Fabrication of the Silicate Containing CaTiO_3 Film with Hydrophilic and Smooth Surface on Titanium to Improve Osteoconductivity

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It has been reported that hydrophilicity and hydrophobicity of implants influenced the bioactivity. However, it is hard to maintain the hydrophilicity in case of being stored in air. So it is critical to find a way to maintain implants' hydrophilicity. In general, silicate has been known to contribute the hydrophilicity. In this study, the silicate containing CaTiO_3 films have been prepared on Ti substrates by two-step treatment for biomaterial applications. The hydrophilicity, osteoconductivity and protein adsorption of treated specimens have been investigated. The 1st step treatment for Ti is to form TiO_2 as precursors, either by anodizing in sulfuric acid solution at 298 K, liquid phase oxidation in nitric acid solution with hydrogen peroxide at 353 K, or thermal oxidation at 673 K in air. Hydrothermal treatment in silicate containing alkaline solution is the 2nd step to convert TiO_2 to silicate containing CaTiO_3 films. The SEM, XRD, XPS, WCA (water contact angle) investigations and protein adsorption measurements have been carried out to characterize the surface properties. This surface maintained 10 deg. in WCA after 7 d exposure in air, while the specimen without silicate has WCA of more than 40 deg. The osteoconductivity is evaluated based on the contact ratio of formed hard tissue on the implanted specimens after 14 d implantation in rats' tibia *in vivo* test. The as-prepared film not only has exhibited smooth and superhydrophilic surface, but also has achieved high osteoconductivity and great protein adsorption capacity.

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1. Introduction

By virtue of excellent mechanical strength, corrosion resistance and biocompatibility to the *in vivo* environment, Ti alloys were regarded as the fundamental materials in orthopedic surgeries in heavy load conditions, e.g. bone screws and plates. However, Ti alloys have relatively poor bone-forming properties. To clear the obstacles from the slow reaction rate in bone formation between implants and bone tissues, it is a feasible and promising approach to enhance osteoconductivity by bioactive coatings on implants. Several bio-coatings have been investigated to improve bioactivity with osteoblast, using coating materials such as titanium oxide,^{1,2)} HAp,³⁾ and so forth.

It has been reported that titanium oxide otherwise calcium titanate had bioactivity to hard tissue, compared with titanium oxide, henceforth, many surface modifications are proposed to fabricate calcium titanate (CaTiO_3) compounds on Ti substrates. The CaTiO_3 coatings have been reported to tremendously accelerate apatite nucleation on Ti, and their relevant surface features make an attractive scaffold for the enhancement of apatite adherence on metallic load bearing prostheses.⁴⁻⁸⁾ Wei *et al.* report that fibronectin can be adsorbed easily onto hydrophilic surface and albumin onto hydrophobic surface, respectively.⁹⁾

Anodizing treatment provides a uniform and stable protective film on the valve metals. The previous studies showed that osteoconductivity was obviously affected by hydrophilicity and surface roughness because of their strong influence on protein adsorption and cell adhesion after implantation into the body. Yamamoto *et al.* assessed the influence of surface roughness and hydrophilicity on the osteoconductivity of TiO_2 film by anodizing. They found that

the better hydrophilic performance gave rise to the higher osteoconductivity of TiO_2 coatings on a smooth surface ($\text{Ra}/\mu\text{m} < 0.1$).¹⁰⁾ They also found that changing the anodizing condition altered the hydrophilicity of TiO_2 film. However, the water contact angle (WCA) did not decrease to below 20 deg. and degraded to over than 40 deg. after storing in air for 7 d.¹¹⁾

Titanium and its alloys cannot form calcium phosphate only by the immersion test in the solution containing calcium and phosphate ions. On the other hand, it was said that the calcium phosphate precipitation was much faster on Ca^{2+} -implanted titanium surface than on the Ti surface, resulting in earlier bone formation on the Ca^{2+} -implanted Ti compared to as-polished Ti.¹²⁾

