BRIEF REPORT

Effect of hydrothermal treatment of titanium in high concentration of AgNO_3 solution on surface morphology and roughness [version 1; peer review: 2 approved]

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Abstract

Development of silver (Ag) modified titanium (Ti) as an antibacterial dental implant has recently been growing. Ag demonstrated an excellent antibacterial property without the risk of bacterial resistance. Hydrothermal treatment using AgNO_3 solution is one of the facile and promising methods to modify Ti surface with Ag. However, the effect of high AgNO_3 concentration and the absent of a toxic reduction agent has not been clearly studied. In this study, Ti surface was hydrothermally treated in 0.01 mol/L and 0.1 mol/L AgNO_3 solutions at 150°C for 24 hours. Analysis of surface morphology using scanning electron microscopy with energy dispersive X-ray analysis suggested the formation of non-homogenous Ag coating with a tendency to be aggregated and thicken with the increase of AgNO_3 concentration. The Ag coating deposited on Ti surface were composed of mainly metallic and some oxide forms. Surface roughness of all AgNO_3 treated Ti surface was comparable based on the analysis of surface roughness parameter. In conclusion, hydrothermal treatment of Ti surface in solely AgNO_3 solution at high concentration produced non-homogenous Ag coating on its surface without significantly changed surface roughness.

Keywords: Silver nitrate, titanium, hydrothermal, surface morphology, roughness

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Approval Status

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1. Le Thi Bang , Hanoi University of Science and Technology, Hanoi, Vietnam		
2. John Nicholson , Bluefield Centre for Biomaterials, London, UK		

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Sunarso S:** Conceptualization, Funding Acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Putra RJH:** Investigation; **Theodorea CF:** Writing – Review & Editing; **Pangesty AI:** Funding Acquisition, Resources, Supervision, Writing – Review & Editing

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Introduction

Titanium (Ti) has widely been used clinically for dental implants due to their excellent mechanical properties, biocompatibility and osteoconductivity.^{1,2} Development of Ti implant for dental application is still challenging. Dental implant not only requires osseointegration capability but also antibacterial property.³ Titanium demonstrated satisfactory osseointegration clinically. However, its antibacterial capability is lacking.

Silver coating has emerged as an alternative to prepare antibacterial titanium surface.⁴ Silver is considered a promising element to prevent and combat implant-related infection. Its main advantage is that it would not induce bacterial resistance.⁵ Silver is coated onto Ti surface often in the form of particles by immersion in AgNO_3 solution mixed with a reduction agent.^{6,7} This results in silver particulates being deposited on the Ti surface often in nanoscale, thus called silver nanoparticles (AgNPs). The use of reduction agents is problematic because they are often toxic chemicals such as Sodium borohydride, ammonium formate and hydrazine.^{8,9} A recent study has been conducted to coat Ti surface with silver under hydrothermal without the need for reduction agents.¹⁰

Direct Ag coating onto Ti surface using hydrothermal has not been clearly described especially in high concentration of AgNO_3 solutions and without the addition of toxic reduction chemicals. Therefore, this study aimed to coat the Ti surface with Ag particles using hydrothermal using solely AgNO_3 solution. Surface morphology including the distribution of the Ag coating and the change in surface roughness were then evaluated.

Methods

Sample preparation

Two Ti plates (Maximus Guard, Tokopedia) with a size of 10 cm × 10 cm and thickness of 1 mm were cut into 10 mm × 10 mm using a diamond cutter. A total of fifteen Ti plates (10 mm × 10 mm) were used in this study. The samples were washed ultrasonically with acetone, ethanol, and distilled water before drying. Silver nitrate (AgNO_3 , Merck) solutions with a concentration of 0.01 mol/L and 0.1 mol/L were prepared. Titanium samples were immersed in a 100 ml-size Teflon container with 25 ml of AgNO_3 solutions, which was then placed into a hydrothermal vessel (FBA_Lab, Tokopedia). The hydrothermal vessel was heated in an oven at 150°C for 24 hours. After hydrothermal treatment, the samples were washed with ethanol three times before drying.

Surface chemical composition, morphology and roughness

The elemental composition of titanium surface samples was examined using energy dispersive spectroscopy (Oxford instruments, UK) and analyzed using Oxford Aztec software. Scanning electron microscope (SEM) (Thermoscientific Quanta 650) (accelerating voltage (HV): 12kV, Secondary electron (SE), working distance (WD): 10.3-10.4mm) was used to evaluate the morphology of surface before and after hydrothermal. Surface roughness of titanium samples before and after hydrothermal treatment are measured using roughness tester (Surtronic S128) (Sampling length (l): 7 mm, cut-off (λ_c)/Type: 0.25 mm/2CR, Range: 100 μm). The average values of surface roughness were calculated using Microsoft excel spreadsheet software.

