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Morphological and chemical characterisation of biomimetic bone like apatite formation on alkali treated Ti6Al4V titanium alloy

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ABSTRACT

The present study is an attempt to enhance the apatite-forming ability of titanium metal induced by the alkaline (NaOH) treatment. A cell free culture medium, acellular DMEM solution was utilised to develop bone-like apatite on alkali-treated titanium alloy surface. The main advantage of this process is the development of bone like apatite with essential trace elements on the metallic substrate by using the DMEM culture medium as a soaking medium. The formed apatite deposits were investigated by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDXS). The obtained results suggest that the method utilized in this work can be successfully applied to obtain deposition of uniform coatings of crystalline hydroxyapatite on alkali treated titanium substrates.

1. Introduction

Titanium and its alloys are considered as the best metallic implants for dental, orthopaedic and osteogenic applications, due to their mechanical properties and nontoxic behaviour, high corrosion resistance and excellent general biocompatibility [1,2]. However, the titanium alloys don't bond directly to living bone [3,4] This can lead to encapsulation by dense fibrous tissue in the body. As a consequence, non-appropriate stress distribution at the bone–implant interface appears which could lead to interfacial failure and loosening of the implants. For many years, ceramic coatings on metallic materials have proved to be effective in bone-bonding ability. Nowadays Hydroxyapatite (HAP) is widely employed to develop coatings on metallic implants and coating processes like plasma spraying [5] or electrodeposition are well established [6]. However plasma spraying does not allow a satisfactory control in composition and structure of the coating and thus degradation of the coated HAP layer is inevitable [7]. In case of the HAP coatings prepared by electrodeposition, its bonding with the titanium implant is found unsatisfactory [8]. As an alternative, there has been an increasing interest in the formation of a biomimetic bioactive surface layer on metal substrates during recent years. HAP as a bioactive ceramic is well known for its usage as orthopaedic, dental implants and scaffolds for bone growth [9–12]. Biomimetic deposition of HAP has gained large interest because of its low deposition temperature and good step coverage; however, it demands a substrate with bioactive properties. Commercially pure titanium is not bioactive but it can acquire bioactive properties through various surface treatments. NaOH and heat treatments of titanium metal induce an apatite-forming ability on the metal by producing a bioactive graded sodium titanate structure on its surface. In the past decade, considerable research attention has been paid to the biomimetic approach to produce HAP coatings [13,14]. Usually the biomimetic approach has been employed for the preparation of so-called bone-like apatite that contains essential trace elements in its structure with small crystallites [15–17]. Generally, simulated body fluid (SBF) is used as a medium for the development of biomimetic apatite. In the present study, an attempt has been made for the first time to utilize acellular fluid (DMEM) as a medium for the development of a bone-like apatite layer on the titanium alloy metal surface at physiological temperatures. Since, acellular scaffolds are “designed for purpose”, they offer many potential advantages over conventional scaffold materials, and upon implantation, should be infiltrated and remodelled by host cells into long-lived functional tissue. The aim of this study was to develop a biomimetic apatite from acellular culture medium (DMEM) on surface treated Ti6Al4V titanium alloy.

2. Materials and methods

Commercially available Ti6Al4V titanium alloy (Goodfellow, England) specimens 10x10x1 mm were used. They were polished with SiC paper at different grades (600, 1200, 2400 and 4000), washed with acetone, rinsed with distilled water in an ultrasonic bath and finally

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Table 1
Compositions of the acellular culture medium DMEM and SBF.

Ionic concentrations (mM)	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3	Cl	HPO_4^{2-}	SO_4^{3-}
DMEM	154.56	5.37	1.82	0.8	44	120.5	1.0	0.8
SBF	141.8	5.0	2.5	1.5	4.2	148.0	1.0	0.5
		DMEM			SBF			
pH		7.3			7.4			
Buffer		No			Tris-hydroxymethyl-aminomethane ($(CH_2OH)_3CNH_3$) + HCl at 36.5 °C			
Amino acids (mg/l)		L-Arginine. HCl (84) L-Cystine (48) L-Alanyl-L-Glutamine (862) Glycine (30) L-Histidine HCl. H_2O (42) L-Isoleucine (105) L-Leucine (105) L-Lysine HCl (146) L-Methionine (30) L-Phenylalanine (66) L-Sérine (42) L-Threonine (95) L-Tryptophane (16) L-Tyrosine (72) L-Valine (94)			No			
Vitamins (mg/l)		Calcium panthothenate (4) Choline choryde (4) Folic acid (4) i-inositol (7.2) Nicotinamide (4) Pyridoxine HCl (4) Riboflavine (0.4) Thiamine HCl (4)			No			
Other compounds (mg/l)		D-Glucose (1000) Phenol red (15) Sodium pyruvate (110)			No			