From the viewpoint of application in surgeries, the degradation of hydrophilicity has adverse impact to osteoconductivity when the samples exposed in the air. Hence, it is important to maintain hydrophilicity before surgical operation. In the previous studies, phosphate buffer solution (PBS(-)) was used to maintain hydrophilicity by changing storage environment and period.¹³⁾ In this study, silicate containing films on Ti substrate was adopted. Silicate is widely used as a dehydrating agent for silica gel as a desiccant, preservation of food and semiconductor precision equipment, and it is a substance with high affinity for water, shown as SiO_3^{2-} in human body. The silica surface contains silanol with a hydroxyl group therein, which is known to form hydrogen bond with water molecule. In addition, water-soluble silica is not only contained in trace amounts in the human body, but also contained in hair, nails, blood vessels, bones, joints, cell walls, etc., especially concentrated in the cell layer of osteogenesis. Furthermore, it has been reported that the surface hydrophilicity could be enhanced by the ionic formation of the silicate to the surface of the base material, since it has a complicated surface shape and therefore surface

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Table 1 Treatment conditions of one step process.

Denotation		Conditions	
(a)	AO ₁	0.1 M Na ₂ SiO ₃	80 V, 0.1 V S ⁻¹
(b)	AO ₂	1.0 M Na ₂ SiO ₃	
(c)	AO ₃	0.1 M NaOH	100 V, 0.1 V S ⁻¹
(d)	AO ₄	0.1 M H ₂ SO ₄	

Table 2 Treatment conditions of two step process.

Denotation		1st step conditions	2nd step conditions (HT, 453 K, 3 h)
(e)	AO _a	0.1 M H ₂ SO ₄ 100 V, 0.1 V S ⁻¹	20 mM Ca(OH) ₂
(f)	AO _b		20 mM Ca(OH) ₂ and 10 mM Na ₂ SiO ₃
(g)	TO _a	673 K, 4 K min ⁻¹ , 2h	20 mM Ca(OH) ₂
(h)	TO _b		20 mM Ca(OH) ₂ and 10 mM Na ₂ SiO ₃
(i)	LO _a	5 M H ₂ O ₂ and 0.1 M HNO ₃ , 353 K, 20 min	20 mM Ca(OH) ₂
(j)	LO _b		20 mM Ca(OH) ₂ and 10 mM Na ₂ SiO ₃

polarity is increased.¹⁴⁾ As demonstrated in the past research, silanol groups abundant on the surface of silica gel might be responsible for apatite formation in simulated body environment, which showed strong connectivity with bioactivity.¹⁵⁾

The purpose of this study is to fabricate smooth and hydrophilic surfaces, with better stability in air, by embedding silicate in CaTiO₃ thin film, furthermore, and to investigate the connection between protein adsorption and osteoconductivity of the treated Ti substrate.

2. Materials and Methods

2.1 Preparation of specimens

Commercially pure Ti (cp-Ti) plates with 1.13 cm² in surface area (12 mm φ $\times$ 3 mm) were subjected to surface modification and cylindrical rods with 2 mm in diameter and 5 mm in length were subjected to *in vivo* testing. All the substrates were polished with emery paper up to #2000 and then with buffing using Al₂O₃ particles with 0.05 μ m in particle size to produce smooth surfaces (Ra/ μ m < 0.1). After polishing, the substrates were ultrasonic cleaned in distilled water and dried in air.

2.2 One step treatment of silicate containing titanium compound thin films

In order to find the suitable conditions to form silicate containing TiO₂ thin-films, the as-polished Ti specimens were treated by one step process at first. One step treatment

was anodizing oxidation in several electrolytes, i.e., 0.1~1.0 M Na₂SiO₃ or 0.1 M NaOH solution (M = mol dm⁻³). Ti potential was elevated to 80 V with scan rate of 0.1 V s⁻¹ as shown in Table 1.

2.3 Two step treatment of silicate containing titanium compound thin films

The one step treatment could not realize the formation of silicate. Therefore, two step treatment was tried to fabricate SiO₃²⁻ and CaTiO₃ film. Two step treatment conditions, consisting of 1st step and 2nd step is summarized in Table 2.