Results and discussion

Figures 1 and 2 show the photographs and SEM images of Ti surface before and after hydrothermal in AgNO_3 solutions. Bright particles were observed on Ti surface hydrothermally treated in 0.01 mol/L and 0.1 mol/L AgNO_3 . An area showed more concentrated particles, which may indicate the agglomeration. At higher concentration of AgNO_3 (0.1 mol/L), that concentrated area was larger (Figure 2). The elemental analysis from the surface using energy dispersive X-ray analysis (EDX) indicated that those particles are Ag (Figures 3-5). Many methods have been developed to coat Ag into Ti surface both in the form of particles or ions.^{4,11}

The formation of silver particles on Ti surface under hydrothermal from AgNO_3 solution was still unclear in this study. Several different mechanisms may be responsible for how Ag could be deposited on Ti surface from AgNO_3 under hydrothermal treatment. One possible way is through thermal decomposition.¹² The deposition of Ag particles from only AgNO_3 solution up to 75 $\mu\text{mol/L}$ under hydrothermal conditions was recently reported.¹⁰ However, the exact mechanism on how Ag particles could be deposited on Ti surface was not clearly described. AgNO_3 was also reported to transform into Ag nanoparticles under hydrothermal condition at 121°C.¹³ Hydroxyl ions exist on Ti oxide layer may also play a role on the Ag particles growth on its surface.⁶ It is known that Ti surface naturally forms thin oxide layer which contain Ti-OH groups on its outmost part. Figure 2 has confirmed the formation of Ag particles on Ti surface both in 0.01 mol/L and 0.1 mol/L AgNO_3 solutions. The Ag particles were also observed in the solution after hydrothermal (solution turned darkish color). The Ag particles seem to be non-homogenously distributed on Ti surface. As explained above, there is an area which contain thicker aggregated Ag particles masking the Ti surface. The concentrated Ag area was found to be larger in 0.1 mol/L AgNO_3 than that in 0.01 mol/L AgNO_3 . These findings suggest that at high concentration of AgNO_3 , the Ag coating tends to be aggregated and thicker, thus the use of a lower concentration might be preferred.

Figure 1. Photo of untreated Ti, Ag coated Ti from 0.01 mol/L AgNO_3 , and Ag coated Ti from 0.1 mol/L AgNO_3 .

Figure 2. Scanning electron microscope images of untreated Ti (A and B), Ag coated Ti from 0.01 mol/L AgNO_3 (C and D), and Ag coated Ti from 0.1 mol/L AgNO_3 (E and F).¹⁴

The next question is that whether the Ag coating deposited on Ti surface is in the metallic or oxide forms. One way to find this is using the EDX elemental mapping to the aggregated Ag coating. Figures 3 and 4 show the elemental mapping of Ag coated Ti prepared from 0.01 mol/L and 0.1 mol/L AgNO_3 solutions.¹⁴ In Figure 3, a thick Ag coating area (white dash line) shows a very strong purple color compared to the area in which less Ag coating was observed (white arrows). In Ti and oxygen (O) element mapping (green color and yellow color respectively), the Ag coating area was darker compared

Figure 3. Scanning electron microscope image (A) and energy dispersive X-ray analysis element mapping images of Ag coated Ti from 0.01 mol/L AgNO_3 (B).¹⁴

to the rest. The O elemental mapping provided very important data about the deposited Ag coating. The darker area (white dash line; Figure 3) in O elemental mapping indicated that that area was composed mostly of metallic Ag. Ag oxide also existed since the bright yellow color was also observed sporadically inside the white dash line (Figure 3; O K α 1). A similar trend was shown in Figure 4 where the Ag aggregate coating is larger. The thick Ag coating was most likely composed mainly from metallic Ag and smaller portion of Ag_2O

Surface treatment often changes surface roughness.¹⁵ The change in surface roughness might alter the biological performance of Ti implant. Therefore, it is necessary to evaluate whether the current method of Ag coating changed

Figure 4. Scanning electron microscope image (A) and energy dispersive X-ray analysis element mapping images of Ag coated Ti from 0.1 mol/L AgNO_3 (B).¹⁴

the surface roughness of Ti surface. Surface roughness parameters roughness average (R_a), maximum profile peak height (R_p), maximum profile valley depth (R_v), and mean roughness depth (R_z) were measured from all sample surfaces. Surface roughness texture of the sample surfaces were shown¹⁵ in Figure 6.¹⁶ The surface texture of all Ti samples before and after Ag coating were comparable. This data suggests that no significant changes were observed on Ti surface after Ag coating (Table 1). Comparison of SEM images between Ag coating and untreated Ti surfaces (Figure 2) also support surface texture data. The Ti substrate in which Ag coating deposited was found to be comparable (Figure 2A and B).

