Materials Research Express

PAPER

Surface characteristics and osteocompatibility of titanium preserved in a ZnS@BSA-containing storage solution

Zehua Tang1,2, Kaiming Tang1,2, Yao Liu1,2, Wenqing Zhu1,2 and Jing Qiu1,2,3

1 Department of Oral Implantology, Affiliated Stomatological Hospital of Nanjing Medical University, Nanjing, People’s Republic of China
2 Jiangsu Province Key Laboratory of Oral Diseases, Nanjing, People’s Republic of China
3 Jiangsu Province Engineering Research Center of Stomatological Translational Medicine, Nanjing, People’s Republic of China

E-mail: qiujing@njmu.edu.cn

Keywords: titanium, ZnS, BSA, storage solution, surface, osteocompatibility

Abstract

This study aimed to the preparation of a storage solution containing zinc sulfide particles encapsulated in bovine serum albumin (ZnS@BSA) and its application to improve surface characteristics and osteocompatibility of pure and SLA titanium surfaces. The analysis of surface characteristics including surface topography, elemental distribution and protein amounts confirmed that ZnS@BSA particles were successfully adsorbed on the two titanium surfaces without changing the original morphology. Assays of wettability showed that titanium surfaces preserved in ZnS@BSA solution had superior hydrophilicity compared with control groups. In vitro experiments demonstrated that the titanium surfaces preserved in ZnS@BSA solution significantly promoted the proliferation, adhesion and differentiation of MC3T3-E1 cells. The study therefore concluded that the ZnS@BSA solution could improve the bioactivity of titanium surface and exhibit the potential to be a new type of titanium implant storage solution.

1. Introduction

Implant dentures have been the first choice for restoring missing teeth because of the high success rate and excellent clinical aesthetic outcome [1, 2]. Osseointegration between implant and bone tissue is the key to success of implant denture which can be achieved by implant surface modifications, such as optimization of surface morphology, change in chemical composition, and biochemical modification [3–5]. However, two independent animal experiments indicated that the bone–implant contact percentage of implants with different modifications after osseointegration was only 45% ± 16% and 50%–75% [6, 7], which were far lower than the ideal value of 100%.

Although the reason for incomplete contact between the implant and bone tissue has not been elucidated, it may be partly related to the aging of titanium. Once exposed to the atmosphere, titanium forms a passive and protective surface oxide layer and hydrocarbons unavoidably accumulate on the titanium surface as time goes by [8]. The contamination of carbon causes an aging process of titanium surface from biological activity to biological inertness. This process leads to gradual deterioration during at least 6 months of preparation of the titanium surface [9]. The biological aging of titanium has been reported to be related to the changes in hydrophilicity and hydrocarbon contamination on the titanium surface [10], resulting in a decrease in surface biocompatibility. The super-hydrophilicity of titanium surfaces immediately after processing gradually attenuates and the surfaces become hydrophobic in 2 weeks with a significant increase in contact angle [11]. The protein adsorption, osteoblast adhesion and differentiation of the titanium surface significantly reduced after storage for 4 weeks compared with the freshly prepared titanium surface [8]. In addition, the aging phenomenon occurs on various modified titanium surfaces [12, 13], which has received more attention. How to preserve the bioactivity of titanium implants effectively so as to delay natural aging has become a research focus.

Finished titanium implants are usually preserved by aseptic packaging filled with air or saline in the clinical application. In recent years, it has been proposed to irradiate the titanium surface with ultraviolet rays (UV) to
reduce hydrocarbon contamination and thus maintain surface bioactivity [14]. However, with the passage of
time and the natural release of energy, the UV induced super-hydrophilic surface could not be sustained for a
long time [15]. In contrast, the titanium surface treated with UV remained bioactive through subsequent ddH₂O
or alendronate immersion [16, 17]. Indeed, liquid solution for implant storage is an economical, simple and
feasible anti-aging strategy. It has been reported that isotonic saline solution could significantly promote the
sandblasted with large grit and acid etched (SLA) implant osseointegration rate [18]. Compared with isotonic
saline, the titanium surface preserved in CaCl₂ solution was more conducive to the adhesion, differentiation and
mineralization of osteoblasts due to the calcium ion adsorbed on the titanium surface [19]. It was inferred that
the storage solution containing bioactive ions might help improve the bioactivity of the titanium surface while
resisting surface aging.