During the 1st step to form TiO₂ films as a precursor, anodizing oxidation, thermal oxidation and liquid phase oxidation were employed using the polished Ti substrate. The anodizing oxidation was carried out in 0.1 M H₂SO₄ aqueous solution (pH = 1.0) with an applied voltage that increased from 0 up to 100 V at a rate of 0.1 V s⁻¹. The electrolytic bath was stirred by magnetic stirrer and kept at a constant temperature (298 K) while anodizing. After reaching a potential of 100 V, the applied voltage was quickly turned off and the sample was shifted into a beaker and was ultrasonic cleaned for 5 min. in distilled water and dried in air. The anodizing oxidation treatment is denoted as "AO". As-polished Ti specimens were put into electric furnace for thermal oxidation, where the temperature increased from room temperature to 673 K at a rate of 4 K min⁻¹ in air atmosphere and maintained for 2 h. The specimens were then cooled in the furnace, in the same atmosphere. This thermal

oxidation process is denoted as “TO”. In the method of liquid phase oxidation, Ti specimens were immersed into a beaker containing 50 mL solution with 5 M H_2O_2 and 0.1 M HNO_3 , at 353 K for 20 min. with stirring. The beaker was put into water bath to control the temperature at 353 K. This liquid-phase oxidation process is abbreviated as “LO”.

Then, hydrothermal as the 2nd step process was performed to add silicate ions into titanate compounds. $\text{Ca}(\text{OH})_2$ aqueous solution was chosen in hydrothermal treatment. Here, CaTiO_3 could not generate on the Ti surface directly by merely soaking in $\text{Ca}(\text{OH})_2$ solution with at 298 K.¹⁶⁾ So the two step treatment was carried out to obtain the mixture of CaTiO_3 and SiO_3^{2-} containing compounds. The samples were immersed in teflon bottles with 40 mL aqueous solution of 20 mM $\text{Ca}(\text{OH})_2$ and 0~15 mM Na_2SiO_3 (pH 12.5~13). Teflon bottles were put into stainless container, set at 453 K and maintained at this condition for 3 h. After hydrothermal treatment, the samples were cooled down naturally to room temperature and ultrasonically cleaned in distilled water for 5 min. and dried in air. Finally, they were dried for further evaluations. This hydrothermal treatment is denoted as “HT treatment”.

2.4 Film characterization

The surface morphologies of the treated and untreated samples were observed by scanning electron microscopy (SEM). The crystal structure and chemical composition of Ti substrate were determined by thin-film X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS), respectively. The surface roughness was measured by means of contactless probing using a confocal laser scanning microscope with a measurement area of $150\ \mu\text{m} \times 112\ \mu\text{m}$. The arithmetical mean of the profile deviations (R_a) defined the surface roughness. The water contact angle (WCA) was estimated using a $2\ \mu\text{L}$ droplet of distilled water.

2.5 Protein adsorption

The samples with several WCA were used in the protein adsorption testing. WCA was controlled by the change of storing time and temperature in air after the hydrothermal treatment in two step treatment. Fibronectin or albumin was picked up in this protein adsorption experiment, which was cell adhesive protein and not cell adhesive, respectively. The samples were immersed in 0~1.0 mg mL⁻¹ fibronectin aqueous solution or 0~50 mg mL⁻¹ albumin at 310 K for up to 1 h, respectively. The protein adsorbed samples were subjected to the FT-IR analysis (ATR mode) after the ultrasonic cleaning in the distilled water. The protein content was evaluated from the peptide binding in the protein in the FT-IR spectra.

2.6 In vivo testing

Because the experimental procedure for our *in vivo* study was almost the same as what has been illustrated in previous reports,^{11,17)} so it is described only briefly here. All cylindrical rod samples before and after the two step treatment were subjected to *in vivo* testing. They were implanted in rats' tibia for 14 d. The samples were sliced toward longitude and stained with toluidine blue. Eight-week-old male Sprague Dawley rats (Charles River Japan,

Inc., Tokyo, Japan) were used in our experimental procedures. The interface morphology between implant and the cortical bone or cancellous bone was observed by optical microscope. The bone implant contact ratio of cancellous bone and the cortical bone were recorded as percentage (the bone implant contact ratio, R_{B-I}) between formed hard tissue and the entire implant length, respectively.

