

# EFFECTS OF AN ALKALI TREATMENT ON TNTZ SURFACES TO GROW Ca-P USING IMMERSION IN A SIMULATED BODY FLUID AND A HANKS' BALANCED SALT SOLUTION

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*An alkaline treatment was conducted by immersing TNTZ in NaOH solutions with concentrations of 1, 5, and 10 M at 60 °C for 24 hours. Following the alkali treatment, the samples were immersed in a Simulated Body Fluid (SBF) Kokubo and Hank's Solution for 2, 3, and 4 weeks using an incubator shaker at 37 °C. The treated TNTZ was then analysed using Scanning Electron Microscopy (SEM) and X-ray fluorescence (XRF). The results indicated that TNTZ was subjected to alkaline treatment with NaOH concentrations of 1, 5, and 10 M, along with soaking in SBF Kokubo and Hank's Solution for varying durations. In the presence of calcium (Ca) and phosphorus (P), an apatite layer was also developed, accompanied by a TiO<sub>2</sub> layer. The XRF testing revealed that as the concentration of NaOH and the immersion time in the Hank's Solution and SBF Kokubo increased, the formation of apatite on the TNTZ also increased. This was evidenced by a higher content of the Ca and P elements. Additionally, the TiO<sub>2</sub> layer covered more than 40 % of the TNTZ surface. The presence of TiO<sub>2</sub>, Ca, and P confirms that the simple alkaline treatment process applied to TNTZ can enhance the bioactive properties of the material.*

## INTRODUCTION

As individuals age and the prevalence of diseases increases, the demand for implants to replace hard tissue also rises. Implants are medical devices that consist of specialised metal supports designed to engage directly with bone, thereby ensuring structural stability and facilitating the process of osseointegration within the body. The choice of metal for an implant largely depends on the specific medical application [1, 2]. For safe functionality and longevity without the risk of rejection, the selected metal must possess essential characteristics, including high biocompatibility, resistance to corrosion and wear, appropriate mechanical properties, and the ability to promote osseointegration [3]. Additionally, it is crucial for the metal to exhibit optimal ductility, hardness, and bioactive properties that support the regeneration of bone tissue.

Biomaterials are substances frequently used in the medical field, sourced both naturally and synthetically. They are often employed as human structures and implants to replace missing biological parts. When it comes to osteogenesis, titanium

and its alloys are recognised for having the highest level of biocompatibility compared to other biomaterials, including 316L stainless steel, cobalt-chromium alloys, magnesium alloys, tantalum, and niobium [4-6]. Titanium alloys are widely used in various applications because their properties can be modified by adjusting the composition of the alloying elements. Additionally, these alloys can undergo allotropic transformations, meaning they can change from one crystallographic structure to another.

Titanium alloys can be classified by their crystallographic phase,  $\alpha+\beta$  alloys, and  $\beta$  alloys [7].  $\alpha/\beta$  alloys have better mechanical properties than pure titanium due to the combination of the  $\alpha$  phase, which provides resistance to plastic deformation, and the  $\beta$  phase, which increases the strength, such as Ti-29Nb-13Ta-4.6Zr (TNTZ) [8, 9]. However, the bioinert nature of Ti alloys results in a slower process of osseointegration with the bone. To overcome this, scientists have proven that the most effective surface treatment technology is to improve the bioactivity and biocompatibility of titanium by changing its surface characteristics [10].

Among the various surface treatment methods, the use of strong alkaline solutions has been extensively researched due to their operational ease, practicality, and effectiveness in promoting faster osseointegration compared to conventional titanium (Ti) implants. An alkaline treatment presents many advantages, such as having a low cost, its simplicity, and feasibility for large-scale manufacturing [11, 12]. It is known that the titanium surface will react with NaOH, providing a bioactive surface on the implant due to the formation of a porous sodium titanate hydrogel layer [13]. Simultaneously, after an alkaline treatment, titanium has been demonstrated to induce bone-like apatite depositions. In various studies that have been conducted, scientists have used simulated testing media, with solutions containing compositions like human blood plasma, such as simulated body fluid (SBF) and a balanced salt solution (Hank's Balanced Salt Solution) [14-16]. Both of which contain inorganic components of blood plasma, rich in chloride ions, which are often used to investigate material properties such as bioactivity and corrosion resistance in biological media. It can also be used as an electrolyte source during calcium phosphate (CaP) deposition, amorphous at 37 °C on biomedical materials [17].

Hydroxyapatite (HA) or  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , is a mineral that mimics the natural extracellular matrix of bone due to its similarity to the mineral phases in human bone tissue, as well as its biocompatible, bioactive, and osteoconductive properties [18]. HA plays an active role in ion exchange and bone matrix metabolism and can support adhesion proteins and bone tissue cells, thereby enhancing the continuity of bonding with bone minerals. Numerous studies have focused on improving the bioactivity of titanium through various HA deposition techniques, including plasma spray, sol-gel, and electrophoresis [14].