Recently, soluble and biocompatible zinc sulfide nanoparticles encapsulated in bovine serum albumin
(ZnS@BSA) were prepared for fluorescent probe applications [20, 21]. Zinc, a trace element required by the
human body, plays an irreplaceable role in bone development and bone metabolism [22–24]. Studies confirmed
that zinc could stimulate osteoblast differentiation by activating osteogenesis-related signaling pathways and
reduce the osteolytic ability of osteoclasts [25, 26]. After dental implantation, proteins adsorbed on the titanium
surface prior to other processes, thus affecting subsequent cell adhesion and proliferation [27]. The adsorption
properties of protein on the solid–liquid interface have been widely used in biosensors, gene delivery and
pharmaceuticals [28, 29]. The immobilization of albumin on titanium surface was found to be beneficial to the
osteoblast behavior and improved the corrosion resistance of the biomaterials [30, 31]. In addition, albumin
encapsulation promoted the sustained release of the drug and ameliorated the biocompatibility by reducing its
cytotoxicity [32–34].

Based on the aforementioned background, it was speculated that the ZnS@BSA-containing solution might
be applied for implant storage. Therefore, the present study evaluated the influence of ZnS@BSA storage
solution on the surface properties and biocompatibility of titanium, thus providing different ideas for
developing new implant storage solutions.

2. Materials and methods

Preparation of ZnS@BSA storage solution and titanium surfaces
First, 0.9% NaCl (AR, SCR, China) solution and 5 g l⁻¹ BSA solution were prepared with ddH₂O. According to
previous studies, 0.1 mol l⁻¹ zinc acetate aqueous (C₆H₅O₂Zn 2H₂O, AR, Shanghai Jiuyi, China) solution and
20 g l⁻¹ BSA (purity ≥ 98%, SunShineBio, China) aqueous solution were recombined at the ratio of 1:1.
Further, 0.1 mol l⁻¹ sodium hydroxide (NaOH, AR, SCR, China) was added to adjust the pH of the mixed
solution to 10. After that, 0.1 mol l⁻¹ sodium sulfide (Na₂S·9H₂O, AR, KESHIB, China) was added to the mixture
to obtain the ZnS@BSA solution. All the solutions were sterilized with bacterial filters in a sterile air cabinet
before mixing and all the reactions were performed at room temperature. The starting sizes of the ZnS@BSA
particles were measured using a Zetasizer Nano (ZS90, Thermo, USA) in triplicate.

Commercially pure titanium (TA1, Baoji, China) disks with diameters of 5 mm and 3 cm were polished with
SiC sandpaper (180, 400, 600, 1200, and 1500 grit) as CP-Ti. The SLA-Ti surfaces were prepared by sandblasting
with Al(OH)₃ and etching in HF/HNO₃ solution (H₂O:HF (0.11 mol/L):HNO₃ (0.09 mol l⁻¹) = 1000:2:4) for
10 min at room temperature, followed by etching in H₂SO₄/HCl solution (H₂O:HCl (5.8 mol l⁻¹):H₂SO₄
(8.96 mol l⁻¹) = 2:1:1) for 45 min at 80 °C. After ultrasonic cleaning, the two kinds of newly prepared
specimens were preserved in air, 0.9% NaCl solution, 5 g l⁻¹ BSA solution and ZnS@BSA solution, and kept in
airtight well plates for 2 weeks at 4 °C. Titanium disks with a diameter of 5 mm were stored in 96-well plates with
200 μl of solution, and titanium disks with diameter of 3 cm were stored in six-well plates with 2 ml of solution.

2.1. Surface characterization
Titanium disks stored in different media were washed with phosphate-buffered saline (PBS) three times to
remove the residual storage solution and incompletely adsorbed solute and subsequently air dried. The surface
morphology was observed using a scanning electron microscope (SEM, MAIA3 RISE, TESCAN, Czech) under
the accelerating voltage of 10.0 kV and the working distance of 5 mm. The distribution of different elements on
the titanium surface preserved in four media was detected using a energy-dispersive x-ray spectrometry (EDS,
ULTIM, Oxford, UK) under the same operating conditions.

Three samples preserved in different media for 2 weeks were rinsed with PBS three times to wash away the
unadsorbed solute so as to assay the protein amounts on the surfaces of BSA and ZnS@BSA groups. Afterwards,
the samples were transferred into a new 96-well plate and 200 μl of 1% SDS (Beyotime, China) was added.
Different samples were shaken for 1 h to transfer the protein adsorbed on the samples into the solution, and the

2
suspensions were collected to analyze the amounts of protein adsorbed on different specimens using a BCA protein assay kit (KeyGen Biotech, China).