3. Results and Discussion

3.1 Characteristics of the film obtained by one step fabrication

The films were characterized to examine whether hydrophilicity can maintain in air. As a pre-experiment, simple soaking of Ti substrate for 1 day in Na_2SiO_3 solution was examined. TiO_2 peaks were detected in XRD profile after soaking, while it is failed to detect Si 2p in thin films by XPS. Next, AO in Na_2SiO_3 solution was employed to make hydrophilic films. In respect of anodizing technique using only one step treatment, the change in water contact angle, WCA, with storage time in air after AO and typical XPS results for Ti specimens are shown in Fig. 1. AO were performed in each solutions of 1.0 M Na_2SiO_3 of pH 14.0, 0.1 M Na_2SiO_3 of pH 13.5 and 0.1 M NaOH of pH 13.6. The as-polished Ti showed WCA of 62 deg. and increased to 75 deg. after storage for 7 d in air. The WCA of specimen anodized in 1.0 M Na_2SiO_3 solution showed high values of 50~55 deg. Though the WCA values decreased to less than 30 deg. for the specimens anodized in 0.1 M Na_2SiO_3 or in 0.1 M NaOH solutions, they increased up to more than 50 deg. gradually with time for 7 d. Therefore, only one step AO could not lead to superhydrophilic surface, i.e., WCA < 10 deg. It was confirmed that Si 2p peaks was not detected from XPS for the anodized films as shown in Fig. 1. Hence, silicate could not be directly included into TiO_2 only by one step AO in silicate containing solution. Storage environment in air was incapable for remaining long-term hydrophilicity on these specimens.

As silicate could not be added into the TiO_2 films by AO treatment in Na_2SiO_3 solution, the two step treatment was

Fig. 1 Change in water contact angle of Ti with storage time in air (● as-polished Ti) after anodizing in (a) 0.1 M Na_2SiO_3 , (b) 1 M Na_2SiO_3 , and (c) 0.1 M NaOH solutions. Internal profiles are XPS results for (a) and (c).

Fig. 2 Potential-pH diagrams for Ti-H₂O and Si-H₂O systems at 298 K. Ion concentration = 10^{-6} .¹⁸⁾ Water stable window is between (a) and (b).

applied. Considering titanate and silicate on E -pH diagram,¹⁸⁾ the thermodynamic stabilities of TiO₂ and SiO₃²⁻ are confirmed as shown in Fig. 2, i.e., E -pH diagrams of Ti-H₂O system and Si-H₂O system (ion concentration = 10^{-6} M, 298 K). It is difficult to include silicate into TiO₂ films as silicate ion was not stable in aqueous solution under pH < 10. Both silicate ion and TiO₂ were stable in the pH region between 10 and 12, according to E -pH diagram. However, it seems difficult to control the pH near Ti surface in this region because the pH was partially changed with the process of AO treatment. Also, in the stable pH region on silicate, titanate is stable species, instead of TiO₂. As a result, the two step treatment strategy of the fabrication of silicate containing titanate film to maintain long-term hydrophilicity is highly considered. Namely, we employed two step treatment consisting of AO in H₂SO₄ solution and hydrothermal treatment for converting titanate and doping silicate in alkaline solution, sequentially. Thermal oxidation and liquid phase oxidation processes were also discussed briefly for comparison with the 1st step process.

3.2 Characteristics of the film obtained by two step treatment

At the 1st step, the treatments of AO, TO and LO were employed to as-polished Ti substrate described above. After the 1st step treatment, film thickness was detected to be 120 nm for AO, 120 nm for TO and more than 600 nm for LO. Surface roughness was controlled by $R_a/\mu\text{m} < 0.1$ for AO and TO treatment as 1st step, while it was not able to control $R_a/\mu\text{m} < 0.1$ for LO, where micro cracks were

observed in SEM images. Then, hydrothermal as the 2nd step process was performed to dope Si into CaTiO₃ and SiO₃²⁻ film. It is reported that calcium phosphate could form *in vivo* test on titanium surface which Ca²⁺ ion was adsorbed beforehand.¹⁹⁾

Figure 3 shows the SEM images, optical micrograph of specimens surfaces after two step treatment. Smooth surfaces ($R_a/\mu\text{m} < 0.1$) were maintained by AO and TO as the 1st step treatment, while relative rough surfaces, $R_a/\mu\text{m} = 0.19$ and $R_a/\mu\text{m} = 0.24$ were formed in LO treatment as shown in Fig. 3(e) and (f), respectively. By treatment with these two conditions, the surfaces with concave-convex morphologies were observed in SEM images in Fig. 3(e) and (f). While, in the Fig. 3(a) to (d), the smooth morphologies are shown.