dried in an oven at 40 °C. The clean and polished titanium alloy plates were soaked in 200 ml of 10 M NaOH aqueous solution at 60 °C for 24 h. The NaOH solution was prepared by dissolving commercial sodium hydroxide pellets (ACROS ORGANICS, USA) in deionized water. The alkali treatment was carried out at 60 °C ± 1 °C for 24 h. The samples were soaked in NaOH solution followed by gentle rinsing in deionized water and finally dried in air at room temperature for 24 h. After the alkali treatment, the titanium samples were sterilized before being immersed into the DMEM acellular medium (composition presented in Table 1) for the development of bone like apatite on the treated titanium surface. The standard procedure for the sterilization named as dry heat sterilization (DO) of surgical materials was employed. The dry heat sterilization process was carried at 190 °C for 2 h in a TERRUZI TCA 110P air oven on paper wrapped samples. After sterilization, the metal plates were immersed into 40 ml of Dulbecco's MEM solution (GIBCO) at 37 °C for two different time periods of 96 h and 360 h respectively. The composition of DMEM has been presented in Table 1 along with simulated body fluid (SBF) for comparison purposes. After incubation, the samples were washed with distilled water and dried in an air oven. The surface morphology of the candidate material at different immersion timings were observed by Scanning Electron Microscopy (JEOL JSM-5400LV) in the secondary electron mode and the surface chemical composition was analyzed by energy dispersive X-ray analysis (Si(Li) detector for X-ray measurements EDAX, France) spectroscopy. X-ray diffraction studies on the immersed surface of the passivated metal surface were carried out using a high resolution diffractometer with Cu K^{α} radiation (Rigaku Geigerflex D/Mac, C Series diffractometer). The thickness of the coatings was measured by using the sensing probe of ELCA-D meter (Germany).

3. Results

3.1. Alkali (NaOH) pretreatment on Ti6Al4V substrate before soaking in DMEM acellular solution

Fig. 1 represents the results of the surface treatment. The 600 and 2400 SiC grit polished surface of the titanium alloy shown respectively in Fig. 1a and b had witnessed big ridges and the corresponding EDXS spectrum had clearly revealed the presence of a TiO_2 surface oxide layer. The surface morphology induced by the alkali treatment presented in Fig. 1c had revealed the microporous surface nature and the results are in good agreement with the previous reports [18,19]. The surface morphology of the alkali treated titanium has shown the propagation of the crack network on its surface and the pre-treated surface suggests the probable formation of a crystalline sodium titanate $Na_2Ti_5O_{11}$ on the titanium surface. The structural characteristics of the coatings followed by XRD (figure not shown) indicate the presence of $Na_2Ti_5O_{11}$ on the titanium surface.

3.2. Soaking into the acellular DMEM medium

After the dry sterilization process, the samples were soaked in 40 ml of DMEM at 37 °C. The EDXS spectra presented in Fig. 2 and the related SEM micrographs obtained after 96 h (Fig. 2a and b) had revealed the appearance of calcium and phosphate peaks with a strong reduction in the sodium peak intensity without any change in the surface topography. After 360 h (Fig. 2c and d) a significant change in the surface topography has been observed. Indeed, Fig. 2d EDXS reveals a strong increase in the Ca and P peaks along with Mg and K peaks. Fig. 2d micrograph exhibits a strong alteration in the surface topography: the bone like apatite deposit completely covers the metal surface. Moreover, micrometer size randomly distributed crystals were observed on the surface (Fig. 2d). Coating formation was achieved by soaking the specimens in the solution. The XRD patterns for the as formed biomimetic apatite is presented in Fig. 3 along with the standard (JCPDS File no. 09432) hydroxyapatite patterns for comparison. The average thicknesses of the obtained coatings were observed in the range between 10–15 μm. To increase the coating thickness, the process was repeated for a total of three times. The specimens were then removed from the solution, extensively rinsed with de-ionized water, and dried at 60 °C.

4. Discussion

The results of this paper support the ability of the acellular DMEM solution to induce the nucleation of an apatite layer on the surface of Ti alloy. The NaOH pre-treated titanium metal forms apatite in a biological environment through the following mechanism. The metal releases sodium ions from the surface amorphous sodium titanate layer into the surrounding fluid through an ion exchange process and with the hydronium ions present in the surrounding fluid leads to form Ti–OH groups on its surface (Fig. 1c). The Ti–OH groups induce apatite nucleation, and the released sodium ions accelerate the apatite formation by increasing the pH of the fluid [20].

$$TiO_2 + OH^- \rightarrow HTiO_3$$

$$Ti + 3OH \rightarrow Ti(OH)^{3/4} + 4e^-$$

$$Ti(OH)^{3/4} + e^- \rightarrow TiO_2H_2O + 0.5H_2$$

This biomimetic process, in the case of metallic materials, generally consists of chemical treatment in an alkaline solution, followed by a heat treatment and ending with an immersion in a cell free culture medium (DMEM). The immersion in DMEM can be considered as a first-stage process in assessing the biocompatibility of a biomaterial. Once apatite nuclei are formed, they grow spontaneously by