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Enhancement of Bioactivity of Zr-50Ti Alloys through Sulfuric Acid Treatment followed by Modified Simulated Body Fluid Treatment

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Abstract
Zirconium-titanium (Zr-Ti) alloys are known for their superior strength, corrosion resistance, and biocompatibility, making them promising biomaterials. Among these, Zr-50Ti alloys exhibit a lower elastic modulus and reduced magnetic susceptibility compared to pure titanium (Ti), which diminishes stress shielding and minimizes interference during medical diagnostics. Despite these advantages, Zr-50Ti alloys lack inherent bioactivity, necessitating surface modification to enhance their ability to bond with bone. This study aimed to impart bioactivity to Zr-50Ti alloys through sulfuric acid treatment to create micropores, followed by treatment in a modified simulated body fluid (m-SBF) under moderate conditions (36.5 °C, pH 7.40). The bioactivity of the treated alloys was evaluated by soaking in simulated body fluid (SBF) for 1, 3, and 7 days. The results demonstrated that the sulfuric acid treatment significantly enhanced the calcium phosphate precipitation ability of Zr-50Ti alloys in m-SBF, leading to successful precipitation. This approach under mild conditions suggests the potential for further testing on materials suitable for biological conditions without additional restrictions.
Keywords: zirconium–titanium alloy; calcium phosphate; apatite-forming ability; simulated body fluid; sulfuric acid treatment.

Introduction

Zirconium-titanium (Zr-Ti) alloys exhibit up to 40% higher strength than titanium (Ti), while maintaining comparable corrosion resistance and biocompatibility to pure titanium [1)]. Zirconium (Zr) shares close properties with Ti as a group element akin to Ti, while possessing about one-third of magnetic susceptibility than Ti [2)]. This lower magnetic susceptibility minimizes interference during medical diagnostics. Among Zr-Ti binary alloys, the Zr content with 50-60 atom% demonstrates the lowest elastic modulus, approximately 90 MPa [3)]. Furthermore, the apatite-forming ability of Zr-Ti alloys diminishes significantly when the Zr content exceeds 60 atom% [4)]. These features suggest Zr-50Ti alloys as a promising biomaterial.

However, as a metallic material, Zr-50Ti does not have bioactivity. It is necessary to impart bioactivity to Zr-50Ti alloys through surface modification. Kokubo et al. claimed that bioactivity can be assessed in the simulated body fluid (SBF) environment [5)]. Therefore, we used SBF to evaluate the bioactivity of the Zr-50Ti alloys obtained in this study. When raising the temperature and pH of SBF, fine particle precipitation of amorphous calcium phosphate occurs through homogeneous nucleation. Yao et al. found these particles, named apatite nuclei (AN), were highly bioactive and could induce apatite formation in SBF [6)]. AN is a promising material for imparting bioactivity to various substrates through surface treatment.

Based on our previous research, the surface morphology of Zr-50Ti alloys was modified using a sulfuric acid solution to create micropores on the surface, which can enhance the bonding ability between the apatite coating and Zr-50Ti substrates, and the SBF was modified by removing other ions other than the calcium and phosphate ions, resulting in a modified-SBF (m-SBF). In this study, we used moderate conditions (36.5 ℃, pH 7.40) instead of high temperature and high pH supersaturated conditions to precipitate calcium phosphate (CaP) on the surface of micropore-formed Zr-50Ti alloys in the m-SBF. After this process, bioactivity was imparted to the Zr-50Ti alloys, and it was evaluated by soaking m-SBF treated Zr-50Ti alloys in the SBF for 1, 3, and 7 days while observing their apatite growing performance.

2. Experiment

2.1. Materials

The Zr-50Ti alloys with a 1 to 1 atomic ratio were prepared in plate sizes of 15 × 10 × 2 mm^3 (E-Metals, Osaka, Japan). These alloys were initially ground using #1200 silicon carbide (SiC) abrasive papers and then ultrasonically cleaned in acetone for 10 minutes.