The wettability of the titanium surface was evaluated by the contact angle test. Automatic Contact Angle Meter Model SL200B (Solon, Shanghai, China) was used to measure the contact angle and surface energy with 2 μl droplets on the specimens in triplicate. Titanium samples with a diameter of 5 mm were used in the aforementioned measurements.

2.2. Cell culture
MC3T3-E1 osteoblast-like cells were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). The cells were incubated with α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco, USA) in a constant-temperature incubator (37 °C, 95% relative humidity, 5% CO₂). The culture medium was refreshed every 3 days and the cells were passaged at 80% confluence.

2.3. Cell adhesion assay
MC3T3-E1 cells were seeded at the density of 5 × 10³ cells/well on the specimens with a diameter of 5 mm in 96-well plates. Cells were rinsed with PBS to remove the non-adherent cells, and then fixed with 4% paraformaldehyde at 4 °C over night. To observe early adhesion, the cells cultured for 1 h on different samples were stained with 4′,6′-diamidino-2-phenylindole (DAPI, Beyotime, China) for 30 s and counted. The cells cultured for 8 h on different samples were stained with Rhodamine Phalloidin (Cytoskeleton, USA) for 30 min and with DAPI (Beyotime, China) for 30 s in the dark to observe spreading morphology. The cell adhesion and spreading morphology were observed with a laser scanning confocal microscope (LSM710, Zeiss, GER) at 100× and 400× magnification. All tests were conducted in triplicate.

2.4. Cell proliferation assay
CCK-8 kit was used to quantify cell proliferation in terms of cell density on day 1, day 3, and day 6. MC3T3-E1 cells were seeded at a density of 3 × 10³ cells/well on the surface of the specimen with a diameter of 5 mm after 2 weeks of storage in 96-well plates. After 1, 3 and 6 days of culture, the medium was replaced with 100 μl of fresh medium supplemented with 10 μl of CCK-8 reagent (Beyotime, China), and then incubated at 37 °C for 2 h. The absorbance was measured at 450 nm wavelength with a microplate reader (Spectramax190, MD, USA). Three samples per group were used for this assay.

2.5. Alkaline phosphatase (ALP) activity assay
MC3T3-E1 cells were seeded at a density of 5 × 10³ cells/well on the specimens with a diameter of 3 cm and cultured for 7 days. Cells were rinsed three times with pre-cold PBS and lysed with RIPA buffer (LEAGENE, Beijing, China) at 4 °C for 30 min. The lysates were centrifuged at 12000 rpm at 4 °C and then the supernatants were collected. The ALP activity and protein content were determined using an AKP assay kit (Jiancheng Bioengineering Institute, China) and a BCA protein assay kit (KeyGen Biotech, China) following the manufacturer’s protocol, with samples analyzed in triplicate.

2.6. Western blot analysis
After 7 days of culture, MC3T3-E1 cells (5 × 10⁴ cells/well) seeded on samples with a diameter of 3 cm were rinsed with pre-cold PBS and harvested by lysis in RIPA buffer. BCA protein assay kit was used to determine the total proteins. Protein samples were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, MA, USA) followed by blocking with 5% nonfat milk for 2 h. The blots were then incubated with primary antibodies against Runx2 (12556, CST, USA), Osterix (ab209484, Abcam, USA), OCN (ab10911, EMD Millipore, USA), and GAPDH (60004, Proteintech, USA) overnight at 4 °C. The blots were then probed for 2 h with appropriate secondary antibodies (ZB-2301, goat anti-rabbit IgG; ZSGB-BIO,Beijing, China; AP124P, goat anti-mouse IgG; Millipore) and then visualized using a ECL Western Blot Kit (Millipore, CA, USA). GAPDH was used as an internal control. This assay was repeated in triplicate.

