Novel porous $\text{Al}_2\text{O}_3\text{-SiO}_2\text{-TiO}_2$ bone grafting materials: Formation and characterization

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Abstract

The present article deals with the development of 3D porous scaffolds for bone grafting. They were prepared based on rapid fluid infiltration of $\text{Al}_2\text{O}_3\text{-SiO}_2$ sol into a polyethylene non-woven fabric template structure. Titanium dioxide in concentration equal to 5 wt% was added to the $\text{Al}_2\text{O}_3\text{-SiO}_2$ mixture to produce $\text{Al}_2\text{O}_3\text{-SiO}_2\text{-TiO}_2$ composite scaffolds. The prepared scaffolds are characterized by means of X-ray diffraction, scanning electron microscopy and three-point bending test techniques. The bioactivity of the produced bodies is discussed, including the in vitro and in vivo assessments. The produced scaffolds exhibit mean total porosity of 66.0% and three-point bending strength of 7.1 MPa. In vitro studies showed that MG-63 osteoblast-like cells attach and spread on the scaffolds surfaces. Furthermore, cells grew through the scaffolds and start to produce extra-cellular matrix. Additionally, in vivo studies revealed the ability of the porous scaffolds to regenerate bone tissue in femur defects of albino rats 5 months post surgery. Histological analysis showed that the defect is almost entirely filled with new bone. The formed bone is characterized as a mature bone. The produced bone grafts are intended to be used as bone substitute or bone filler as their degradation products caused no inflammatory effects.

Keywords

Bone grafting, bioceramics, porous materials, in vitro, in vivo

Introduction

Bone not only provides mechanical support but also elegantly serves as a reservoir for minerals in the body, particularly calcium and phosphate. It is a good example of a dynamic tissue, since it has a unique capability of self-regeneration or self-remodeling, to a certain extent without leaving a scar.¹

Over the past four decades, several biomaterials have been developed and successfully used as bone grafts. Ceramics were introduced to orthopedics during the 1960s.²

Alumina was the first clinically used bioceramic material owing to its excellent biocompatibility, hardness, strength to resist fatigue and corrosion resistance.^{3,4} However, as Al_2O_3 is an inert ceramic material, no direct bonding occurs between Al_2O_3 surface and the surrounding bone. Mostly, a layer of connective tissue (CT) is found in the interface, which is responsible for poor osseointegration.^{5,6}

Silica-based bioactive glasses have supplied successful solutions to different bone defects and soft tissue

treatments during the last decades.⁷ The biocompatibility and the positive biological effects of their reaction products after implantation⁸ have made silica-based bioactive glasses one of the most interesting bioceramics. In contrast, the poor mechanical properties of these compounds have limited the range of their clinical applications.

Mullite is a crystalline compound of the binary alumina–silica system, typically $3\text{Al}_2\text{O}_3\cdot 2\text{SiO}_2$ (3/2), and it has been demonstrated to be a biocompatible ceramic phase.⁹ Leivo et al.¹⁰ studied the osteoblast response to sol–gel derived high-purity aluminosilicate ceramic coatings, with and without nanosized transitional

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mullite crystals and with various defined nanoscale structures. Cell culture testing by using rat osteoblasts showed good biocompatibility of aluminosilicates with sustained normal osteoblast functions. Todea et al.¹¹ investigated the potential biomedical applications of aluminosilicate microspheres obtained by spray drying technique. After 48 h of immersion of the microspheres in simulated body fluid (SBF) the ²⁹Si MAS-NMR spectra showed changes in the silica network, as a result of dissolution,¹² network fragmentation and hydrolysis.¹³ This process was shown to lead to the formation of Si-OH groups and further to silica-gel surface layer.¹⁴ Also partial loss of Si as Si (OH)₄ and a structure reconstruction during immersion was seen to occur. The microspheres/SBF interface interactions led to the development of nanocrystals on the microspheres surfaces. These nanocrystals were seen to be of apatite type. They grew by self assembly of calcium (Ca) and phosphorus (P) ions from SBF and they proved the bioactivity of the microspheres.