2.2. Micropore Formation by Sulfuric Acid Treatment

A mixed sulfuric acid solution of H_2SO_4 (FUJIFILM Wako Pure Chemicals, 95 wt%) and ultrapure water, with a volume ratio of 3 to 2, was prepared. The Zr-50Ti alloys were immersed in the solution and subjected to a water bath at 70 °C for 3 hours. After this treatment, the alloys underwent ultrasonic cleaning in ultrapure water for 30 minutes and were subsequently air-dried for 1 day. Micropores were formed on the surface of the alloys after this procedure.

2.3. Preparation of SBF and m-SBF

In order to prepare SBF, reagent-grade NaCl (FUJIFILM Wako Pure Chemicals, 99.5%), $NaHCO_3$ (Hayashi Pure Chemicals, 99.5%~), KCl (Hayashi Pure Chemicals, 99.5%), $K_2HPO_4{\cdot}3H_2O$ (Nacalai Tesque, 99.0%), $MgCl_2{\cdot}6H_2O$ (Hayashi Pure Chemicals, 98.0%), $CaCl_2$

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(FUJIFILM Wako Pure Chemicals, 95.0%) and Na_2SO_4 (Hayashi Pure Chemicals, 99.0%) were dissolved in ultrapure water. The pH value was adjusted to 7.40 using tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_2$, FUJIFILM Wako Pure Chemicals, 95.0%) and 1 M HCl (FUJIFILM Wako Pure Chemicals) at 36.5 °C, following the ISO 23317 standard method [7]. This process resulted in an aqueous solution, named SBF, with inorganic ion concentrations similar to those in human blood plasma.

The m-SBF was derived from SBF by removing all components except for $K_2HPO_4 \cdot 3H_2O$ and $CaCl_2$. The pH was then adjusted to 7.40 by dissolving $(CH_2OH)_3CNH_2$ and 1 M HCl at 36.5 °C, which favors the precipitation of AN. The ion concentrations of human blood plasma, SBF, and m-SBF are shown in Table 1.

Table 1. The ion concentration of human blood plasma, SBF, and m-SBF.

	Ion Concentration (mM)		
	Blood Plasma	SBF	m-SBF
Na^+	142.0	142.0	0.0
K^+	5.0	5.0	2.0
Mg^{2+}	1.5	1.5	0.0
Ca^{2+}	2.5	2.5	2.5
Cl^-	103.0	147.8	5.0
HCO_3^-	27.0	4.2	0.0
HPO_4^{2-}	1.0	1.0	1.0
SO_4^{2-}	0.5	0.5	0.0
pH	7.2–7.4	7.4	7.4
Temp. (°C)	36.5	36.5	36.5

2.4. m-SBF Treatment

Zr-50Ti alloys were soaked in m-SBF and incubated at 36.5 °C for 24 hours to form calcium phosphate on the surface. Following this treatment, calcium phosphate was deposited on the surface of acid-treated and untreated Zr-50Ti alloys.

2.5. The Evaluation of Bioactivity

The bioactivity, also known as the apatite-forming ability, was evaluated by soaking the bioactive substrates in SBF for 1, 3, and 7 days at 36.5 °C. The surface morphology of the Zr-50Ti alloys was observed using thin film X-ray diffraction (TF-XRD, X'Pert PRO, PANalytical, Almelo, The Netherlands), using CuKα X-ray with tube voltage and current of 45 kV and 40 mA. Additional characterization methods included scanning electron microscopy (SEM, SU6600, Hitachi High-Tech, Tokyo, Japan), energy dispersive X-ray spectroscopy (EDX, XFlash 5010, Bruker, Billerica, MA, USA), and Fourier transform infrared spectroscopy with diamond ATR techniques (FTIR, FT/IR-4700, JASCO, Tokyo, Japan).