2.7. Statistical analysis
The results were analyzed using SPSS 22.0 (SPSS, Inc., IL, USA) with one-way analysis of variance, followed by the Student–Newman–Keuls post hoc test. *P < 0.05 indicated a statistically significant difference.
3. Results

3.1. Synthesis of ZnS@BSA solution
BSA contains abundant active groups including amine, carboxyl, sulphydryl and thiol groups, which can induce the formation of metal ion complex due to strong affinity\cite{35}. Upon mixing zinc ions (Zn$^{2+}$) with BSA solution, the BSA initially seized the Zn$^{2+}$ to form protein–metal complexes through electrostatic interactions\cite{36}. The alkaline environment could promote BSA to unfold the tertiary configuration\cite{37} and release active sulfur anions (S$^{2-}$) arising mainly from the disulfide bonds\cite{38}. The introduction of Na$_2$S into the mixture triggered the nucleation of ZnS particles to seed at the binding sites through the precipitation reaction between Zn$^{2+}$ and S$^{2-}$ in the expansive albumin cavities\cite{35}, which were much efficient to confine and regulate the crystal growth and encapsulation of ZnS particles\cite{39,40}. Figure 1 shows that the size of ZnS@BSA particles mainly ranged from 300 nm to 2 $\mu$m (average size, 929 nm).

3.2. Microstructural observation
The surface morphologies of specimens using different storage methods are shown in figure 2. After using different storage methods, CP-Ti and SLA-Ti showed their inherent characteristic structures under different magnifications: neat mechanical scratches on the CP-Ti surface and uniform micron-sized pit structure on the SLA-Ti surface. These SEM results indicated that different storage methods did not change the original microstructure of titanium surfaces. Particularly, microspheres were observed on the two titanium surfaces stored in the ZnS@BSA solution.

3.3. Surface analysis
The distribution of different elements on the two types of titanium surfaces was observed by EDS mapping. Figure 3 reveals the presence of titanium, oxygen, and carbon elements on the surface of each group. Sodium and chlorine elements overlapped in the normal saline group, indicating that sodium chloride crystals were scattered on two types of titanium surfaces after storage. Compared with the air group and normal saline group, the distribution of carbon and oxygen elements on the surfaces of the BSA group was denser and overlapped with nitrogen, suggesting that BSA mainly formed from carbon, oxygen, and nitrogen was adsorbed on the titanium surfaces. The distribution of zinc, sulfur, carbon, oxygen, and nitrogen elements overlapped with the microspheres on the two surfaces, illustrating that BSA-coated particulates containing ZnS were successfully assembled and uniformly adsorbed on the titanium surfaces.

Figure 4 represents protein amounts on CP-Ti and SLA-Ti disks preserved with BSA and ZnS@BSA solution. Compared with the air and 0.9% NaCl groups, protein was detected in the BSA and ZnS@BSA groups, confirming that proteins in the storage solution successfully adsorbed on two titanium surfaces. Moreover, the protein amounts on SLA-Ti was significantly higher than that of CP-Ti.
3.4. Hydrophilic property

Figure 5 displays the contact angles and surface energy of the two titanium surfaces. The titanium surfaces preserved with air tended to be hydrophobic with low surface energy. However, the normal saline group and BSA group had lower contact angles and higher surface energy, which might be related to the reduction of carbon pollution. Titanium surfaces stored in the ZnS@BSA solution exhibited minimal contact angles with the maximal surface energy compared with other storage methods. Particularly, SLA-Ti preserved in the ZnS@BSA solution had super-hydrophilic properties with a contact angle close to 0 degrees. These results showed that the wettability of the titanium surface could be effectively improved when stored of ZnS@BSA solution.
3.5. Cell adhesion and proliferation

Figure 6 shows the numbers of cells attached to the four samples after culturing for 1 h. The quantitative data measured by counting the cellular nuclei indicated that there were significantly more adhered cells on the surfaces preserved in the ZnS@BSA solution. Meanwhile, all the samples stored in different solutions exhibited higher adhesion numbers compared with the air group. The spreading morphology of MC3T3-E1 cells cultured on the surfaces of the four groups for 8 h is shown in figure 7. Compared with the air group, the cells spread more evenly across the titanium surfaces soaked in the ZnS@BSA solution. No clear morphological differences were observed between cells grown on samples stored in different solutions. The cell proliferation was measured using CCK-8 assay on day 1, 3, and 6 as shown in figure 8. The cell density was not significantly different on the third day of culture on the surfaces of CP-Ti and SLA-Ti after storage using different methods. However, on the first and sixth day of culture, the cell density of the two surfaces immersed in the ZnS@BSA solution was
Figure 6. Laser scanning confocal microscopy was used to count the adhesion numbers of MC3T3-E1 cells on CP-Ti and SLA-Ti preserved in different methods after 1 h of incubation (magnification: 100×, n = 3). Data are means ± SD. *P < 0.05. **P < 0.05 versus Air; ***P < 0.05 versus 0.9% NaCl; †P < 0.05 versus BSA; ‡P < 0.05 versus ZnS@BSA.