Sultan et al.¹⁵ prepared bauxite-based mullite-rich hard ceramic systems by mixing fly ash with different concentrations of bauxite. The test of antimicrobial activity of the prepared bodies revealed activity against both pathogenic Gram-positive and Gram-negative bacteria. A reduction of the growth of the pathogenic bacteria by more than 90%, by decomposing the cell membrane and causing internal damage of bacteria was observed.

The influences of titanium oxide (TiO₂) on the sintering temperature, solubility in the mullite lattice and mechanism of solid solution formation have been the subject of several studies. Murthy and Hummel¹⁶ showed that the solubility limit of TiO₂ at 1600°C ranges between 2 and 4 wt% and decreases with temperature, while according to Baudin and Moya¹⁷ at the same temperature TiO₂ solubility ranges between a considerably narrower concentration region; 2.9 ± 0.2 wt%. Schneider and Rager¹⁸ found that there is not only morphologically distinguished mullite but also titanium in large mullite grains is not distributed uniformly. Tkalčec et al.¹⁹ studied the distribution of titanium and aluminium in sintered mullite. They stated that mullite appears in two morphological and compositional crystals. The prismatic mullite has constant Al₂O₃ values, independent of the sintering temperature and added TiO₂. If >1 wt% TiO₂ is added, titania is not only distributed between mullite crystals and the glassy matrix but also recrystallizes in the form of needle-shaped rutile crystals. It has been shown that TiO₂ is an attractive filler material for biodegradable polymer matrices since it enhances cell attachment and proliferation on the composite surfaces.^{20,21} Li et al.²² reported that hybrid organic-inorganic PCL/TiO₂ composites prepared by in situ sol-gel process exhibit faster

degradation rate than pure Polycaprolactone (PCL) itself due to suppression of PCL crystallization. It has also been shown^{20,23} that in vitro bioactivity of Poly D, L lactic acid (PDLLA)/TiO₂ and PDLLA/TiO₂-Bioglass® foams depends on the distribution of TiO₂ nanoparticles and the presence of Bioglass® in the samples. The formation of hydroxyapatite after 28 days immersion of Poly lactic-co-glycolic acid (PLGA)/5 wt% TiO₂ nanoparticle-filled composites in SBF was demonstrated by Torres et al.²⁴ Tamjid et al.²⁵ showed that among different studied morphologies, the highest bioactivity was attained for spherical TiO₂ nanoparticles due to their high amount of anatase phase and distribution on the film surface.

In the present study, the design and development of porous Al₂O₃-SiO₂ bone grafting materials were carried out. TiO₂ was added to the Al₂O₃-SiO₂ mixture to increase the bioactivity of the produced bodies. The physical and mechanical properties of these porous materials were measured, and in vitro and in vivo tests were conducted to address the effect of the synthetic graft materials on bone tissue formation and bone cell function.

Materials and methods

Starting materials

In the present study, the following starting materials were used:

Chemically pure aluminum nitrate, tetraethyl orthosilicate (TEOS), chemically pure titanium oxide (TiO₂>99%, Panreac, made in EU), synthetic sponge and reagent-grade NaCl, NaHCO₃, KCl, Na₂HPO₄·2H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄.

Methods of preparation

A set of prismatic specimens with dimensions of 1 cm × 12 cm × 2 cm was cut from pieces of high-density polyethylene non-woven fabric. Specimens were immersed in an aqueous solution containing aluminum nitrate equal to 80 wt% of alumina and tetraethylorthosilicate equal to 20 wt% of silica under vacuum for 3 h to form by firing porous mullite/alumina composite. Titanium dioxide powder in concentration equal to 5 wt% was added to the Al₂O₃-SiO₂ mixture to produce Al₂O₃-SiO₂-TiO₂ composite. The soaked samples were dried slowly at 50°C for 6 h, then at 80°C for 6 h and finally at 110°C overnight. To accelerate the oxidation of the formed carbon produced by the fabric fiber and to sweep the gases resulted from the nitrates disintegration; the samples were fired up to 600°C under static air. A slow heating rate was adopted at low temperature to prevent bodies cracking during the burnout

process, i.e., a rate of 1°C/min from room temperature to 600°C followed by a higher rate of 5°C/min to the sintering temperature. The samples were fired at 1500°C up to 1700°C with a firing interval of 50°C and a soaking time of 1 h.