Results

Fig. 1 presents the SEM images, EDX mappings, and EDX spectra of Zr-50Ti alloys pretreated with or without sulfuric acid solution and then soaked in m-SBF. The changes in surface morphology due to sulfuric acid treatment have been previously reported in our research [8]. In the SEM image of the sample labeled "m-SBF (G)," no significant CaP was observed on the surface. This finding is corroborated by the EDX mapping and spectra, which showed barely detectable levels of calcium (Ca). In contrast, for the sample "m-SBF (S)", treated with the sulfuric acid solution, the SEM images clearly indicate the presence of CaP on the Zr-50Ti alloy surface. EDX mapping revealed extensive CaP precipitates, and although the phosphorus peak overlapped with the zirconium peak in the EDX spectra, the distinct Ca peaks confirmed the presence of CaP precipitates, indicating a higher CaP precipitation ability compared to grinding-only samples.

Fig. 2 presents the SEM images, EDX mappings, and EDX spectra of sulfuric acid-treated Zr-50Ti alloys subsequently soaked in m-SBF and then soaked in SBF. After soaking in SBF, the SEM images and EDX mappings show a substantial increase in calcium phosphate precipitates. The EDX spectra further confirm this, with a marked intensification of the Ca peak. These findings suggest that the CaP formed in m-SBF is capable of further growth when soaked in SBF.

Fig. 3 presents the magnified SEM images and EDX spectra from the center of the corresponding points in Fig. 1 and Fig. 2. The CaP precipitates were not crystallized flake-like hydroxyapatite in the sample "m-SBF (G)". In contrast, the sample "m-SBF (S)" exhibited a morphology indicative of crystallization. After soaking in SBF, although the Ca peaks in EDX spectra were intensified, the size of the CaP particles initially decreased before increasing. Additionally, the presence of a magnesium peak was detected.

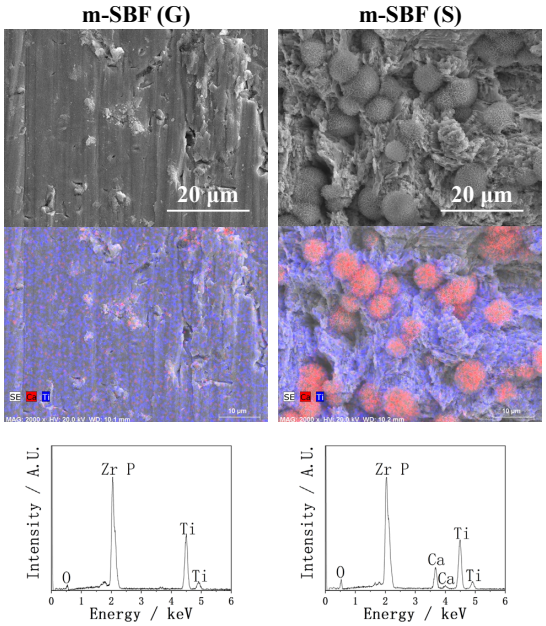

Figure 1. The SEM images, EDX mappings, and EDX spectra of Zr-50Ti alloys pretreated with or without sulfuric acid solution and then soaked in m-SBF. The "m-SBF (G)" signifies grinding-only Zr-50Ti alloys soaked in m-SBF for 1 day, and the "m-SBF (S)" signifies sulfuric acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day.