Figure 7. Laser scanning confocal microscopy was used to observe the spreading morphology of MC3T3-E1 cells cultured on the substrates of CP-Ti and SLA-Ti preserved by different methods after 8 h of incubation (magnification: 400×).
significantly higher than that in the air group and saline group, indicating that the two titanium surfaces submerged in the ZnS@BSA solution formed a more favorable environment for cell proliferation.

### 3.6. Osteoblast differentiation

The ALP activity of osteoblasts on the titanium surfaces preserved by different methods for 2 weeks is shown in figure 9. The ALP activity of osteoblasts on the two titanium surfaces was dramatically affected by the storage method. After culturing for 7 days, the cells on CP-Ti and SLA-Ti preserved in the BSA and ZnS@BSA solutions showed significantly higher ALP activity compared with the other two groups, especially the ZnS@BSA group showed the best activity.

The osteogenic protein expression levels of Runx2, Osterix, and OCN were determined by Western blot analysis to evaluate the differentiation ability of osteoblasts on the titanium surface. MC3T3-E1 cells cultured on the CP-Ti and SLA-Ti preserved in the ZnS@BSA solution showed the best osteogenic differentiation ability with the highest protein expression of Runx2, Osterix, and OCN. However, the osteogenesis-related proteins Runx2, Osterix, and OCN on the two titanium surfaces stored in the air were expressed at the lowest level (figure 10).

### 4. Discussion

Either pure titanium or SLA titanium surfaces have aging phenomena characterized by the loss of hydrophilicity and protein adsorption as time goes on[41]. In this study, the titanium surfaces were stored in a protein-containing aqueous solution to form a biologically active protein layer on the surface of the implant in advance. SEM results demonstrated that the storage methods would not change the initial morphology of titanium surfaces, which was consistent with previous findings[42]. In the ZnS@BSA group, microspheres ranging from
300 nm to 2 μm (figure 1) were observed both on CP-Ti and SLA-Ti surfaces (figure 2). As detected by EDS mapping (figure 3), the distribution of zinc, sulfur, carbon, oxygen and nitrogen elements overlapped with the microspheres on the two surfaces, indicating that ZnS@BSA was successfully assembled and adsorbed to titanium surfaces. The protein amount assay confirmed that the protein in the storage solution was successfully adsorbed on the two titanium surfaces (figure 4). Meanwhile, the amounts of proteins on the surface of SLA-Ti were much higher than that on CP-Ti, which might be related to the initial porous structure of SLA-Ti, thereby adsorbing more ZnS@BSA. This was consistent with the general conclusion that the adsorption of protein on the surface was affected by the physicochemical properties of the titanium surface[43].

As previously reported[18], compared with traditional storage in air, sodium chloride solution could reduce carbon pollution on the titanium surface, thereby improving the hydrophilicity and the surface bioactivity which was consistent with the findings of the present study (figure 5). The hydrophilicity of the titanium surface affected the total amount and binding strength of proteins, thus further affecting the geometry of the adsorbed molecules[44, 45]. Interestingly, the titanium surfaces preserved in the ZnS@BSA solution possessed the best hydrophilicity and the highest surface energy compared with other groups, which might facilitate the subsequent adsorption of other pro-osteogenic proteins after implantation, such as osteopontin, bone sialoprotein, and bone morphogenetic protein[46].

When implants are in contact with the physiological environment, water molecules first reach the material surface, followed by protein molecules, and finally the cells reach the material surface[47]. The cells adhere to the implant surface through the interaction of integrins on the cytomembrane surface and adhesion proteins on the implant surface. Therefore, in vivo and in vitro, the protein layer on the implant surface rather than the initial surface interacts with cells, thus affecting cell adhesion and differentiation[48]. Moreover, protein adsorption can be drastically enhanced by surface wettability, thereby improving the initial host contact[49]. Based on these, it is believed that the presence of protein and the improved hydrophilicity may lead to better adhesion of osteoblasts on the samples stored in the ZnS@BSA solution (figure 6).