Material characterization

Bulk density and apparent porosity of the fired specimens were evaluated using the Archimedes method (ASTM C-20). The phase composition of the samples was analyzed using X-ray diffraction (XRD) using monochromated Cu K α radiation (D 500, Siemens, Mannheim, Germany). The microstructure of the samples was investigated via scanning electron microscopy (SEM; Model XL 30, Philips, Eindhoven, Netherlands). Bending strength was measured using a three-point bending test on a universal testing machine (Model 4204, Instron Corp., Danvers, MA) at a cross-head speed of 1 mm/min and support distance of 40 mm. At least 10 specimens with nominal dimensions of 50 mm $\times$ 10 mm $\times$ 7 mm were measured for each data point. Mercury porosimetry (Model pore sizer 9320, Micromeritics, USA) was used to measure the samples average pore size.

In vitro bioactivity test

For in vitro acellular test, mullite-alumina-titania porous bodies were immersed in SBF.²⁶ The samples were immersed in SBF with ion concentration nearly equal to that of human blood plasma¹¹ under static conditions at a concentration of approximately 0.01 g/mL of solution. One mg of the sample was immersed in 100 mL SBF at pH 7.4 and the temperature was kept at 37°C during the test. After immersing for a pre-determined period of time, the composition of the solution was analyzed by ICP-AES. The microstructure of the immersed samples was observed by SEM attached to energy-dispersive X-ray analysis (EDS). Three specimens were measured for each data point.

Cell culture study

To evaluate the cell behaviour on porous scaffolds, MG-63 osteoblast-like cells (Sigma-Aldrich, Germany) were used. This cell line was cultured at 37°C in a humidified atmosphere of 95% air and 5% CO₂, in DMEM medium (Gibco, Germany) containing 10 vol% fetal bovine serum (FBS, Sigma-Aldrich, Germany) and 1 vol% penicillin/streptomycin (Sigma-Aldrich, Germany). Cells were grown to confluence in 75 cm² culture flasks (Nunc, Denmark), harvested using Trypsin/EDTA (Sigma, Germany), counted by a hemocytometer (Roth, Germany) and diluted to a

concentration of 100,000 cells/mL cell culture medium. Before cell seeding, the samples were cleaned by soaking in Extran (Merck, Germany) and sodium dodecyl sulphate (SDS, Sigma-Aldrich, Germany) solutions. Afterwards they were sterilized at 121°C in an autoclave (Systec, Germany). Then the samples, four replicates of each sample type for several experiments, were incubated with cell for 7 and 14 days. For cell morphology characterization, cells on samples were fixed in 3 vol.% paraformaldehyde, 3 vol.% glutaraldehyde (Sigma-Aldrich, Germany) and 0.2 M sodium-cacodylate (Sigma-Aldrich, Germany). After a dehydration through incubation with a series of graded ethanol (10, 30, 50, 75, 90, 95, 98 and 100 vol%), the samples were critical point dried with CO₂ (Samdri PVT-3; Tousimis Research Corp., USA) and sputtered with gold (Cressington, UK). The cell morphology was analyzed by SEM (Quanta FEI 200, Netherlands). Afterwards samples were broken into two to analyze the cross section.