In research conducted by Prochor and Mierzejewska (2021), using PEEK GRF 30, Ti-6Al-4V (SLM), and commercial Ti-6Al-4V materials, the formation of apatite layers in each sample material using the alkali treatment method was carried out using SBF and HBSS solutions at 37 °C. The alkaline immersion varies with the times of 2, 7, 14, 21, and 28 days. The results showed that the highest Ca/P hydroxyapatite layer was formed in the 28-day immersion period with the immersion in the SBF solution. Then, the research conducted by Aviles et al. (2020) using five groups of materials as experimental materials, namely, uncoated titanium, NaOH-treated Ti, Ti coated with SiO<sub>2</sub>, Ti coated with TiN, and Ti coated with quaternary TiN, using an alkali treatment and an SBF solution which is replaced every 48 hours for 28 days. The highest Ca/P ratio results were obtained for the uncoated Ti sample [19].

A study was conducted to investigate the effect of NaOH and heat treatment on nanotubes formed on anodised Ti-7.5Mo under various alkali treatment conditions, aiming to improve the apatite formation ability. The nanotubes created on Ti-7.5Mo through anodisation at 10 V in an  $\text{NH}_4\text{F}/\text{NaCl}$  electrolyte were initially amorphous. However, when treated with an NaOH solution, the anodised Ti-7.5Mo exhibited enhanced bioactivity and developed a nanoscale porous network structure-features that were not present on the surfaces that did not undergo the NaOH treatment. When a specimen treated with 5 M NaOH was immersed in simulated body fluid for 6 hours or less, nano-sized apatite particles coated the entire porous surface. After 14 days of immersion in the simulated body fluid, the thickness of the calcium-phosphate (Ca-P) layer on the surfaces of the anodised Ti-7.5Mo treated with either 0.5 M or 5 M NaOH measured approximately 418 and 439 nm, respectively. In contrast, the thickness of the Ca-P layer on the anodised Ti-7.5Mo surface that was not treated with alkali was around 415 nm. The preliminary in vitro cell culture results indicated that the anodized Ti-7.5Mo alloy, which underwent NaOH and heat treatment, demonstrated good biocompatibility and effectively supported cell adhesion [17].

In this experiment, immersions in HBSS and SBF Kokubo were used as the media to evaluate the hydroxyapatite deposition on the TNTZ samples by the alkaline treatment method. This research is related to forming Ca-P layers on the TNTZ surface. However, few reports are available on the effectiveness of alkali treatments in stimulating hydroxyapatite formation with SBF and HBSS components. This study aimed to compare the adequate molar bonding variation of NaOH in the alkaline treatments on the TNTZ substrates with the formation of Ca-P layers in two solutions (HBSS and SBF Kokubo).

## EXPERIMENTAL

The material used in this study consisted of TNTZ bars, which were manually cut into four samples with the assistance of a vise. The samples had a diameter of 10 mm and a thickness of 2 mm. After cutting, the TNTZ samples underwent a grinding or sanding process to level the rough surfaces. This was accomplished using a belt sanding machine equipped with 100 mesh sandpaper. Once the samples were sanded, they were polished to achieve a smooth surface. The polishing involved a multistage sanding process, progressing through 400, 600, and finally 1000 mesh sandpaper using a Nanofin tool. Following polishing, the TNTZ samples were cleaned in succession with acetone, alcohol, and distilled water for 10 minutes each, using an ultrasonic cleaner. Next, the TNTZ specimens were immersed in a 44 ml NaOH solution, following the ASTM

G31-72 guidelines (with a recommended solution volume of 0.2 to 0.4 ml per mm<sup>2</sup>) for 24 hours at 60 °C in a water bath. The variations in the study included TNTZ samples without an alkali treatment, and those treated with the NaOH solution at concentrations of 0 (no treatment), 1, 5, and 10 M.

The morphology and elemental analysis were identified by a scanning electron microscope (SEM) JSM-IT200(LA) and XRF PANalytical Epsilon 3.

well-coated, but features fine cracks. It is evident that there is a significant difference in the layers and white particles formed at 1 M and 5 M, with both the thickness and size increasing as the NaOH molarity increases. Therefore, the excessive film rupture associated with the increasing thickness can be attributed to the continuous growth of the white particulates. This phenomenon is particularly noticeable on the sample exposed to a 10 M NaOH solution. According to Shinji Fuji-