Nanoparticles have a wide range of medical applications. Although many studies have reported the cytotoxicity of nanoparticles[50], their biocompatibility can be obtained by adjusting the size of nanoparticles, reducing the exposure dose, improving the purity, modifying the nanoparticle surface and so on[51, 52]. In the present study, ZnS nanoparticles and microparticles ranging from 300 nm to 2 μm were encapsulated in BSA and the dose was strictly controlled so as to obtain acceptable biosafety. No cytotoxicity of the two titanium surfaces preserved in the ZnS@BSA solution was observed in the cell proliferation assay (figure 7). Meanwhile, the amounts of proteins on the surface of SLA-Ti were much higher than that on CP-Ti, which might be related to the initial porous structure of SLA-Ti, thereby adsorbing more ZnS@BSA. This was consistent with the general conclusion that the adsorption of protein on the surface was affected by the physicochemical properties of the titanium surface[43].

The osteogenic differentiation ability of MC3T3-E1 cells on the titanium surfaces preserved by different methods was evaluated by the expression of osteogenic markers ALP, Runx2, Osterix and OCN. ALP is involved in the maturation and calcification of osteoblasts, and is one of the early osteogenic markers[54]. The ALP activity of the MC3T3-E1 cells cultured on the ZnS@BSA preserved titanium surfaces was upregulated (figure 9). Moreover, MC3T3-E1 cells cultured on the two titanium surfaces stored in ZnS@BSA highly expressed Runx2, Osterix and OCN (figure 10). The high expression of Runx2 and Osterix in the early stage of osteogenesis determines the differentiation of pre-osteoblasts into osteoblasts[55]. In addition, as a hormone-like polypeptide secreted by osteoblasts, OCN is regarded as a late marker of osteoblast differentiation[56]. The aforementioned results indicated that the titanium surfaces preserved in ZnS@BSA had superior biological
activity to boost the differentiation of osteoblasts. The pre-formed protein layer was believed to increase the biological activity of the titanium surface, thereby promoting the proliferation, adhesion and differentiation of osteoblasts on the titanium surface\cite{53, 57}.

At present, sodium chloride storage solution has been widely used in preserving clinical implants to delay their aging. This study found that storing the two types of titanium surfaces in a solution containing ZnS@BSA could improve the wettability and biological performance of the titanium compared with other groups, thus providing a new idea for the development of implant storage solutions. The binding force between the protein and the surface of the implant can be enhanced using some surface treatments, such as the introduction of chemical groups or nano-modification on the titanium surface\cite{58, 59}. Further optimization of the types of proteins and active particles in the implant storage solution is expected to form a protein-based bioinspired implant surface\cite{60}. Despite the application potential of the present study, whether the surface activity of implants preserved in this way can change over time requires further in vivo and in vitro exploration.

5. Conclusions

ZnS@BSA storage solution could improve the hydrophilicity of different titanium surfaces and enhance adhesion, proliferation and osteogenic differentiation abilities of MC3T3-E1 cells, suggesting its potential to serve as a new type of implant storage solution.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Project Number: 81870799), the Jiangsu Provincial Key Research and Development Program (Project Number: BE2019728), the Jiangsu Provincial Medical Youth Talent (Project Number: QNRC2016850), the Nanjing Medical University-SUYAN Group Intelligent Innovation Research and Development Project (Project Number: NMU-SY201806), the Southeast University-Nanjing Medical University Cooperative Research Project (Project Number: 2242017K3DN14), the Science and Technology Development Foundation of Nanjing Medical University (Project Number: NMUB2019072), and the Foundation of Priority Academic Program Development of Jiangsu Higher Education Institutions (Project Number: 2018–87).

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Author contributions

ZHT contributed to design, data acquisition and analysis, and drafted the manuscript. KMT and YL contributed to data acquisition and analysis. WQZ contributed to design and data analysis. The corresponding author JQ contributed to conception, design, data interpretation, and critically revised the manuscript. All authors give final approval and agree to be accountable for all aspects of the work.

Conflict of interest

All authors declare that they have no conflicts of interest with the contents of this article.