Surgical procedure

Eight adult male albino (*Sprague Dawely Strains*) rats with average weight (200–250 g) were used as experimental animals. Al-Si-Ti scaffold was implanted in femur defects of four rats to evaluate its ability to regenerate bone tissue. The other four rats were not subjected to surgery. They served as a control group for comparison regarding blood analysis. The surgical procedure on animals was performed in accordance with ethical guidelines for Animal Care and Ethics Committee of the National Research Center of Egypt. Animals were anesthetized with sodium thiopental with dose 40–45 mg/kg body weight given into the intraperitoneal cavity. The surgical site was shaved and prepared with a solution of betadine (povidone-iodine) and alcohol. Under the sterile surgical conditions, the skin over the anteromedial aspect of each femoral diaphysis was incised and the skin and periosteum were retracted so that the femur bone can be exposed. A mid-shaft femoral cortical bone defect (2 mm in diameter) was created in the anteromedial aspect of each femur using a low-speed dental burr under continuous rinsing by cold physiologic saline solution to minimize heat-related damage, and to remove all the bone particles. The bone marrow chamber was evacuated by repeated washings with saline solution through a syringe introduced into the defect space. The surgical site was packed with gauze until bleeding subsided. Immediately following, the grafting materials were placed in sufficient amount to fill the holes, then the periosteum, muscle and skin were sutured in layers. After the grafting materials were implanted, the animals were slaughtered for evaluation after 5 months.

Post-operative evaluation

a. Clinical examination

Each animal was monitored closely for any local or general complications after the operation and during the scheduled experimental period.

b. Scanning electron microscope/energy-dispersive X-ray analysis

Newly formed bone (nb) was analyzed using SEM coupled with EDS analysis to determine the Ca/P atomic ratio. Those ratios were compared with the ratio of bone femur extracted from the control group. After the animals were slaughtered, the segment of the femur including the bone defect was excised and fixed in 10% phosphate-buffered formalin solution for 7 days. The fixed specimen was dehydrated in serial ethanol concentration without being decalcified. Several cross sections were prepared using a band saw. The surfaces of the sections were polished and coated with a thin layer of carbon. They were then analyzed using SEM.

c. Histological evaluation

For a histological examination, the specimens were fixed in 10% buffered formalin, decalcified in a decalcifying solution (Plank-Rychlo solution) and stained with hematoxylin and eosin (H&E) for the optical microscopic examination.

d. Biological studies for safety evaluation of implanted materials

The implantation of biomaterials in the body may sometimes cause some side effects due to their degradation product. Liver and kidney are responsible for the elimination of those products from the body. They could be damaged due to the toxicity of those products. In addition, the degradation products may have an oxidative effect causing the liberation of oxygen radicals which destroy the tissues of the body or they may have carcinogenic effect. Therefore, it is important to study the biological behavior of materials inside the animals and to evaluate their safety to be used as biomaterials. Blood analyses including the following tests were carried out:

- (1) Liver and kidney functions tests.
- (2) Measurement of tumour markers.
- (3) Determination of free radical biomarker known as lipid peroxides (Malondialdehyde).

- (4) Determination of nitric oxide production in serum as an indicator of the inflammatory effect of implanted materials.
- (5) Statistic analysis.

Results and discussion

Characterization of the Al_2O_3 - SiO_2 - TiO_2 composite

Figure 1 shows the microstructure of the native non-woven polymeric fabric used as template in the present study. The image indicates the highly porous and randomly situated pores of the starting material. Because of the large interstitial spaces, the composite precursor can penetrate the native fiber more easily.

Figure 1. Microstructure of the native synthetic non-woven fabric used as template to fabricate porous alumina-silica-titania composites.

Figure 2. Apparent porosity of the fired alumina-silica-titania composite.

Figure 2 shows the apparent porosity of the composite samples fired at different temperatures. It is observed that the apparent porosity of the samples decreases with the increase in firing temperature from 84% for the samples fired at 1500°C to 44% for the samples fired at 1700°C. A temperature of 1600°C was chosen as the firing temperature for the samples used in the upcoming tests.

