

The Role of Zamzam Water in Enhanced Apatite Growth - An *In Vitro* Study

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Abstract

Zamzam water has been scientifically proven to contain very high levels of Calcium and Magnesium, the water positively affects the human body, especially the bones as they absorb the vitamins and benefit from them for better growth. Zamzam water encourages the growth of healthy bones. The effect of Zamzam water for enhanced apatite growth and adhesion strength have been studied in the work by biomimetic method and were analysed using SEM-EDX, ICP-AES and potentiodynamic polarisation studies.

Keywords: Adhesion Strength; Apatite Growth; Biomimetic Method; Healthy Bones; Zamzam Water

Introduction

The biomimetic method [1,2] is one of the most promising techniques for the production of apatite crystals on bioactive surfaces. This method consists of soaking the implants under mild conditions of pH and temperature into simulated body fluid (SBF) solutions that have similar inorganic contents as human blood plasma [3,4]. The biomimetic approach has four main advantages: (1) it is a low temperature process (2) it forms bonelike apatite crystals having high bioactivity and good resorption characteristics (3) it is evenly deposited on, or even into pores or complex implant geometries and (4) it can incorporate bone-growth-stimulating factors [5,6].

Producing bioactive titanium surface by immersing titanium (Ti) in a NaOH solution with a subsequent heat treatment have been invented by Kokubo, *et al* [7,8]. Apatite coatings have been successfully formed by the immersion of the chemically treated Ti substrates [9,10] in SBF solution. Several investigators carried out many studies to establish the influence of various ions present in SBF solution to apatite growth [11,12].

Water is one of the main dietary components. Its quality plays an important role for the safety of food particularly for infants. The water from wells of Saudi Arabia is often high in mineral contents [13]. According to Arab historians, the Zamzam well has been in use for around 4000 years. The well is located within the Holy Mosque at about 20 km east of the Kaaba in Makkah [14]. Analysis of this water in the European laboratories give exemplary results that they contain high quantity of calcium and magnesium salts and also this water contains fluorides that they have an effective germicidal action. Moreover, they established that this water was fit for drinking [15]. This fact has also been proven by a group of Pakistani researchers. The present study aims to investigate whether Zamzam water can favor apatite growth and thereby establishing that regular intake of this water favors osseointegration.

In 1973 and 1989, great international specialized chemical companies carried out very accurate chemical analysis of Zamzam water. It was found that this water was absolutely free from all kinds of germs or viruses that causes water contamination due to high fluoride content. This water has never been chemically

treated or chlorinated as in the case of water pumped into cities. Biological growth and vegetation usually take place in most wells. But in the case of the Zamzam water well, there was not any sign of biological growth. The antibacterial effect of the Hydroxyapatite (HAp) coated implants from SBF solution prepared in Zamzam water is also undertaken.

Materials and Methods

Commercially pure Ti (Sigma-Aldrich, England) substrates were cleaned with 150 grit SiC paper, washed with ethanol, acetone and finally deionized water for 10 minutes. Immediately after words, the substrates were etched in 5M NaOH solution for 24 hours at 60°C [1,16]. Two SBF solutions were prepared (1) n-SBF (SBF solution prepared in normal water) and (2) ZZ-SBF. n-SBF were having the same ionic composition as that of human blood plasma and the composition in mM as Na⁺: 142.0, K⁺: 5.0, Mg²⁺: 1.5, Ca²⁺: 2.5, Cl⁻: 103.0, HCO₃⁻: 4.2, HPO₄²⁻: 1.0 and SO₄²⁻: 0.5 [17]. The n-SBF were prepared by adding salts of NaCl (Nice-99.9%), NaHCO₃ (Loba Chemie- 99.5-100%), KCl (CDH-99.8%), K₂HPO₄·3H₂O (Spectrum-98%), MgCl₂·6H₂O (Nice-98%), Na₂SO₄ (Nice -99.5%) and tris buffer (CH₂OH)₃CNH₂) (SRL-99.9%) to the deionised water.