ORCID iDs

Jing Qiu @ https://orcid.org/0000-0001-8001-7432

References

[1] Fobbe H, Rammelsberg P, Lorenzo Bermejo J and Kappel S 2019 The up-to-11-year survival and success of implants and abutment teeth under solely implant-supported and combined tooth-implant-supported double crown-retained removable dentures Clin Oral Implants Res 30 1134–41
[2] Kntha K, Boek A, Peters F, Heitzer M, Modabber A, Kntha H, Holzer F and Mohllhenich S C 2020 Aesthetic aspects of adjacent maxillary single-crown implants- influence of zirconia and titanium as implant materials Int J Oral Maxillofac Surg 49 1489–96
[3] Li Q and Wang Z 2020 Involvement of FAK/P38 signaling pathways in mediating the enhanced osteogenesis induced by nano-graphene oxide modification on titanium implant surface Int J Nanomater 15 4659–76

[4] Sakatsume H, Takahashi M, Kanyi M W, Shimizu Y and Takada Y 2020 The process of magnesium ion modification of titanium surface and the sustained-release of magnesium ions from its surface Dent Mater J 39 509–16

[5] Teng F Y, Tai I C, Ho M L, Wang J W, Weng L W, Wang Y J, Wang M W and Tseng C C 2019 Controlled release of BMP-2 from titanium with electrodeposition modification enhancing critical size bone formation Mater. Sci. Eng. C Mater. Biol. Appl. 105 109879

[6] Weinsaeber M, Kenney E B, Lekovic V, Beumer J 3rd, Moy P K and Lewis S 1992 Histomorphometry of bone apposition around three types of endosseous dental implants Int J Oral Maxillofac Implants 7 491–6

[7] De Maertu M A, Bracera I, Alava J and Gay-Escoda C 2008 Improvement of osseointegration of titanium dental implant surfaces modified with CO ions: a comparative histomorphometric study in beagle dogs Int J Oral Maxillofac Surg 37 441–7

[8] Att W, Hori N, Takeuchi M, Ouyang J, Yang Y, Anpo M and Ogawa T 2009 Time-dependent degradation of titanium osteoconductivity: an implication of biological aging of implant materials Biomaterials 30 5352–63

[9] Minamikawa H, Att W, Ikeda T, Hirot& M and Ogawa T 2016 Long-term progressive degradation of the biological capability of titanium Materials (Basel) 9 102

[10] Shi X, Xu L, Wang Q, Sunarso and Xu L 2017 Hydrothermal sterilization improves initial osteoblast Responses on sandpaper-polished titanium Materials (Basel) 10 812

[11] Suzuki T, Hori N, Att W, Kubo K, Iwasa F, Ueno T, Maeda H and Ogawa T 2009 Ultraviolet treatment overcomes time-related degrading bioactivity of titanium Tissue Eng Part A 15 3679–88

[12] Suzuki T, Kubo K, Hori N, Yamada M, Kojima N, Sugita Y, Maeda H and Ogawa T 2010 Nonvolatile buffer coating of titanium to prevent its biological aging and for drug delivery Biomaterials 31 4818–28

[13] Ueno T, Takeuchi M, Hori N, Iwasa F, Minamikawa H, Igarashi Y, Anpo M and Ogawa T 2012 Gamma ray treatment enhances bioactivity and osseointegration capability of titanium J Biomol Mater Res B Appl Biomater 100 2279–87

[14] Nauzan Z, Rajson Z A B, Malitha S, Harizy P, Muhammad Q S and Noor H A R 2019 Ultraviolet A and ultraviolet C light-induced reduction of surface hydrocarbons on titanium implants Eur J Dent 13 114

[15] Aita H, Hori N, Takeuchi M, Suzuki T, Yamada M, Anpo M and Ogawa T 2009 The effect of ultraviolet functionalization of titanium on integration with bone Biomaterials 30 1015–25

[16] Jeon C, Oh K C, Park K H and Moon H S 2019 Effects of ultraviolet treatment and alendronate immersion on osteoblast-like cells and human gingival fibroblasts cultured on titanium surfaces Sci. Rep. 9 2581

[17] Choi S, Jeong W, Cha J, Lee J, Lee K, Yu H, Choi E, Kim K and Hwang C 2017 Overcoming the biological aging of titanium using a wet storage method after ultraviolet treatment Sci. Rep. 7 3833

[18] Buser D, Broggini N, Wieland M, Schenk R K, Denzer A J, Cochrans D L, Hoffmann B, Lussi A and Steinemann S G 2004 Enhanced bone apposition to a chemically modified SLA titanium surface J. Dent. Res. 83 529–33

[19] Lu H, Zhou L, Wan L, Li S, Rong M and Guo Z 2012 Effects of storage methods on time-related changes of titanium surface properties and cellular response Biomol. Mater. 7 200506

[20] Wu D, Chen Z and Liu X 2011 Study of the interaction between bovine serum albumin and ZnS quantum dots with spectroscopic techniques Spectrochim Acta A Mol Biomol Spectrosc 84 178–83