The total porosity and average pore size of the studied samples fired at 1600°C are illustrated in Table 1. The results show that the fired bodies possess an average pore diameter of 83.97 μm , while its mean total porosity is 66.0%. It is well-known that the addition of TiO_2 promotes the densification process by two ways: (1) it increases the cation mobility by the formation of cation vacancy resulting from the substitution of Al^{+3} by Ti^{+4} and (2) it forms a liquid phase at the firing temperature, which increases the diffusion or mass transport of reactants through it.²⁷ The decrease in the apparent porosity of the samples fired at 1550°C and up to 1650°C is attributed to the formation of mullite phase. Since mullite has a low lattice and grain-boundary diffusion coefficient,²⁸ the mullite formation tends to stabilize the porous framework.²⁹ Thus, only a slight decrease in porosity was observed at the sintering temperature of 1550°C. However, densification becomes much easier at 1650°C, leading to a noticeable reduction in porosity. The fired samples fired at the selected firing temperature show a thermal expansion coefficient of $0.65 \times 10^{-6}/^\circ\text{C}$ between 20 and 1000°C, and a three-point bending strength of 7.1 MPa. The relatively low level of fracture strength is attributed to the high matrix porosity. The mechanical properties of continuous fiber ceramic matrix composites (CFCCs) are sensitive to the level and distribution of matrix porosity. As the matrix porosity decreases, the damage tolerance decreases and the extent of correlated fiber failure increases. The low coefficient of thermal expansion values of the textile-based bodies (Table 1) may be due to orientation of the alumina crystals parallel to the textile fibers plane.

Figure 3(a) shows the porous nature of the fired bodies. The composites maintained the initial porous morphology of the starting material. The mullite particles appeared as elongated particles, Figure 3(b). The

small white rounded particles that appear in Figure 3(b) are assumed to be rutile grains. The presence of TiO_2 in the form of rutile favours the formation of mullite. On the other hand, the mullite formation encapsulates and isolates sub-micron rutile crystals due to the very limited rutile reaction with mullite. The formation of elongated mullite crystals and the presence of rutile phase are confirmed by the results of Bouzidi et al.³⁰ and Hong and Messing.³¹ They found that titania-doped mullite samples contained both equiaxial and elongated grains. The quantity of elongated grains increased as the titania concentration increased. They reported that samples containing 5 wt% TiO_2 strongly developed elongated mullite grains. The XRD pattern of samples sintered at 1650°C showed the presence of rutile. This means that the limit of solubility of TiO_2 in mullite had not been reached and therefore some content of unreacted titania could produce regions of lower viscosity which caused the anisotropic grain growth.

The XRD pattern of the prepared bodies fired at 1600°C for 1 h is shown in Figure 4. It reveals the presence of major mullite sharp peaks together with minor alumina and rutile peaks as a secondary phase. The abovementioned results are in good agreement with the microstructure findings. The Rietveld XRD analysis showed that the studied bodies contain 82.1% mullite, 14.5% alumina and 3.4% rutile.

In vitro bioactivity results

Figure 5 shows the mean value of Ca and P ions concentrations (mg/L) as a function of incubation time in SBF. The results reveal an increase in Ca^{+2} ions concentration with time until the third week. Then the concentration decreases up to the fifth week. The consumption of calcium ions indicates the formation of apatite on the surface of the samples. The figure also shows a decrease in the P^{+3} concentration with time up to 4 weeks. For the following week, a nearly fixed value was observed. The decrease of P^{+3} ions is due to the precipitation of hydroxyapatite on the sample surface.

The microstructure of the Al-Si-Ti bodies immersed in SBF for 14 days is shown in Figure 6. A coating layer of small globules, characterizing the formation of a carbonate apatite layer,^{32,33} was formed on the surface of the bodies after immersing in the SBF solution, Figure 6(a). SEM/EDS analysis of the indicated region confirmed the presence of Ca, P and Al ions. The Ca and P ions indicate the formation of calcium deficiency hydroxyapatite phase ($\text{Ca/P} = 1.47$), Figure 6(b). The ability of TiO_2 nanoparticles to stimulate formation of apatite crystals when incorporated in PDLLA matrices has been reported.²³ Titanium hydroxide (Ti-OH) groups that form by reacting with water molecules lead to a negatively charged TiO_2

Table 1. Average pore diameter, total porosity, thermal expansion coefficient (TCE) and bending strength of the sintered alumina/mullite bodies fired at 1600°C.