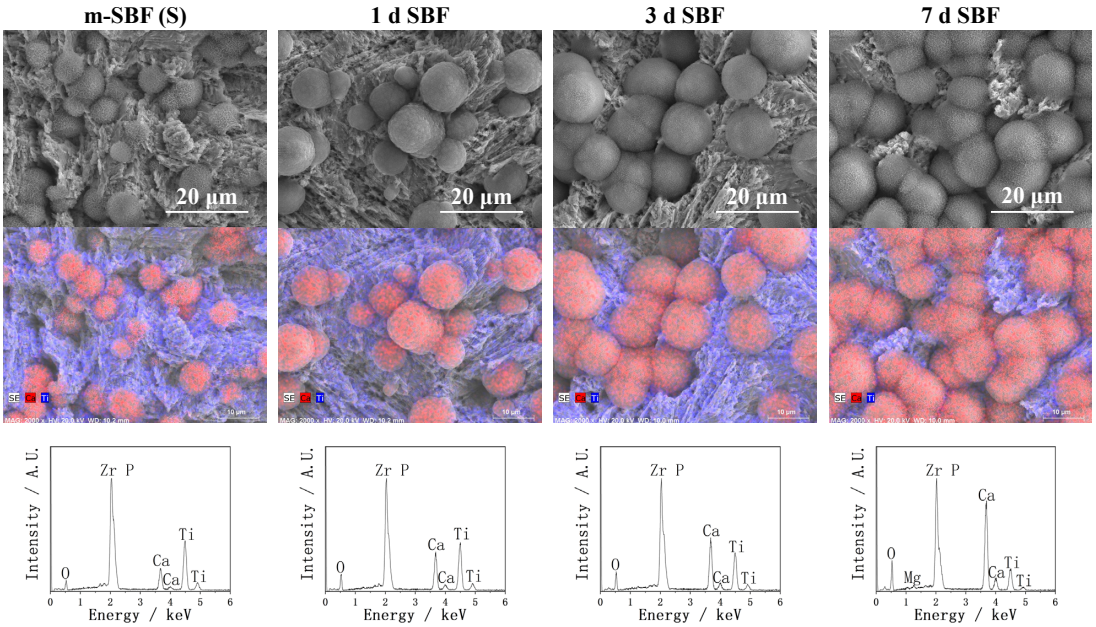

Figure 2. The SEM images, EDX mappings, and EDX spectra of sulfuric acid-treated Zr-50Ti alloys subsequently soaked in m-SBF and then soaked in SBF. The "m-SBF (S)" signifies sulfuric acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day, the "1 d SBF", "3 d SBF", and "7 d SBF" signifies acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day and then soaked in SBF for 1, 3, and 7 days, respectively.

