

Investigation on Hard-Tissue Compatibility of TiN Surface Formed by Atmospheric-Pressure-Plasma Nitriding

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In this study, we demonstrated the improvement in the biocompatibility of titanium alloy by atmospheric-pressure-plasma nitriding, in which the pulsed-arc plasma jet is sprayed onto a titanium alloy to form TiN surface. The deposition properties of calcium phosphate on different samples were compared after immersion in simulated body fluid to investigate hard-tissue compatibility. It was determined that the growth of the calcium phosphate layer on the nitrided sample was the most rapid. This result suggests that atmospheric-pressure-plasma nitriding has the potential to easily improve the hard-tissue compatibility of titanium alloy.

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In recent years, new surface treatment methods have been developed to improve biological characteristics of biomaterials such as Ti alloy, which is used in the medical field. Several studies have reported that TiN coatings and nitriding of Ti alloys improved biocompatibility [1–14]. For example, Lin *et al.* determined that a TiN layer can inhibit the adhesive property of mutans streptococcus [1]. Moreover, Zhao *et al.* demonstrated that a TiN layer improves the adhesive property of osteoblast cells, which synthesize bones [2]. The latter report indicates that a titanium nitride surface improves hard-tissue compatibility as well as mechanical strength. The mechanism of calcium phosphate formation on TiN surfaces has not yet been revealed. However, Hashimoto *et al.* proposed that changes in the zeta potential increases the formation ability of calcium phosphate [15]. Furthermore, Zhao *et al.* and Kokubo *et al.* reported that a positively charged surface showed high forming ability of calcium phosphate in simulated body fluid [15–18].

For practical applications of TiN formation, conventional plasma nitriding methods have several disadvantages owing to large vacuum systems [19–31], which complicate treatment processes and cause difficulties in self-manufacturing in medical/dental fields. To resolve these disadvantages, this study investigated a novel nitriding method using atmospheric-pressure plasmas. Atmospheric-pressure-plasma nitriding has various advantages, e.g., easy operation and the lack of a large vacuum system, which will easily facilitate self-manufacturing in the medical/dental field. Nitriding methods, using the

Pulse-Arc (PA) plasma jet, have been developed in previous studies. The nitriding of steel and nitro-quenching were successful in the atmospheric pressure [32–34]. For the present, a circular area of 20 mm in diameter is treatable at least. In particular, this original nitriding method can form a titanium nitride layer on a titanium surface [35]. Moreover, by performing narrow-hole treatment, it was demonstrated that this nitriding method has high treatment ability for complex-shaped workpieces [36]. This indicates the potential of this method to treat implant screws used in medicine and dentistry. However, no research has been performed to compare the nitrided layer formed by this new method with the previous studies on biocompatibility. Hence, in this study, the hard-tissue compatibility of a Ti alloy nitrided by atmospheric-pressure plasma was investigated for the first time. Here, the immersion test was carried out with simulated body fluid to chemically examine the hard-tissue compatibility.

The sample was titanium alloy Ti-6Al-4V (ASTM F316), which is widely used in medical field. The sample size was $15 \times 15 \times 4 \text{ mm}^3$. The samples were polished with waterproof abrasive papers of #500, #1000, and #2000, and mirror polished with alumina powder ($1 \mu\text{m}$). The polished samples were ultrasonically cleaned with acetone.

A thin layer of TiN was formed by the PA plasma jet shown in Fig. 1. The operating gas was a N_2/H_2 gas mixture with the flow ratio of 99 : 1 [33], and the total flow rate was 20 slm. Low-frequency voltage pulses (5 kV, 21 kHz), shown in Fig. 2, were applied to the internal electrode, and the external electrode was grounded. The discharge gap was ca. 20 mm. The maximum discharge current was ap-

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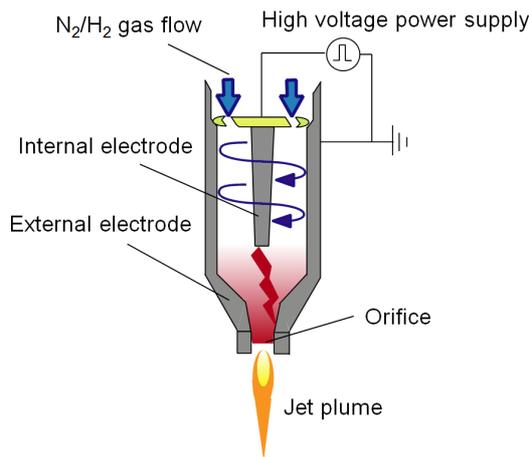


Fig. 1 Schematic of pulsed-arc plasma jet nozzle.