Figure 4 shows XRD patterns of treated specimens by two step treatment. CaTiO₃ peaks were newly detected in XRD patterns of all specimens after two step treatment. However, XRD peaks corresponding to TiO₂ were rarely observed on the specimens after two step treatment. Meanwhile, the Si-containing compound peaks were not found in XRD results.

In order to examine the SiO₃²⁻ existence in the thin-films, XPS arrangement was carried out for the specimens treated in Ca(OH)₂ solutions containing various concentration of Na₂SiO₃ in HT treatment, and the result is shown in Fig. 5. Si 2p peaks were detected by examination on AO_b, TO_b and LO_b at 103 eV. The ratio of Si to Ti was increased with the increase in Na₂SiO₃ concentration. This ratio was approach steady state when the concentration of Na₂SiO₃ was over than 10 mM. The specimens after LO as 1st step and HT as 2nd step showed the highest Si/Ti value, i.e., 0.73. Si 2p peak was detected by XPS, nevertheless no peaks of silicate compounds were detected in XRD in Fig. 4.

3.3 WCA change of treated samples

Time-dependence of WCA under air atmosphere for specimens treated by two step treatment is shown in Fig. 6. It was found that the WCA values of the as-treated specimens after HT treatment were decreased from 23 deg. to under 10 deg. for as-anodized Ti, unrelated with the existence of Na₂SiO₃ in aqueous solution. SiO₃²⁻-containing thin film was expected to maintain hydrophilic properties in air environment for 7 days. It was obvious that SiO₃²⁻-containing specimens maintained hydrophilic surfaces WCA < 22 deg. even after the exposure in the air for 7 days, as shown in Fig. 6(h), (f) and (j). And there was positive correlation between Si/Ti ration and WCA after 7 days in air.

It was reported that hydrocarbon (C-H) always adsorbed on the solid surfaces in the air and the aqueous solutions containing organic contaminants.²⁰⁾ The hydroxyl group is another factor affecting the surface hydrophilicity, in a similar manner as adsorbed C-H. Therefore, surface functional group was analyzed for the treated specimens and the ratio of intensity area of -OH, C-H to intensity area of O-Ti in XPS was compared, respectively. Figure 7(1) shows the typical XPS profiles, the peak attributed to the Si-O bond can be found at approximately 103 eV. The change in hydroxyl group and hydrocarbon group with time for Ti treated by two step treatment was shown in Fig. 7(2). The amount of -OH was clearly high for the specimens after hydrothermal treated in the solution containing Na₂SiO₃. This suggested that

Fig. 3 Surface morphology, optical micrograph and surface roughness (Ra) of Ti treated by two step process. The 2nd step process of HT (a) AO_a (c) TO_a (e) LO_a in 20 mM Ca(OH)₂ solution, (b) AO_b (d) TO_b (f) LO_b in 20 mM Ca(OH)₂ + 10 mM Na₂SiO₃ solution.

silicate containing film was effective to maintain hydrophilicity. There is no obvious difference of XPS intensity ratios between AO and LO treatment. It is shown that $-OH$ and $C-H$ intensity ratio was increased for the specimen exposed in the air after 3 d, comparing with 0 d exposure of as-treated specimens. Combined the results of Fig. 6 and Fig. 7, it showed that there was no obvious difference of the $C-H$ amount between AO_a and AO_b or LO_a and LO_b, however, there is remarkable increase of $-OH$ amount, which was considered as the reason of WCA increase in Fig. 6 from AO_a and LO_a to AO_b and LO_b.

3.4 *In vivo* test and protein adsorption test

In vivo test was carried out for the specimens treated by two step treatment after storage for 3 d in air. The values are shown in Fig. 8. The results of previous work¹¹⁾ using Ti by controlling the WCA and roughness ($Ra/\mu m < 0.1$) are also accompanied with this work. It was found that R_{B-I} values of previous works were described as the V-like line, i.e., the smaller WCA the higher R_{B-I} , in the region beneath of 60 deg. The R_{B-I} of the two step treated specimens increased with decreasing the WCA by in spite of containing silicate. The R_{B-I} value became higher by 40%–45%, compared with that of the treated samples without silicate, 30%–35%.