[21] Hsieh M, Li J, Lin C, Huang S, Sperling R, Parak W and Chang W 2009 Tracking of cellular uptake of hydrophilic CdSe/ZnS quantum dots/hydroxyapatite composites nanoparticles in MC3T3-E1 osteoblast cells J. Nanosci. Nanotechnol. 9 2758–62

[22] Ceylan M N, Akdas S and Yazihan N 2021 Is zinc an important trace element on bone-related diseases and complications? A meta-analysis and systematic review from serum level, dietary intake, and supplementation aspects Biol. Trace Elem. Res. 199 545–59

[23] O’Connor J P, Kanjilal D, Teitelbaum M, Lin S S and Cottrell J A 2020 Zinc as a therapeutic agent in bone regeneration Materials (Basel) 13 2211

[24] Huang Y, Yan G and Guan M 2020 Zinc Homeostasis in bone: zinc transporters and bone diseases Int. J. Mol. Sci. 21 1236

[25] Park K H, Choi Y, Yoon D S, Lee K M, Kim D and Lee J W 2018 Zinc promotes osteoblast differentiation in human mesenchymal stem cells via activation of the cAMP-PPA-CREB signaling pathway Stem. Cells Dev. 27 1125–35

[26] Yusa K, Yamamoto O, Iino M, Takano H, Fukuda M, Qiao Z and Sugiyama T 2016 Eluted zinc ions stimulate osteoblast differentiation and mineralization in human dental pulp stem cells for bone tissue engineering Biomaterials 28 3120–30

[27] Yang Y, Deng H, Huang C, Lu Z, Wang X, Zeng X, He H and Rao H 2019 Research of protein adsorption on the different surface topography of the zinc oxide Surf. Interface Anal. 47 245–52

[28] Caglioni R, Gatto F and Bardi G 2019 Protein Adsorption: a feasible method for nanoparticle functionalization? Materials 12 124911

[29] Bhakta S A, Evans E, Benavidez T E and Garcia C D 2015 Protein adsorption onto nanomaterials for the development of biosensors and analytical devices: a review Anal. Chim. Acta 872 7–25

[30] Gomes O P, Feltran G S, Ferreira M R, Albano C S, Zambuzzi W F and Lisboa-Filho P N 2020 A novel BSA immobilizing method on modified titanium surface ameliorates osteoblast performance Colloids Surf B Biointerfaces 190 110888

[31] Talha M, Ma Y, Kumar P, Lin Y and Singh A 2019 Roles of protein adsorption in the bio corrosion of metallic implants - a review Colloids Surf B Biointerfaces 176 494–506

[32] Zhang J, Hao G, Yao C, Yu J, Wang J, Yang W, Hu C and Zhang B 2016 Albumin-mediated biominalization of paramagnetic NIR Ag25 QDs for tiny tumor bimodal targeted imaging in vivo ACS Appl. Mater. Interfaces 8 16612–21

[33] Ziaaddini V, Saeidifar M, Esfandi- Moghadam M, Saberi M and Mozafari M 2020 Improvement of efficacy and decrement cytotoxicity of oxaliplatin anticancer drug using bovine serum albumin nanoparticles: synthesis, characterisation and release behaviour IET Nanobiotechnol. 14 105–11

[34] Fattahian Khorsh N, Saediifar M, Ramshini H and Saboury A A 2020 Interaction, cytotoxicity and sustained release assessment of a novel anti-tumor agent using bovine serum albumin nanocarriers J. Biomol. Struct. Dyn. 38 2546–58

[35] Yang T et al. 2016 Protein-nanonear-assisted synthesis of semiconductor nanocrystals for efficient cancer theranostics Adv. Mater. 28 5923–30

[36] Yang T et al. 2017 Size-dependent Ag5 nanodots for second near-infrared fluorescence/photocautious imaging and simultaneous photothermal therapy ACS Nano 11 1848–57

[37] Yang W et al. 2016 Albumin-bioinspired GdCu5 nanothoracic agent for in vivo photocaloustic/magnetic resonance imaging-guided tumor-targeted photothermal therapy ACS Nano 10 10245–57

[38] Sheng J, Wang L, Han Y, Chen W, Liu H, Zhang M, Deng L and Liu Y N 2018 Dual roles of protein as a template and a sulfur provider: a general approach to metal sulfides for efficient photothermal therapy of cancer Small 14 1101082