Average pore diameter, μm	Total porosity, %	TCE $\times 10^{-6}/^\circ\text{C}$ (20–1000°C)	Bending strength, α MPa
83.97	66.0 $\pm$ 0.8	0.65	7.1 $\pm$ 0.2

Figure 3. Scanning electron microscopy (SEM) micrographs of the fired $\text{Al}_2\text{O}_3\text{-SiO}_2\text{-TiO}_2$ composite. T: rutile, M: mullite.

Figure 4. X-ray diffraction (XRD) pattern of the studied bioceramic bodies.

surface in SBF (pH = 7.25). Then positively charged calcium ions are attracted to the TiO_2 surface resulting in the formation of calcium phosphate.

Cell culture tests

In Figure 7, SEM-images of MG-63 osteoblast-like cells on the scaffold surface after 7 and 14 days are shown. As shown in these images, cells are attached and well spread. Furthermore, the cultured cells are homogeneously distributed over the whole surface and smaller pores are already covered by the developed cell layer.

After 14 days of cultivation, extracellular matrix (ECM)-production of the cells started already. The analysis of the cell morphologies confirmed no toxic effect of the material on the cells.

An important question using 3D geometries for tissue engineering is the ingrowth of cells into the structure. Therefore, cross sections have been used to study cell morphology inside the scaffolds (Figure 8). On the top of the scaffold, the cell layer can be observed and the large pores inside the scaffold are clearly visible.

At higher SEM magnification, cell attachment onto the scaffold is shown (Figure 9). After 7 days of

Figure 5. Ca^{+2} and P^{+3} ions concentration in solution as a function of incubation time periods in simulated body fluid (SBF).

Figure 6. (a) Scanning electron microscopy (SEM) image showing the microstructure and (b) energy-dispersive X-ray analysis (EDS) analysis of mullite-alumina bodies immersed in simulated body fluid (SBF) for 14 days.

incubation, only isolated cells can be detected, however, after 14 days a dense cell layer with spread cells are observed.

Biological and surgical evaluation

Clinical examination. The rats recovered quickly from the operation, walked freely and continued to gain weight throughout the observation period. All animals survived without any local or general complications for the scheduled experimental period. During the experimental period, the animals remained free of infected wound or disturbed wound healing at the surgical site. The surgical incisions made on the rats rapidly healed. No hematoma or festers occurred. In addition, no abscess or inflammation of the peripheral osseous tissues at the implantation site was observed. These observations reveal that grafting material implanted in the bone defects did not lead to histopathology or exhibit negative biocompatibility with the peripheral osseous tissue.

Scanning electron microscopy/energy-dispersive X-ray analysis. Figure 10(a) and (b) shows a back scattered electron micrograph of the cross section of bone defect grafted with Al-Si-Ti, 5 months post surgery, at different magnification. The defect was mainly filled with new bone (nb), Figure 10(a), however few residues of the Al-Si-Ti sample can be seen in the figure. Some of them were covered with nb as shown by the dotted circles in the figure indicating that the material was highly bioactive. This high bioactivity is attributed to the presence of silica (20 wt%) in the structure of the samples as well as titania (5 wt%). Silica and titania could form Si-OH and Ti-OH groups on the surface of implanted Al-Si-Ti samples.^{26,34–36} Those active groups will in turn induce the formation of an apatite layer on the surface of the samples and will improve their in vivo bioactivity. The ability of a material to form HA-like surface layer when immersed in SBF, in vitro, is often taken as an indication of its bioactivity.³⁷ Furthermore, it has been suggested that in vitro bioactivity is an indication of the bioactive potential of a material in vivo. Moreover, several studies have indicated that the dissolution of silica ions from bioactive materials such as bioactive glass or calcium silicate is responsible for stimulating several genes of bone cells towards a path of regeneration and self-repair.^{38–42} Therefore, the release of Si ions from Al-Si-Ti in vivo could be responsible for its high bioactivity. Figure 10(c) and (d) shows the EDS analysis of the remaining Al-Si-Ti (c) and the nb (d). The EDS spectrum of Al-Si-Ti showed peaks of aluminum, titanium and silicon, which are the main components of the material investigated. Also, the spectrum

Figure 7. Scanning electron microscopy (SEM)-images of osteoblast-like MG-63 cells on the scaffold surfaces after 7 d (left) and 14 d (right) incubation.