According to previous reports,^{21,22)} surface hydrophilicity influenced the adsorption of cell adhesive proteins containing Ag-Gly-Asp (RGD) sequences, such as fibronectin, on the

implant surface, and thus enhanced the adhesion and spreading of osteoblast precursors on these surfaces. Our results were consistent with those of previous *in vitro* and *in vivo* researches^{23–25)} that detected osteoblast cell adhesion, differentiation, and early hard tissue formation on implant surfaces were significantly enhanced on hydrophilic surfaces compared with hydrophobic surfaces. In case of the given WCA, the R_{B-I} and adsorption of protein were estimated accordingly.

Both fibronectin and albumin adsorption on the surfaces of thin films with silicate were about two times higher than those without silicate as shown in Fig. 9. The trend of protein was agreed well with the R_{B-I} value of the samples without the protein adsorption. LO_a and LO_b were slightly not agreed with the other lines, which was probably because $Ra/\mu m$ of these specimens was higher than 0.1, the rough surfaces showed high hydrophilicity, but it had no benefits on the protein and cell adsorption.

4. Conclusions

In this research, new method to maintain superhydrophilicity in air was adopted by using two step process, i.e., anodizing oxidation, thermal oxidation and liquid phase oxidation as 1st step, and hydrothermal treatment as 2nd step.

After 3 d stored in air, specimens with the surface of silicate containing CaTiO₃ film exhibit superhydrophilicity

Fig. 4 XRD patterns of Ti treated by two step process. The 2nd step process of HT (e) (g) (i) in 20 mM Ca(OH)_2 solution, (f) (h) (j) in 20 mM $\text{Ca(OH)}_2 + 10 \text{ mM Na}_2\text{SiO}_3$ solution.

Fig. 5 Ratio of Si to Ti area in XPS analysis for Ti treated two step process. 1st step: AO, TO, LO. 2nd step: HT in the solution of 20 mM Ca(OH)_2 with 0–15 mM Na_2SiO_3 .

and show higher osteoconductivity than that of CaTiO_3 film without silicate doping. The silicate containing CaTiO_3 film retained high rate of $-\text{OH}$ groups on the surface of specimens. At 1st step, the specimens treated by liquid phase

Fig. 6 WCA time-dependence of Ti treated by two step process. The 2nd process of HT (e) (g) (i) in 20 mM Ca(OH)_2 solution, (f) (h) (j) in 20 mM $\text{Ca(OH)}_2 + 10 \text{ mM Na}_2\text{SiO}_3$ solution. (d) was as-anodized Ti sample in H_2SO_4 .

Fig. 7 (1) Graph of typical XPS profiles, (2) change in hydroxyl group and hydrocarbon group with time for two step process treated Ti. The 2nd process of HT (e) (i) in 20 mM Ca(OH)_2 solution, (f) (j) in 20 mM $\text{Ca(OH)}_2 + 10 \text{ mM Na}_2\text{SiO}_3$ solution. $\square$ 0 d exposed or $\square$ 3 d exposed in air after 2nd step treatment.

oxidation show best hydrophilicity, although it has the worst osteoconductivity compared to other treatments, since its $R_a/\mu\text{m}$ is around $0.24 \mu\text{m}$ with doping silicate and $0.19 \mu\text{m}$ without silicate, while surface roughness significantly affects wettability and osteoconductivity.

In general, the combination of anodizing in acidic solution and hydrothermal treatment in alkaline solution with silicate is an effective method in order to maintain hydrophilicity in air for a long period and enhance the osteoconductivity of Ti. It is also found that silicate containing surface lead to increase in protein adsorption. Moreover, rough surfaces are

Fig. 8 Relationship between the WCA and R_{B-I} values of cortical bone of two step treated Ti samples and $\circ$: previous works of as-polished Ti and as-anodized Ti in H_2SO_4 .

Fig. 9 Effect of water contact angle on protein adsorption on Ti controlled surface water contact angle. $\circ$: previous works of as-polished Ti and as-anodized Ti in H_2SO_4 .

considered as negative effect to the protein adsorption, which is in accordance with the roughness to osteoconductivity.

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