Figure 5. Element composition of untreated Ti (A), Ag coated Ti from 0.01 mol/L AgNO₃ (B), and Ag coated Ti from 0.1 mol/L AgNO₃ (C) obtained from energy dispersive X-ray analysis.¹⁴

Figure 6. Representative of surface roughness texture of untreated Ti (A), Ag coated Ti from 0.01 mol/L AgNO₃ (B), and Ag coated Ti from 0.1 mol/L AgNO₃ (C) generated from roughness tester.¹⁶

Table 1. Surface roughness parameter obtained from roughness tester. The values were calculated from three independent samples.¹⁶

Samples	Surface roughness parameter							
	Roughness average (μm)		Maximum profile peak height (μm)		Maximum profile valley depth (μm)		Roughness depth (μm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated Ti	0.44	0.01	1.38	0.09	1.45	0.05	2.84	0.04
Ag-Ti 0.01	0.44	0.03	1.34	0.12	1.39	0.08	2.73	0.20
Ag-Ti 0.1	0.48	0.02	1.48	0.04	1.45	0.05	2.94	0.02

Table 1 shows the quantitative values of surface parameter obtained from the roughness tester. The surface Ra and Rv values of all samples were relatively similar which indicating its comparable average surface height and deepest valley of the substrate. A slight increase of Rp and Rz values were recorded in Table 1. Surface roughness Rp shows the maximum peak height which come from the Ag coating aggregate and was larger when a solution of 0.1 mol/L AgNO_3 was used. The slight increase of Rp was followed by slight increase of Rz. Taken together, all surface roughness parameter demonstrated comparable values between untreated Ti (UnTi) and Ag coated Ti samples. This result suggests that hydrothermal treatment of Ti in 0.1 mol/L (Ag-Ti 0.1) and 0.01 mol/L AgNO_3 (Ag-Ti 0.01) did not cause a notable change in the surface roughness.

Conclusion

This study reported the effect of hydrothermal treatment of Ti surface in AgNO_3 solution on the surface morphology and roughness. After hydrothermal treatment, an Ag coating was observed in all treated Ti surfaces. EDX mapping suggests that Ag coating composed of mainly metallic Ag and in smaller quantities, Ag oxide. Using a higher AgNO_3 solution concentration resulted in more Ag aggregates that mask Ti surface, creating a non-homogenous coating. Surface roughness of treated Ti surface did not change significantly when coated. Nevertheless, a slight increase of Rp and Rz was observed; this might be due to Ag coating aggregates.

Data availability

Underlying data

Figshare: SEM and EDX <https://doi.org/10.6084/m9.figshare.17159234>¹⁴

This project contains the following underlying data:

- RAW SEM image (Fig 2A).jpg
- RAW SEM image (Fig 2B).jpg
- RAW SEM image (Fig 2C).jpg
- RAW SEM image (Fig 2D).jpg
- RAW SEM image (Fig 2E).jpg
- RAW SEM image (Fig 2F).jpg
- RAW EDX Mapping Data for 0.01M AgNO_3 treated Ti (Fig. 3)
- RAW EDX Mapping Data for 0.1M AgNO_3 treated Ti (Fig. 4)
- RAW EDX Map sum element spectrum for untreated Ti (Fig. 5A)
- RAW EDX Map sum element spectrum for 0.01M treated Ti (Fig. 5B)
- RAW EDX Map sum element spectrum for 0.1M treated Ti (Fig. 5C)

Figshare: Surface roughness <https://doi.org/10.6084/m9.figshare.17215958>¹⁶

This project contains the following underlying data:

- Output files for roughness testing
 - o 0.01 M-1_1.jpg, 0.01 M-1_2.jpg, 0.01 M-1_3.jpg, 0.01 M-1_4.jpg (Roughness testing for 0.01 M AgNO₃ treated Ti (specimen 1))
 - o 0.01 M-2_1.jpg, 0.01 M-2_2.jpg, 0.01 M-2_3.jpg, 0.01 M-2_4.jpg (Roughness testing for 0.01 M AgNO₃ treated Ti (specimen 2))
 - o 0.01 M-3_1.jpg, 0.01 M-3_2.jpg, 0.01 M-3_3.jpg, 0.01 M-3_4.jpg (Roughness testing for 0.01 M AgNO₃ treated Ti (specimen 3))
 - o 0.1 M-1_1.jpg, 0.1 M-1_2.jpg, 0.1 M-1_3.jpg, 0.1 M-1_4.jpg (Roughness testing for 0.1 M AgNO₃ treated Ti (specimen 1))
 - o 0.1 M-2_1.jpg, 0.1 M-2_2.jpg, 0.1 M-2_3.jpg, 0.1 M-2_4.jpg (Roughness testing for 0.1 M AgNO₃ treated Ti (specimen 2))
 - o 0.1 M-3_1.jpg, 0.1 M-3_2.jpg, 0.1 M-3_3.jpg, 0.1 M-3_4.jpg (Roughness testing for 0.1 M AgNO₃ treated Ti (specimen 3))
 - o UnTi 1_1.jpg, UnTi 1_2.jpg, UnTi 1_3.jpg, UnTi 1_4.jpg (Roughness testing for untreated Ti (specimen 1))
 - o UnTi 2_1.jpg, UnTi 2_2.jpg, UnTi 2_3.jpg, UnTi 2_4.jpg (Roughness testing for untreated Ti (specimen 2))
 - o UnTi 3_1.jpg, UnTi 3_2.jpg, UnTi 3_3.jpg, UnTi 3_4.jpg (Roughness testing for untreated Ti (specimen 3))