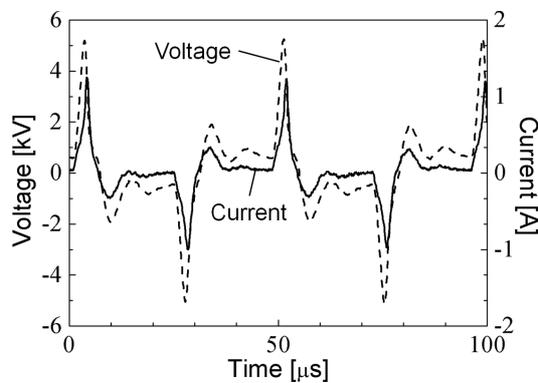


Fig. 2 Applied voltage and discharge current.

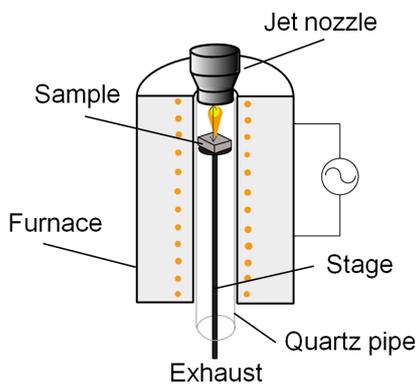


Fig. 3 Schematic of experimental setup.

proximately 1.2 A. As shown Fig. 3, PA plasma jet nitriding was performed in a quartz pipe to purge oxygen gas. A furnace surrounded the quartz pipe to maintain uniform treatment temperature. Each sample was nitrided by spraying the jet plume onto the sample surface, where the irradiation distance was 30 mm, and the treatment temperature was approximately 1000°C. After a 2-hour treatment, a TiN layer of 10 μm formed on the sample surface [35].

To evaluate the formation ability of calcium phos-

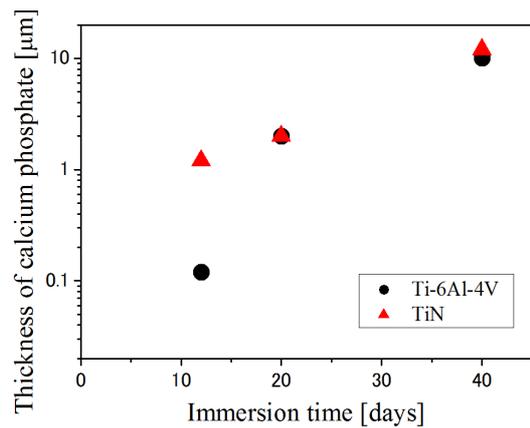


Fig. 4 Time evolution of thickness of calcium phosphate layer.

phate in vitro, samples were immersed in the simulated body fluid (Hanks' solution: KOJINBIO, HBSS+) in an incubator at 37°C. The components of the solution are listed in Table 1. The immersion test is a typical method for examining the hard-tissue compatibility of materials in vitro [14,37–42], in which the deposition properties of calcium phosphate are compared. The volume of the solution for each sample was 12 mL. Immersed samples were masked to measure the thickness of the calcium phosphate layer. Hanks' solution and the petri dish were changed at intervals of 2 days. After the immersion test, the sample was dipped into distilled water, then dried in the atmosphere. Energy dispersive X-ray spectrometry (EDX) analysis proved that the layer formed on the sample surface was calcium phosphate. The thickness of the calcium phosphate layer was measured by a laser microscope (KEYENCE, VK-9700SP). The surface morphology of the layer was observed by a scanning electron microscope (JOEL, JSM-7400F).

First, the time evolution of the calcium phosphate layer was observed. The nitrided and untreated samples were immersed in simulated body fluid for 12, 20, and 40 days. Figure 4 shows the relationship between the thickness of calcium phosphate layer and immersion days. After 12 days, the thicknesses of the layers formed on the nitrided and untreated samples were 1.2 μm and 0.12 μm, respectively. Here the calcium phosphate on the nitrided sample is 10 times thicker than untreated samples. Conversely, there was only a trivial difference in the thickness of the calcium phosphate layer after those 12 days. These facts indicate that the thickness after the 12 days still holds influences from the early stages of deposition when the calcium phosphate reacted directly with the sample surface. Eventually, the early stage influences disappear because the calcium phosphate merely reacted with the previously deposited calcium phosphate. Thus, subsequent immersion tests were performed for 10 days.

In general, plasma surface treatment hydrophilizes and roughens the surface. Therefore, it is necessary to ex-

Table 1 Components of Hanks' solution.