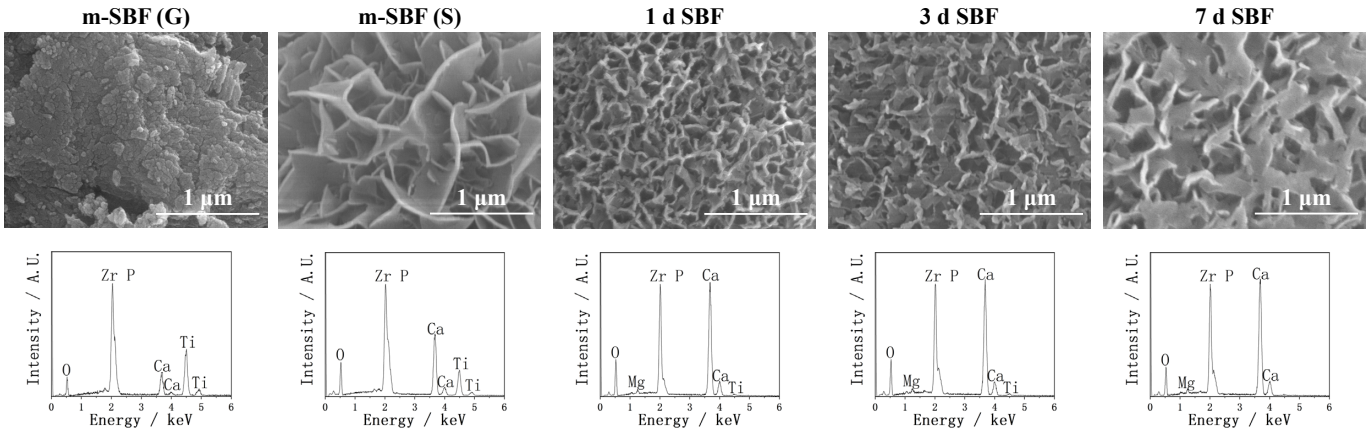

Figure 3. The SEM images and EDX spectra of Zr-50Ti alloys magnified from the center point of the corresponding place in Fig. 1 and Fig. 2. The "m-SBF (G)" signifies ground-only Zr-50Ti alloys soaked in m-SBF for 1 day, the "m-SBF (S)" signifies sulfuric acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day, the "1 d SBF", "3 d SBF", and "7 d SBF" signifies acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day and then soaked in SBF for 1, 3, and 7 days, respectively.

Fig. 4 presents the XRD spectra of Zr-50Ti alloys. In sample "m-SBF (G)" of Fig. 4 (a), no discernible characteristic peaks were observed apart from the peaks corresponding to Zr-50Ti. However, in sample "m-SBF (S)", characteristic peaks specific to hydroxyapatite were detected, indicating the crystallization of calcium phosphate. In Fig. 4 (b), after soaking in SBF, the intensity of the existing characteristic peaks increased, and new characteristic peaks of hydroxyapatite emerged. The longer the soaking time in SBF, the more characteristic peaks appeared, and the higher their intensity.

Fig. 5 presents the FTIR spectra of Zr-50Ti alloys. Unlike the XRD spectra, the sample "m-SBF (G)" of Fig. 5 (a) exhibits very weak absorption bands of PO_4^{3-} compared with the sample "m-SBF (S)". In Fig. 5 (b), after SBF soaking, the intensity of the PO_4^{3-} absorption bands increased; however, although not as significant as observed in the XRD spectra.

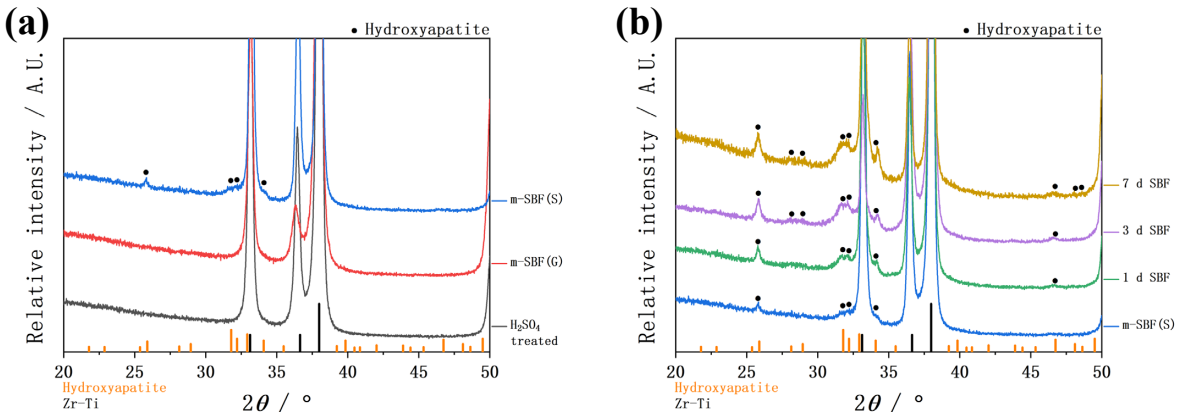

Figure 4. The XRD spectra of Zr-50Ti alloys. (a): Zr-50Ti comparison with or without sulfuric acid treatment, (b): sulfuric acid treated Zr-50Ti alloys before and after SBF soaking. The "H_2SO_4 treated" signifies ground Zr-50Ti alloys treated by sulfuric acid solution, the "m-SBF (G)" signifies ground-only Zr-50Ti alloys soaked in m-SBF for 1 day, the "m-SBF (S)" signifies sulfuric acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day, the "1 d SBF", "3 d SBF", and "7 d SBF" signifies acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day and then soaked in SBF for 1, 3, and 7 days, respectively.