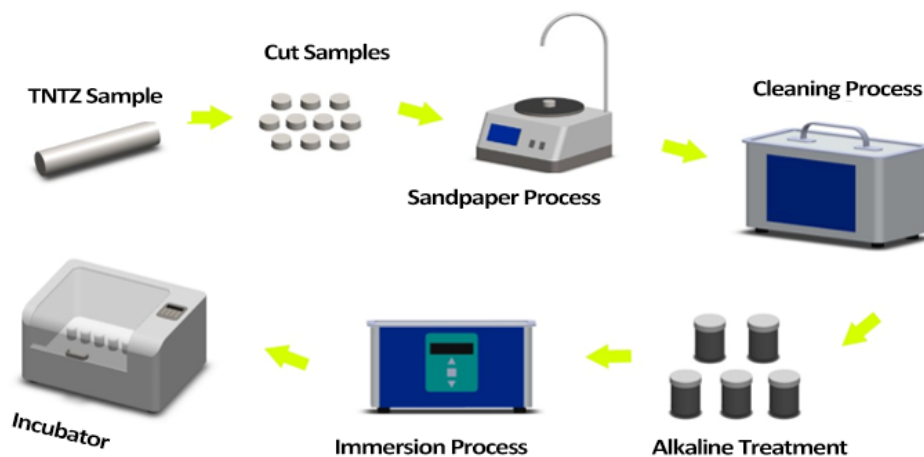


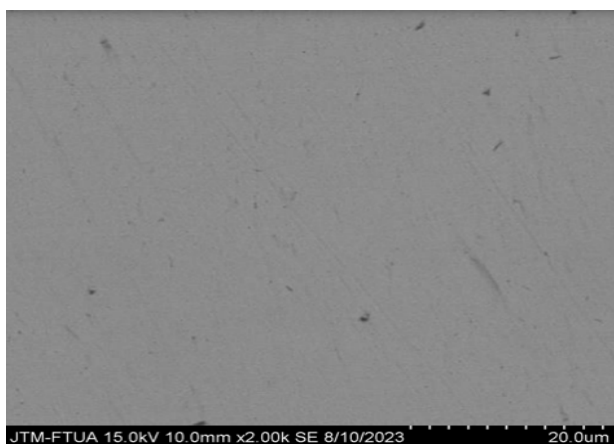
Figure 2. Schematic of alkaline treatment with variations of molar NaOH and immersion in variation SBF (Hank's and Kokubo).

## RESULTS AND DISCUSSION

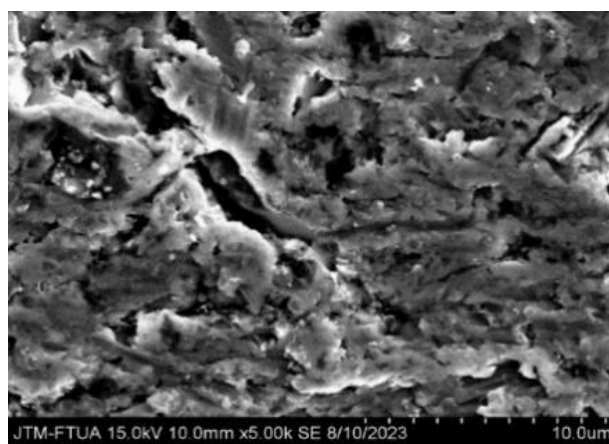
Figure 2 shows the morphology after 24 hours of exposure to varying molarities of NaOH. The surface displays some cracks that begin around the particles. After 24 hours, the sample with a 1 M NaOH solution exhibits a rough and irregularly coated surface. In contrast, the surface coated with 5 M NaOH appears

moto's research, these white particles are tantalum-enriched particulates, and their growth actively contributes to the cracking of the sample surface [20].

The localised enrichment of tantalum occurs during the alkali treatment process. In their study, Kokubo et al. have found that the formation of Na<sub>2</sub>Ta<sub>4</sub>O<sub>11</sub>, sodium tantalate, on a tantalum substrate after immersion in 0.5 M NaOH at 60 °C for 24 hours [21, 22].