ZZ-SBF were prepared by adding the required amounts of the salts to the Zamzam water. The alkaline and heat-treated Ti substrates were immersed in both n-SBF and ZZ-SBF at 36.5°C and pH: 7.4. The ratio of specimen surface area to soaking solution volume, S/V was 0.05 cm⁻¹ [18] for 42 days. The surface potential change during biomimetic growth were monitored for 14 days by OCP measurements. The mechanical stability of the biomimetically grown apatite to the substrates were carried out by anodic polarization studies in 0.9% NaCl solution. The phases of the coatings before and after biomimetic growth were determined by X-ray diffraction (XRD) (model: X-Pert Pro). In the XRD measurements CuKα radiation at 40 KV and 30 mA was used as the X-ray source and the samples were scanned from 10 - 70° 2θ at a scan rate of 1.2° per min. The surface morphology and elemental composition of the coatings were evaluated using a scanning electron microscope (SEM) (JEOL-JSM-840A) coupled with energy dispersive spectroscope (EDS) and also by optical microscope (Olympus SZ61, Magnification X40). Elemental analysis of the SBF solution was carried out by Inductively coupled plasma spray atomic emission spectroscopy (ICP-AES) and spectrophotometry.

Results and Discussions

Figure 1 shows the surface potential change of the alkaline and heat-treated Ti during biomimetic growth in both n-SBF and ZZ-SBF at 36.5°C and pH: 7.4. In both the SBF's the initial potential was highly negative. Many researchers have given explanation for this. Ti metal is generally covered with a thin TiO₂ layer, and hence is chemically durable. It has been found that the alkaline treated Ti forms sodium titanate (Na₂Ti₅O₁₁) surface layer. This sodium titanate could form many Ti-OH groups in SBF. When exposed to SBF, the Na⁺ ions in the surface layer of Na₂Ti₅O₁₁ are exchanged with H₃O⁺ ions present in the fluid facilitating the formation of Ti-OH groups. Because of the negatively charged ions at the surface, the initial surface potential during biomimetic development in SBF solution was extremely negative [19]. The positively charged calcium (Ca²⁺) ions from the SBF solution are preferentially attracted to this negatively charged surface, and the potential is shifted to the anodic direction. The negatively charged phosphate (HPO₄²⁻) ions are attracted to this positively charged surface, forming an amorphous CaP and the potential shifts negative. This CaP develops into crystalline apatite on its own. Because the SBF is significantly supersaturated in relation to apatite even under normal circumstances, the apatite develops spontaneously by up taking the calcium and phosphate ions in the fluid once created [20,21].

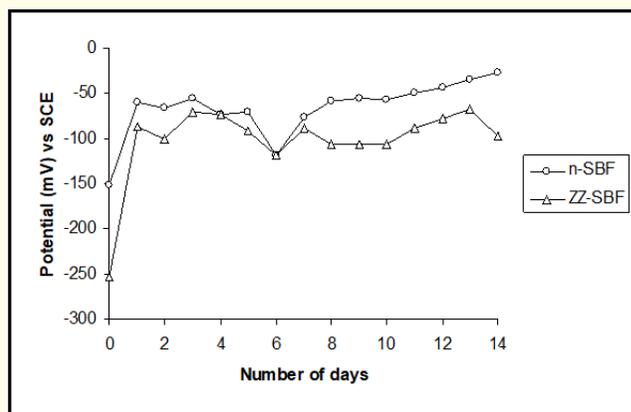


Figure 1: Surface potential change of the alkaline and heat-treated Ti during biomimetic growth in (○) n-SBF and (Δ) ZZ-SBF at 36.5°C and pH:7.4.