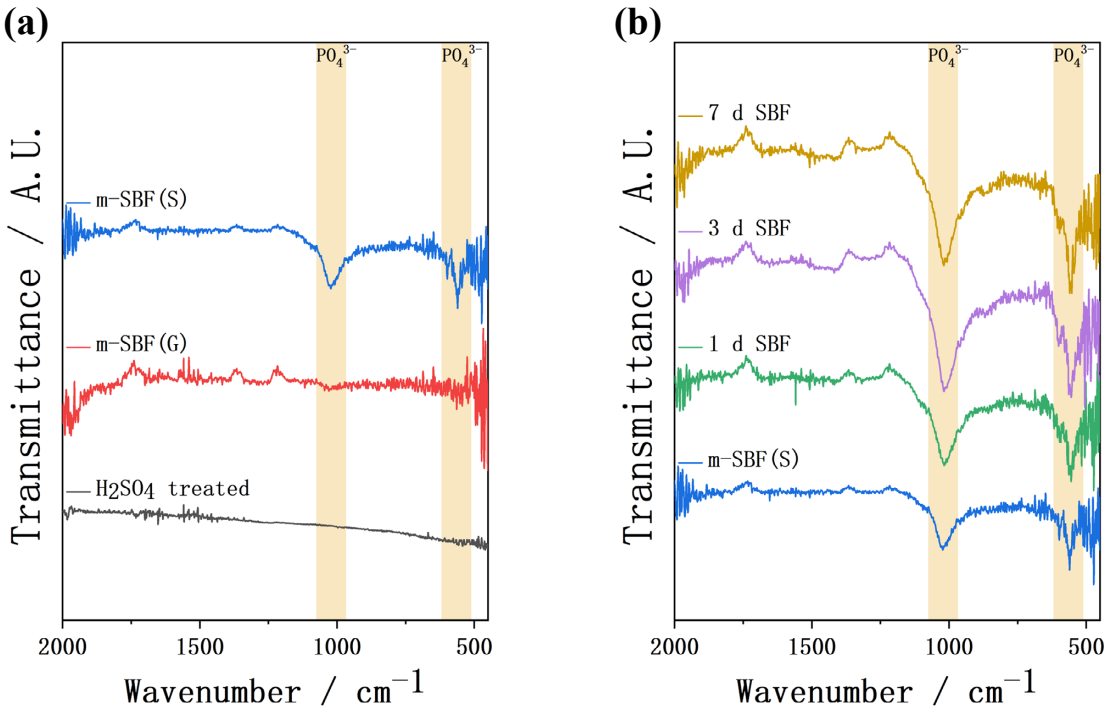

Figure 5. The FTIR spectra of Zr-50Ti alloys. (a): Zr-50Ti comparison with or without sulfuric acid treatment, (b): sulfuric acid treated Zr-50Ti alloys before and after SBF soaking. The "H_2SO_4 treated" signifies ground Zr-50Ti alloys treated by sulfuric acid solution, the "m-SBF (G)" signifies ground-only Zr-50Ti alloys soaked in m-SBF for 1 day, the "m-SBF (S)" signifies sulfuric acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day, the "1 d SBF", "3 d SBF", and "7 d SBF" signifies acid-treated Zr-50Ti alloys soaked in m-SBF for 1 day and then soaked in SBF for 1, 3, and 7 days, respectively.

Discussion

As a metallic material, Zr-50Ti alloys are bioinert, making direct bonding with apatite challenging. Consequently, the Zr-50Ti alloys only ground by abrasive paper precipitated minimal calcium phosphate on the surface, as shown in Fig. 1, despite the m-SBF removed interfering ions which favored the precipitation [9]. After sulfuric acid treatment, it enabled Zr-50Ti alloys to precipitate apatite on the surface, indicating that the sulfuric acid treatment enhanced the CaP precipitation ability of Zr-50Ti alloys.