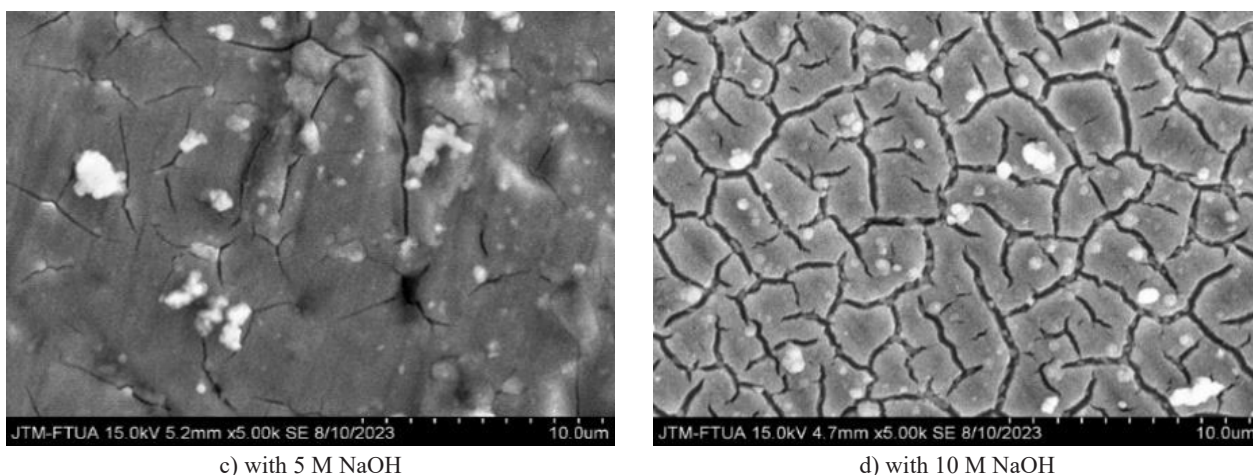
a) without the alkali treatment



b) with 1 M NaOH

Figure 2. Microstructures of the TNTZ surface.

*continued*



c) with 5 M NaOH

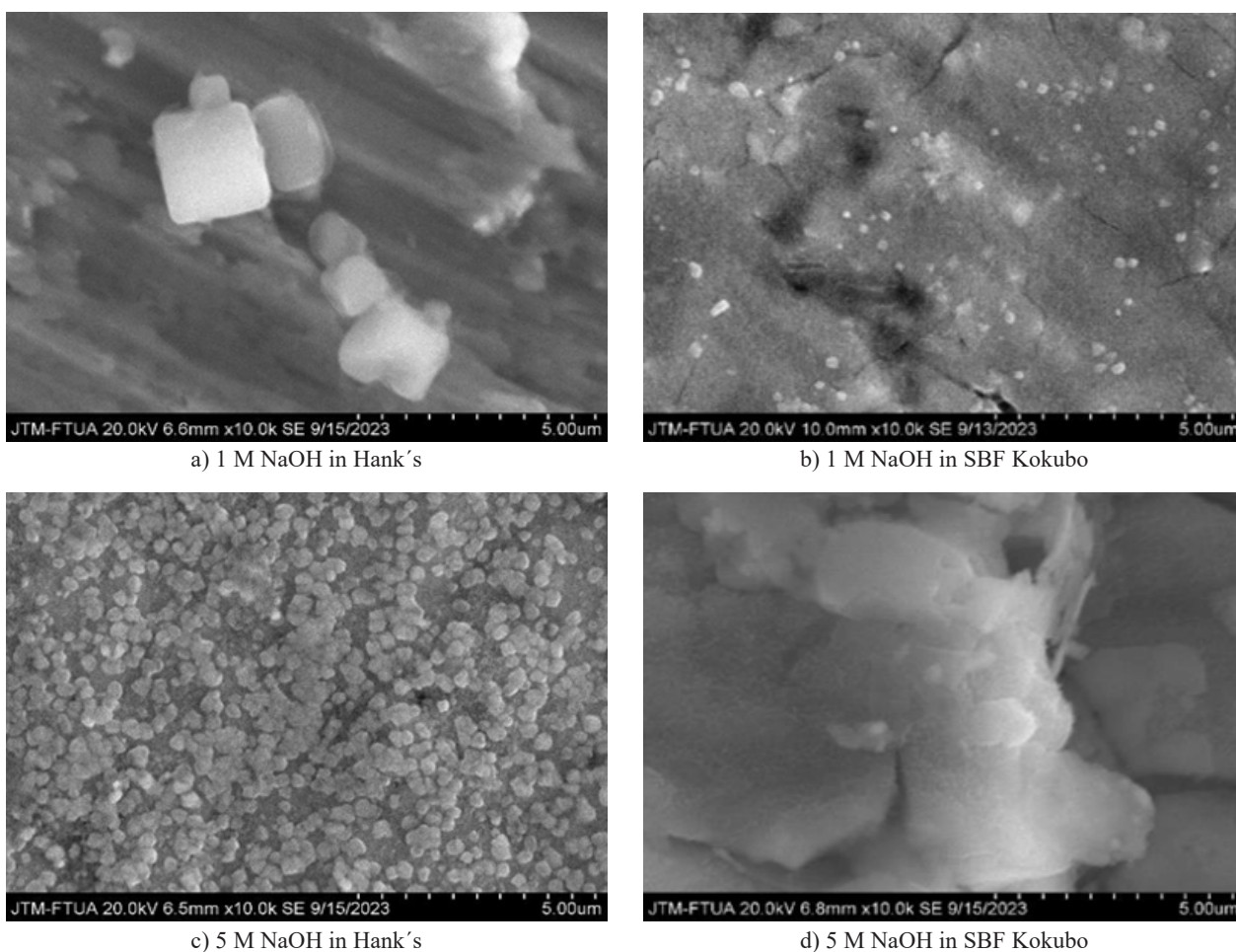
d) with 10 M NaOH

Figure 2. Microstructures of the TNTZ surface.