Table 1 shows the mass % of Ca and P on the surface of alkaline and heat-treated Ti after immersion in SBF solutions for 14 and 42 days at 36.5°C. The mass % was found to be higher for the alkaline and heat-treated Ti immersed in ZZ-SBF for the two immersion times and this shows that higher amount of CaP crystals on the surface. This is an evidence for the influence of ZZ-water for enhanced apatite growth in living body environment also.

Immersion time	Physiological solution	Mass %	
		Ca	P
14 days	n- SBF	0.15	0.32
	ZZ-SBF	0.36	0.96
42 days	n-SBF	0.27	0.81
	ZZ-SBF	0.69	1.33

Table 1: Mass % of Ca and P on the surface of alkaline and heat-treated Ti after immersion in SBF solutions.

Figure 2 shows the SEM micrograph and associated EDS pattern of (a) alkaline and heat-treated Ti; after biomimetic growth in n-SBF and ZZ-SBF for 20 days and pH: 7.4. This shows the enhanced biomimetic growth character of the ZZ-SBF solution. The first CaP film deposited on the Ti surface acted as a seeding surface for further apatite growth. The high mass% Ca and Mg in the ZZ-SBF increases the ionic activity product of the apatite in the narrow gap between the substrate and the bone surface.

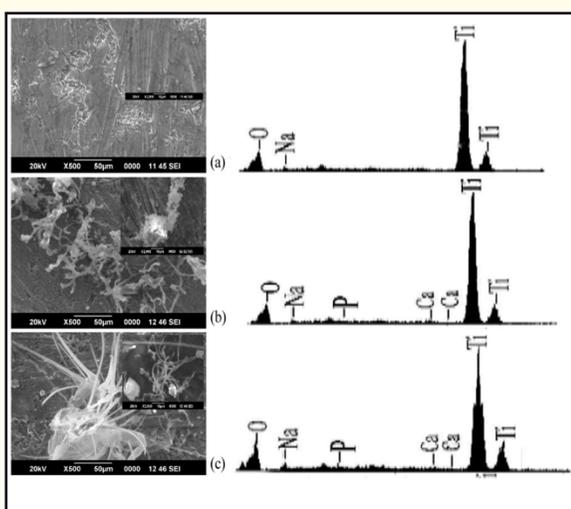


Figure 2: SEM micrograph and associated EDS pattern of (a) alkaline and heat-treated Ti; after biomimetic growth in (b) n-SBF (c) ZZ-SBF for 20 days and pH: 7.4.

Figure 3 shows the potentiodynamic anodic polarization curves of the HAp coatings on Ti in 0.9% NaCl at 36.5°C and pH:7.4. From the polarization studies, the HAp coatings developed in ZZ-SBF could withstand aggressive conditions without delamination and shows the adhesive strength of the HAp coatings.

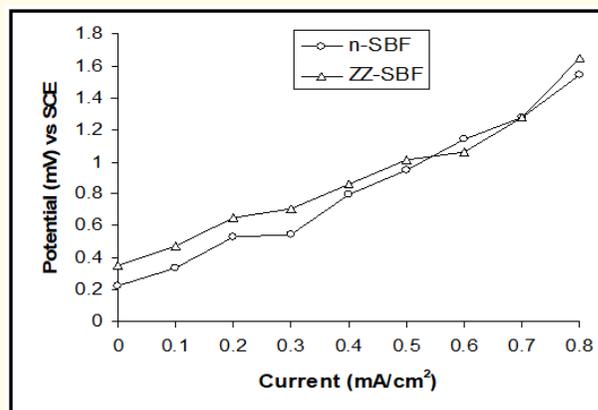


Figure 3: Potentiodynamic anodic polarization curves of the HAp coatings on Ti in 0.9% NaCl solution at 36.5°C and pH:7.4. The coating developed in (○) n-SBF and (Δ) ZZ-SBF.

Conclusion

The alkaline and heat-treated Ti shows enhanced apatite growth in SBF prepared in Zamzam water. The HAp coatings developed in ZZ-SBF shows an enhanced adhesion strength even under aggressive conditions.

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