After soaking in SBF, the increased coverage area of apatite as shown in Fig. 2, along with the intensified EDX spectra in Fig. 1, Fig. 2, and the enhanced peaks in XRD and FTIR in Fig. 4 and Fig. 5 respectively, confirmed that the apatite-forming ability was imparted to Zr-50Ti alloys during the m-SBF treatment. This apatite-forming process can predict bioactivity *in vivo* environment according to the report from Kokubo et al. [5]. However, the crystal size did not grow directly; it first decreased before increasing. LeGeros et al. claimed that the Mg^{2+} ions reduce the crystallite size of apatite [10], in Fig. 3, the EDX spectra of magnified apatite morphology on the Zr-50Ti alloys detected Mg peaks after SBF soaking, suggesting that Mg^{2+} ions decreased the apatite crystal size after 1 day of SBF soaking.

In the XRD results shown in Fig. 4 (a), the sample "m-SBF (G)" may have had such a small amount of precipitation that characteristic peaks were absent in the XRD. However, in the FTIR results shown in Fig. 5 (a), weak absorption bands of PO_4^{3-} were present, which did not correspond with the XRD. Combining this with the formation of flake-like apatite, which suggests crystallization in sample "m-SBF (S)", and the completely different morphology of calcium phosphate precipitates in sample "m-SBF (G)", it is reasonable to suspect that the precipitates in sample "m-SBF (G)" were amorphous CaP.

According to Chen et al. [11], moderate surface roughness facilitates the apatite-forming ability, although adhesive strength was not mentioned. Compared to other research [12], where Miyazaki et al. imparted bioactivity to Zr-50Ti using NaOH followed by $CaCl_2$ treatment, the bonding strength between the apatite layer and Zr-50Ti alloys was very low. This study imparted bioactivity to Zr-50Ti alloys by sulfuric acid treatment followed by m-SBF treatment, which not only successfully precipitated apatite on the surface of Zr-50Ti alloys but also ensured a strong bond between the apatite and Zr-50Ti alloys, as reported previously [8].

In this study, the apatite-forming ability was successfully imparted to Zr-50Ti alloys through sulfuric acid treatment followed by m-SBF

treatment under moderate biological conditions (36.5 ℃, pH 7.40). Unlike the high temperature and high pH used in our previous study, these milder conditions suggest that subsequent tests can be performed on materials suitable for biological conditions without other restrictions.

The authors acknowledge a limitation in this study: evaluation of bone-bonding ability on the samples by animal test was not carried out. Although this study did not include data from animal experiments, according to the report from Kokubo et al. [5], the apatite-forming process in SBF solution can predict bioactivity *in vivo* environment. In this experiment, a significant amount of apatite formed on the surface of the sulfuric acid-treated Zr-Ti alloys within one day, and this apatite continued to grow in the SBF solution, suggesting that the material has bioactivity. Furthermore, cytotoxicity tests and animal implantation experiments will be conducted in the future.

Conclusion

This study successfully imparted bioactivity to Zr-50Ti alloys through a combination of sulfuric acid treatment and m-SBF treatment under moderate conditions (36.5 °C, pH 7.40). The sulfuric acid treatment effectively created micropores on the alloy surface, moreover, it significantly facilitates the precipitation of apatite in the subsequent m-SBF treatment. Unlike methods requiring high temperatures and pH levels, this approach utilized milder conditions that are more suitable for biological applications. SEM, EDX, XRD, and FTIR analyses confirmed the formation and growth of apatite on the treated alloys, demonstrating high apatite-forming ability. The bioactivity achieved under these conditions suggests potential for further testing and application in biological environments. This method offers a promising approach to enhance the performance of Zr-50Ti alloys in medical applications, particularly for improving bone integration ability.

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