*continued*

The tantalum-enriched particles observed in this study may be the localised formation of sodium tantalite crystals. These small cracks propagate and the film fragments into many pieces as the thickness of the coating increases [23].

In Figures 3a, c, and e, the differences in the surface morphology of the TNTZ can be observed based on variations in the alkali treatment and immersion in Hank's solution. In the TNTZ treated with 1 M NaOH, a small amount of apatite is present, though it exhibits



a) 1 M NaOH in Hank's

b) 1 M NaOH in SBF Kokubo

c) 5 M NaOH in Hank's

d) 5 M NaOH in SBF Kokubo

Figure 3. Microstructures of the TNTZ surface subjected with variations in the molar NaOH (1, 5, and 10 M) after 4 weeks immersion in Hank's solution and in SBF Kokubo.

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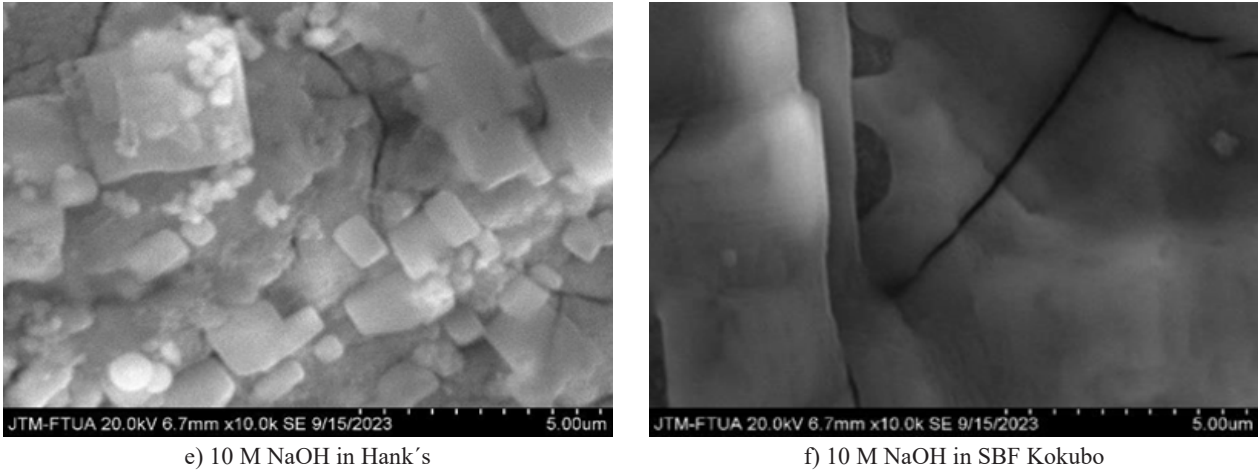


Figure 3. Microstructures of the TNTZ surface subjected with variations in the molar NaOH after 4 weeks immersion in Hank's solution and in SBF Kokubo. *continued*

a larger particle size. In contrast, the TNTZ treated with 5 M NaOH shows a significantly greater apatite deposition, almost fully covering the surface with a uniform particle size distribution. Meanwhile, in the TNTZ subjected to 10 M NaOH treatment, a substantial apatite

layer is also observed, nearly covering the entire surface. However, the apatite particles do not exhibit uniformity in size, and cracks are visible on the TNTZ surface. Based on Figures 3b, d, and f, the formation of apatite on the TNTZ surface after four weeks of immersion