Component	Formula	Concentration [mg/L]
Calcium chloride	CaCl ₂	140.0
Calcium chloride hexahydrate	CaCl ₂ ·6H ₂ O	100.0
Magnesium sulfate heptahydrate	MgSO ₄ ·7H ₂ O	100.0
Potassium chloride	KCl	400.0
Potassium phosphate	KH ₂ PO ₄	60.0
Sodium chloride	NaCl	8000.0
Sodium hydrogen carbonate	NaHCO ₃	350.0
Disodium hydrogen phosphate	Na ₂ HPO ₄	47.9
D-glucose	C ₆ H ₁₂ O ₆	1000.0

Table 2 Titanium alloy samples of four properties.

Sample name	Preparation	TiN surface	Hydrophilicity (Contact angle)	Surface roughness [μm]
Control	untreated	-	Low (70.1±0.3°)	Low (0.08±0.00)
TiN	nitrided & buffed	Available	Low (39.1±0.5°)	Middle (0.58±0.05)
Hydrophilic	hydrophilized	-	High (7.3±1.3°)	Low (0.04±0.36)
Rough	sandblasted	-	Low (62.3±1.8°)	High (0.92±0.04)

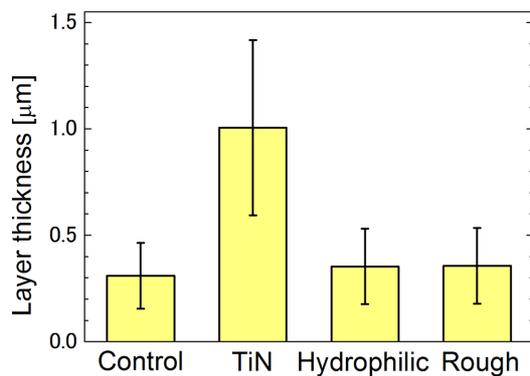


Fig. 5 Comparison of thickness of calcium phosphate layer.

amine the effects of the TiN surface, hydrophilicity, and surface roughness on the deposition separately. As shown in Table 2, four types of samples were prepared. The Control sample was a simple, polished material. The TiN sample was a sample buffed with alumina suspension after nitriding to decrease its hydrophilicity and surface roughness. The Hydrophilic sample was a sample sprayed for 2 s by a PA jet to increase the hydrophilicity only. The Rough sample was a sample sandblasted (#220) to increase the surface roughness.

Figure 5 shows the thicknesses of the calcium phosphate layers deposited on the four samples. The error bars correspond to the standard deviation. The thicknesses of calcium phosphate on the Control, TiN, Hydrophilic, and Rough samples were 0.31 μm, 1.06 μm, 0.35 μm, and

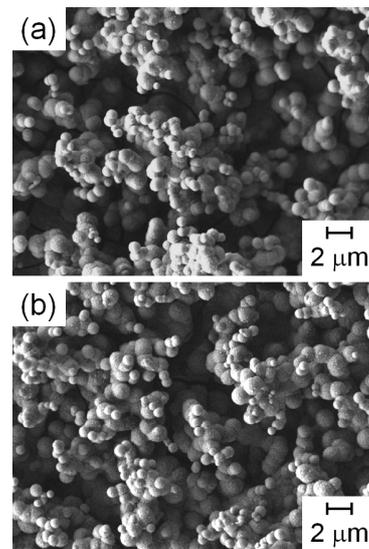


Fig. 6 Surface morphology of calcium phosphate layer deposited (a) on Control sample and (b) on TiN sample.

0.36 μm, respectively. It can be observed that the TiN sample demonstrates clear superiority. Although the standard deviation for the TiN sample is relatively large, the average thickness of the TiN sample is approximately three times larger than that of the other samples, so it must be non-trivial. These results may reflect the ability of the atmospheric-pressure plasma treatment to upgrade the biocompatibility of titanium alloy.

Figures 6 (a) and 6 (b) show the surface morphology of

the calcium phosphate layer deposited on the Control and TiN samples. A number of spherical grains of around 1 μm in diameter compose the outermost layer, where the two samples show no considerable difference. To clarify the growth difference of the layer, it is necessary to investigate the microstructures in deeper layers close to the substrate.

In summary, improving the biocompatibility of titanium alloy by nitriding with an atmospheric-pressure plasma jet was initially examined. The properties of the calcium phosphate layer were compared through an immersion test to examine the hard-tissue compatibility. The growth of the calcium phosphate layer on the nitrided sample was the most rapid among the four samples. This result implies the potential of atmospheric-pressure plasma nitriding to improve the affinity of titanium alloy for hard tissues, such as bones.

To rely on this potential, biological investigations with osteoblast cells and animals need to be conducted. In addition, it would also be worthwhile to enlarge the treatable area by introducing a larger heating system and plasma scanning.

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