Figure 8. Scanning electron microscopy (SEM)-image of the cross section of the scaffold cultivated with MG-63 cells after 7 d (left) and 14 d (right) incubation.

showed peaks of calcium and phosphorous, indicating that the material was covered with bone. In addition, the Ca/P atomic ratio of the nb surrounding the implant was 1.62. This ratio is similar to that of the normal bone of the control group (1.62), which indicates that the nb was well mineralized.

Biological studies for safety evaluation of implanted materials

a. Liver function tests

Liver function tests in terms of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities of animals grafted with Al-Si-Ti implant together with those of the control group (animals left with no surgery) are

given in Table 2. Statistical analysis was carried out using standard analysis of student's *t*-test to evaluate if the difference between liver function activities in serum of grafted group to those of the control group was significant. The level of significance is set at $p < 0.05$. The results showed that animals grafted with Al-Si-Ti implant had lower ALT activity than that of control group. Statistical analysis results showed that the difference between ALT activities in serum of animals grafted with Al-Si-Ti scaffolds to those of control group was not significant $p > 0.05$ ($P = 0.122$). The results indicated that AST activity in serum of the grafted group is similar to that of the control group. Statistical analysis results showed that the difference between AST activities in serum of animals grafted with Al-Si-Ti scaffolds to those of control group was

Figure 9. Scanning electron microscopy (SEM)-images of osteoblast-like MG-63 cells in the scaffold after 7 d and 14 d incubation.

Figure 10. Back scattered electron micrographs of a cross section of bone defect grafted with Al-Si-Ti scaffold; 5-month post surgery; at different magnifications (a) and (b) and the energy-dispersive X-ray analysis (EDS) analysis of the remaining implanted Al-Si-Ti material (c) and the newly formed bone (d). Blue circles show some particles of Al-Si-Ti covered with new bone (nb).

not significant $p > 0.05$ ($P = 0.89$). It can be noticed that ALP activity is higher in serum of grafted animals. The statistical analysis showed that the difference between ALP activities in serum of grafted animals and those of

the control group was not significant $p > 0.05$ ($P = 0.85$).

Liver function tests showed that the grafted animals had normal liver function as compared to the control

Table 2. The liver and kidney functions, tumor markers, nitric oxide and lipid peroxide level measured in serum of animals grafted with Al-Si-Ti as compared with control group.

Sample code	Alkaline phosphatase (IU/L)	ALT (U/mL)	AST (U/mL)	Urea (g/dL)	Creatinine (mg/dL)	Nitric oxide (mmole/L)	Lipid peroxide (nmol/mL)	Arginase (U/L)	α -L-Fucosidase (U/L)
Al-Si-Ti	356 $\pm$ 99	33 $\pm$ 11	93 $\pm$ 7	38 $\pm$ 11	5.30 $\pm$ 0.05	33 $\pm$ 15	17 $\pm$ 3	132 $\pm$ 23	2.7 $\pm$ 0.8
Control Group	345 $\pm$ 106	46 $\pm$ 3	93 $\pm$ 3	40 $\pm$ 2	5.50 $\pm$ 0.06	36 $\pm$ 7	13 $\pm$ 5	126 $\pm$ 4	3.4 $\pm$ 0.5

ALT: alanine aminotransferase activities; AST: aspartate aminotransferase activities

group. This could indicate that the degradation products of the Al-Si-Ti scaffolds did not cause liver disorder.