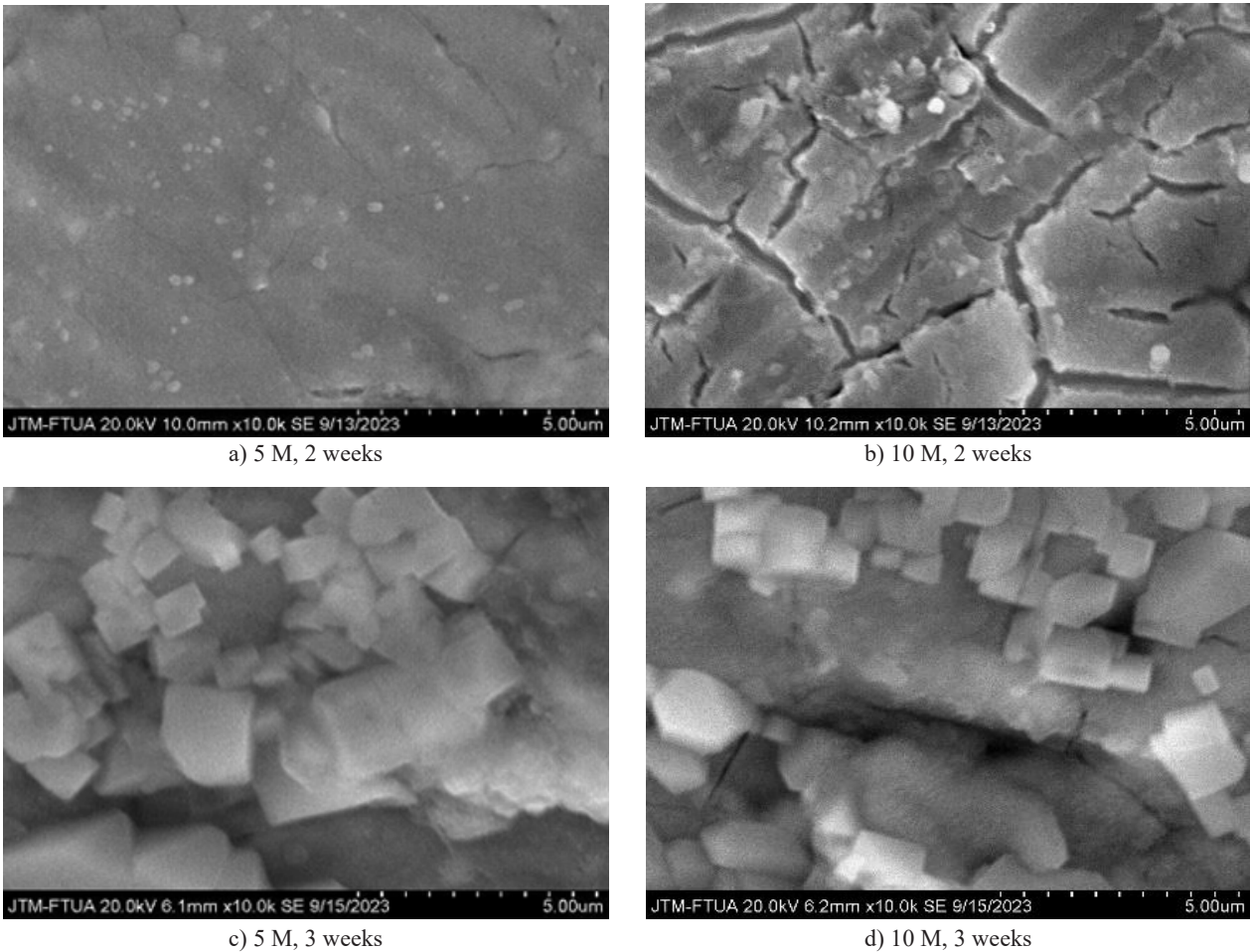


Figure 4. Microstructures of the TNTZ surface subjected to immersion in SBF Kokubo after four weeks with variations in the molar NaOH concentrations (5 and 10 M) and during alkaline treatment (2, 3, and 4 weeks). *continued*

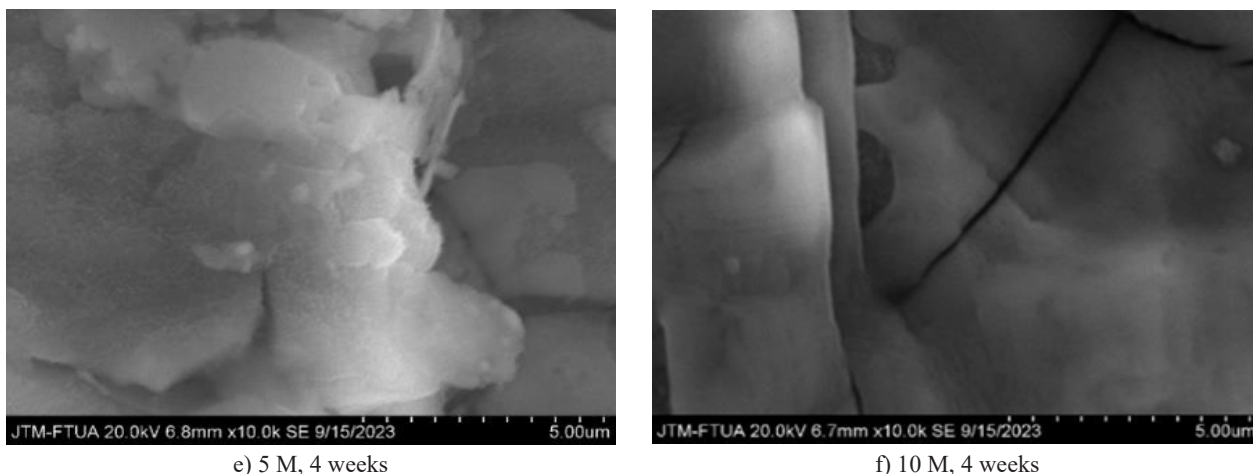


Figure 4. Microstructures of the TNTZ surface subjected to immersion in SBF Kokubo after four weeks with variations in the molar NaOH concentrations (5 and 10 M) and during alkaline treatment (2, 3, and 4 weeks). *continued*

