The background hydroxyl radical concentration was estimated to be ~1.4 mmol/m$^3$.

![Figure 6](image-url)
Therefore, a concentration of hydroxyl radicals of 1.1 (= 2.5–1.4) mmol/m³ was generated by the visible light photocatalysis of AL-Cu-HT. We measured the H₂O₂ concentration to clarify the formation route of the hydroxyl radicals. The H₂O₂ concentrations of AL-HT and AL-Cu-HT, measured by colorimetry, were 9.6 \times 10^{-2} and 8.6 \times 10^{-2} mmol/m³, respectively. These concentrations were lower than those expected and above the lower limit of our colorimetric measurement. Therefore, it is reasonable to consider that the solution contained almost no H₂O₂. The formation route of reactive oxygen species was presented in Section 2.3.5. According to the hydroxyl radical ESR spectroscopic measurement and the H₂O₂ colorimetric measurement, hydroxyl radical concentrations were much higher than the H₂O₂ concentrations in AL-Cu-HT. Therefore, hydroxyl radicals were likely to have been formed via formation route 1, namely, the reaction of hydroxide ions (OH⁻) and holes (h⁺). The hydroxyl radicals formed from hydroxide ions and holes likely contributed to the antibacterial properties of AL-Cu-HT.

The antibacterial properties of TiO₂ materials were studied, and it was reported that such materials exhibited photocatalytic antibacterial properties not only for E. coli, but also *Staphylococcus aureus* and *Streptococcus mutans* under visible light [12,15,27–29]. Therefore, based on the previous reports, our materials are also expected to exhibit antibacterial properties for *Staphylococcus aureus* and *Streptococcus mutans*. We wish to evaluate the antibacterial properties of our materials for such bacteria in a future research work.

4. Conclusions

We prepared Cu-doped TiO₂ by the chemical and heat treatment of Ti metal, and evaluated the surface structure, apatite-forming ability, release behavior of Cu ions from sample specimens, antibacterial activity under visible light irradiation, and photocatalysis of the sample. The findings obtained in this study are listed as follows:

(1) We successfully prepared Cu-doped TiO₂ on a Ti chip surface, with a Cu content of 7 at.%. The crystalline phase of TiO₂ was a mixture of anatase and rutile.

(2) Apatite formation was observed on the Cu-doped TiO₂ surface in the SBF. Hence, Cu-doped TiO₂ is expected to have bone-bonding properties.

(3) The antibacterial properties of Cu-doped TiO₂ were higher than those of non-doped TiO₂ under visible light irradiation. The enhanced antimicrobial effect in the short bacterial culturing period was caused by the visible-light-responsive photocatalysis of the Cu-doped TiO₂. Antibacterial property expression derived from the antibacterial Cu ions from the sample is expected for long-term bacterial culturing because the sample showed a sustained Cu release behavior. The long-term antibacterial properties of the Cu-doped TiO₂ will be evaluated in future research.

(4) The reactive antibacterial properties of Cu-doped TiO₂ were hydroxyl radicals, formed by the reaction of hydroxide ions (OH⁻) and holes (h⁺).

Disclosure statement

The authors declare that there are no potential conflict of interests in this study.

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