b. Kidney function tests

Kidney function tests in terms of serum creatinine and serum urea are given in Table 2. The serum creatinine level of animals grafted with Al-Si-Ti scaffold is lower than that of the control group. The difference is statistically not significant $p > 0.05$ ($P = 0.39$). The serum urea level of animals grafted with Al-Si-Ti scaffolds is lower than that of the control group. The difference is statistically not significant $p > 0.05$ ($P = 0.78$). It can be concluded that the grafted animals had normal kidney function as the control group. This could indicate that the degradation products of Al-Si-Ti scaffolds did not cause kidney failure.

c. Measurement of tumor markers

Tumor markers in terms of α -L-Fucosidase (AFU) and arginase activities in serum of animals grafted with Al-Si-Ti scaffolds are given in Table 2. The results show that AFU activity in the serum of the grafted animals is lower than that of the control group. Statistical analysis showed that Al-Si-Ti scaffold has no carcinogenic effect over the animals as compared with the control group. It has been recorded that the difference between AFU activities in the serum of the grafted animals and those of the control group is not statistically significant $p > 0.05$ ($P = 0.25$). On the other hand, the arginase activities in serum of the grafted animals is higher than those of the control group. However, statistical analysis indicates that the difference was not statistically significant $p > 0.05$ ($P = 0.67$).

d. Determination of free radical biomarker known as lipid peroxides (Malondialdehyde)

Free radical biomarker known as lipid peroxides (Malondialdehyde) in serum of animals grafted with Al-Si-Ti scaffolds are given in Table 2. The results

show that the level of serum lipid peroxide of the grafted animals is higher than that of the control group. This test provides a measure of total serum lipid peroxidation, which is a well-established mechanism of cellular injury in animals that occurs in vivo. Statistical analysis showed that the level of lipid peroxide in serum of grafted animals was in the normal range as compared with the control group ($p > 0.05$ ($p = 0.44$)). The obtained result indicates that the degradation products of grafted materials has no oxidative effect and does not cause the liberation of oxygen radicals that could destroy the tissues.

e. Determination of nitric oxide production in serum as an indicator of the inflammatory effect of implanted materials

Table 2 shows the level of nitrite in the serum of animals grafted with Al-Si-Ti scaffolds and that of the control group. Results showed that the serum nitrite level of grafted animals was lower than that of the control group. However, the difference was not statistically significant $p > 0.05$ ($P = 0.80$). The analysis suggested that the grafted materials caused no inflammatory effect.

Histological analysis. Histological analysis of the bone defect grafted with Al-Si-Ti scaffolds 5 months post surgery (Figure 11) showed that the defect was almost entirely filled with nb. The formed bone was characterized as a mature bone, which is highly vascularized. The arrows point to the several blood vessels containing red blood cells indicating the vascularization of the regenerated bone tissue. Moreover, Haversian canal systems centered around blood vessels were also seen indicating the maturation of the nb, as shown by the dotted rectangles. In addition, osteocytes (oc) could be seen in the mature mineralized tissue. Few remains of Al-Si-Ti scaffolds were still found in the defect, as shown by the dotted circles. They were in direct contact with nb without any fibrous encapsulation and almost completely covered with bone indicating that Al-Si-Ti scaffolds were highly bioactive as well as biocompatible.

Figure 11. Light micrographs of bone defect grafted with Al-Si-Ti scaffolds 5 months post surgery stained with Hematoxylin-eosin ($\times 100$ (a), $\times 200$ (b)).

Conclusions

- (1) The prepared bodies showed a peculiar microstructure mimicking the native template (fibrous) structure.
- (2) All animals survived without any local or general complications which means that the grafting materials implanted in the bone defects did not lead to histopathology or exhibit mal-biocompatibility with the peripheral osseous tissue.
- (3) The grafting materials implanted in the bone defects are highly bioactive. This higher bioactivity is attributed to the presence of silica (20 mol%) in

the structure of the material as well as to the small amount of titania added (5 mol%).

- (4) The Ca/P atomic ratio of the nb surrounding the scaffold was 1.62. This ratio is similar to that of normal bone of the control group (1.62), which suggests that the nb was well mineralized.
- (5) The produced materials may be used as bone substitute or bone filler as they are bioactive and their degradation products caused no inflammatory effects.

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