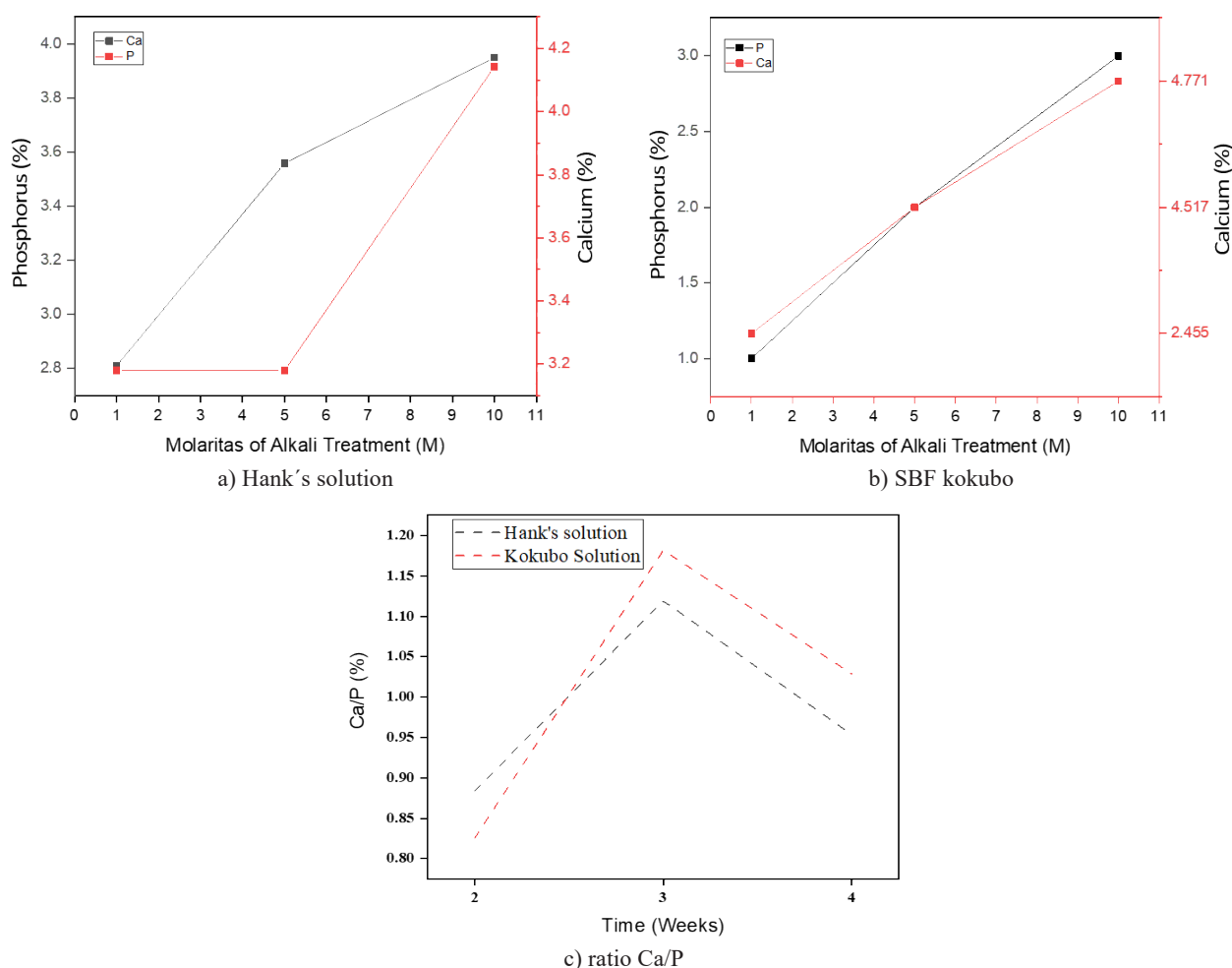


Figure 5. Percentage of calcium (Ca) and phosphorus (P) on the TNTZ surface subjected with variations in the molar NaOH 1, 5, and 10 M after 4 weeks immersion in Hank's solution, SBF Kokubo and ratio Ca/P.

in Kokubo's SBF is also influenced by the NaOH concentration. At 1 M NaOH, apatite particle formation occurs, but voids are present due to the uneven distribution of the apatite layer. At 5 M NaOH, a thicker apatite layer is observed, although some areas remain

incompletely coated. At 10 M NaOH, the apatite layer appears even thicker than that of TNTZ immersed in Hank's solution; however, cracks are also present on the surface [24].