Extended data

Figshare: SEM and EDX <https://doi.org/10.6084/m9.figshare.17159234>¹⁴

This project contains the following extended data:

- Photograph Fig 1.jpg

RAW EDX Mapping for untreated Ti

Figshare: Surface roughness <https://doi.org/10.6084/m9.figshare.17215958>¹⁶

This project contains the following extended data:

- Summary roughness.xlsx (Aggregated data from surface roughness testing)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Author contributions

Conceptualization and methodology, S.; validation, S.; investigation, R.J.H.P.; resources, S.; writing—original draft preparation, S.; writing—review and editing, S., C.F.T. and A.I.P.; visualization, S.; supervision, S. and A.I.P.; funding acquisition, S. and A.I.P.

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John Nicholson

Bluefield Centre for Biomaterials, London, UK

The paper reports a useful and well-planned study of the effect of hydrothermal treatment of titanium with silver nitrate, with the aim of developing a new type of implant surface with inherent anti-bacterial properties.

Surface analysis of the finished metals showed that a non-homogeneous silver coating is deposited on the titanium, which is thicker with higher concentrations of silver nitrate in the treatment solution. Roughness of the surface was almost unchanged by the treatment. The finished coating is a potentially useful modification for clinical application in dental implants.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dental materials, oral biocompatibility, alloys, cements.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 April 2022

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Le Thi Bang

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This research work is the investigation of Ti surface morphology and topography under hydrothermal treatment of Ti in solely AgNO₃ at different concentrations for the antibacterial application. Method in this study is facile and effective for surface modification of any implant shape. The results showed that Ag coating composed of mainly metallic Ag and in smaller quantities, Ag oxide with higher agglomeration at higher Ag concentrations. And the coating does not alter the surface roughness significantly.

The study design is appropriate and the work is technically sound. However, some aspects have to be addressed:

1. There are several grammatical errors needed to be revised, for some examples:

“the absent of a toxic reduction...” should be “the absence of....”

“This results in silver particulates being deposited on the Ti surface often in nanoscale, thus called silver nanoparticles (AgNPs)”- verb of the sentence is missing.

“Figures 1 and 2 show the photographs and SEM images” should be “Figures 1 and 2 show the photographs and SEM images, respectively”. In addition, please give some explanation in the text for Fig. 1.

“The concentrated Ag area was found to be larger in 0.1 mol/L AgNO₃ than that in 0.01 mol/L AgNO₃. These findings suggest...” should be “The concentrated Ag ,,,, this finding suggests...”

Further comments:

1. Although the coating layer was analyzed by the EDX mapping, and it seems that the author speculates the phase of the coating. Do the authors confirm the phase identification of the Ti sample and the precipitated powder after hydrothermal treatment by XRD?
2. Why is the reduction agent needed to be used for the silver nanoparticle coating in previous studies? For example, for a homogeneous coating? What is the positive effect of using solely AgNO₃ coating to produce the antibacterial property to the Ti implant in comparison to the

previous study?

3. In Page 2 the authors stated that "It is known that Ti surface naturally forms thin oxide layer which contain Ti-OH groups on its outmost part" please revise this because the negative charge of Ti-OH was only obtained after treatment in alkali solution (see ¹)?
4. Did the coating particles just precipitate on the Ti surface or there is any possibility of bonding between the coating and the substrate?
5. In the SEM of the coating sample, the coating particles are not in nanoscale size? Can the author give some possibility of agglomeration of the particles?
6. In page 3, the author stated that "The concentrated Ag area was found to be larger in 0.1 mol/L AgNO₃ than that in 0.01 mol/L AgNO₃. These findings suggest that at high concentration of AgNO₃, the Ag coating tends to be aggregated and thicker, thus the use of a lower concentration might be preferred" please explain why? What is the minimum amount Ag requirement for the antibacterial effect and the maximum Ag amount that is safe for the application? Do the homogenous and entirely cover coating is needed for the application?

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biomaterials, calcium phosphate based bioceramic, metal implant, surface modification

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