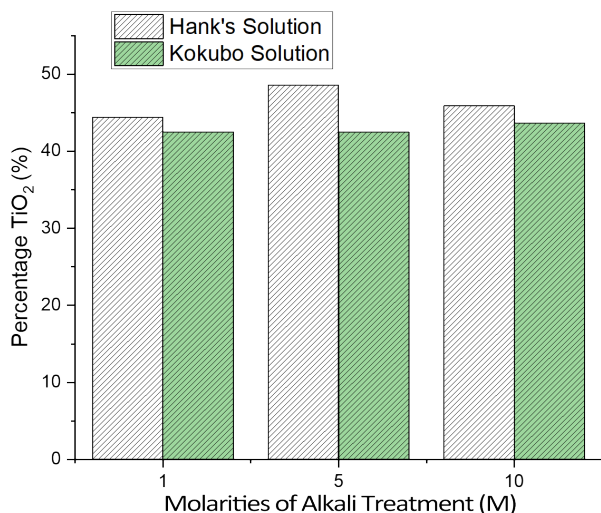


Figure 6. The percentage of  $\text{TiO}_2$  on the TNTZ surface subjected with variations in the molar NaOH concentrations 1, 5, and 10 M after 4 weeks immersion in Hank's solution and SBF Kokubo.

Based on the weight percentages of the obtained calcium (Ca) and phosphorus (P), it is evident that the percentages of both Ca and P have increased. This increase is influenced by the higher concentration of NaOH and the longer immersion time of TNTZ in the Kokubo body simulation solution. After determining the surface morphology and chemical composition of TNTZ, it is essential to understand the mechanism of apatite formation. Initially, a layer of sodium titanate hydrogel forms following the alkali treatment. During this process, sodium ions ( $\text{Na}^+$ ) from the sodium titanate hydrogel are released through exchange with the hydronium ions ( $\text{H}_3\text{O}^+$ ) in the body simulation solution. Consequently, Ti-OH groups are generated on the titanate surface, which then combine with the positively charged calcium ( $\text{Ca}^{2+}$ ) ions and negatively charged phosphate ( $\text{PO}_4^{3-}$ ) ions. This leads to the onset of apatite formation above the surface layer. As the immersion time in the body simulation solution increases, the former apatite integrates to create a thicker apatite layer. This demonstrates that a higher NaOH concentration results in a denser sodium titanate hydrogel layer, leading to the release of more sodium ions and an increase in apatite formation [25].

Figure 6 shows the percentage of  $\text{TiO}_2$  on the TNTZ surface subjected with variations in the molar NaOH concentrations 1, 5, and 10 M after 4 weeks immersion in Hank's solution and SBF Kokubo. Since the isoelectric point of  $\text{TiO}_2$  is approximately 5–6 at a neutral pH, when immersed in the simulated body fluid (SBF) with a pH of around 7.4, the  $\text{TiO}_2$  surface acquires a slight negative charge due to the presence of deprotonated acidic hydroxyl groups. This negatively charged interface selectively attracts positively charged  $\text{Ca}^{2+}$  ions, facilitating the formation of calcium titanate. As

the concentration of  $\text{Ca}^{2+}$  ions increases, the surface charge gradually shifts to positive, promoting the adsorption of negatively charged  $\text{PO}_4^{3-}$  ions, which subsequently leads to the nucleation of apatite [26]. The apatite nuclei continue to interact with the  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions in the calcium phosphate (CaP)-saturated solution, leading to the growth of a calcium phosphate layer. The micro/nano-textured surface possesses a larger specific surface area compared to the nano-textured surface and the untreated Ti surface, thereby expanding the nucleation sites for apatite precipitation. This observation aligns with the surface morphology after the alkali treatment, where the 10 M NaOH treatment results in more consistent, slab-shaped cracks resembling river channels, which facilitate the growth of apatite nucleation.

## CONCLUSIONS

The alkaline treatment of the TNTZ surfaces using an NaOH solution can enhance the material's bioactivity. The optimal variation involves a 5 M NaOH solution, with an immersion period of 4 weeks. This treatment results in a CaP ratio that closely resembles that of bone, an apatite layer covering nearly the entire surface of TNTZ without cracks, and the highest percentage of  $\text{TiO}_2$  oxide layer compared to both the untreated TNTZ and that treated with other concentrations of the NaOH solution, whether immersed in Hank's solution or SBF Kokubo.

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