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The effect of Zirconia/Yttria/Silver substitutions on mechanostructure and cell viability of the synthesized bioceramic bone grafts

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ABSTRACT

Hydroxyapatite (HA) based biografts were synthesized by substitution of Zirconia (Zr), Yttria (Y_2O_3) and Silver (Ag) using the sol-gel method. Fabricated bone grafts (H40ZrYt and H50Yt10) were compared with a control biograft. The effects of Zr and Y_2O_3 and Ag additions on the morphological, mechanical and cell viability of the synthesized biografts were analyzed. The biografts were characterized by X-ray Diffractometer (XRD) and Scanning Electron Microscope (SEM). All biografts were then embedded into the drilled holes (D=3.2 mm) in rabbit femurs. The rabbits were then sacrificed after twelve weeks. Three-point bending (3-PB) tests were conducted on defected and grafted regions. Flexural strength values were found to be 347, 149 and 180 MPa, for the H40ZrYt, H50Yt10 and the control/commercial graft, respectively. Cell viability results indicated that the percentage viability ratios slightly increased after the substitution of Ag into the H50Yt10 biograft at 0.1 and 0.3 µg/ml concentrations and into the H40ZrYt biograft at a 0.1 µg/ml concentration. Although the cell viability was a bit lower in the H50Yt10 graft, the H40ZrYt graft achieved better results compared to the counterpart grafts. Furthermore, the hardness of both of the novel fabricated grafts were found to be higher than the control/commercial graft, however, as the flexture strength of H40ZrYt was higher but H50Yt10 was lower than the control/commercial graft.

1. Introduction

Calcium phosphate containing ceramics are prefered as promising materials due to their excellent biocompatibility [1]. Hydroxyapatite (HA) is the primary inorganic component of natural bone where commonly used in medical applications. It is also an excellent biocompatible material and has a similar chemical structure to that of human bone [2,3]. However, the fracture toughness of human bone is between 2 and 12 MPa√m, while the fracture toughness of HA is a maximum of 1 MPa√m [3,4]. The use of pure HA is limited in dental, crano-facial and orthopedic applications and therefore need some substitutions to improve its mechanostructure and biocompatibility properties. Researchers have tried to enhance its mechanical properties by using various reinforcement materials [4–11]. The addition of some cations, such as Mg^{2+}, Zr^{2+}, Cd^{2+} and Y^{3+} into HA has caused changes in the microstructure, as well as the hexagonal lattice parameters of HA [12]. Also, with minor additions of MgO, SrO, ZnO, or TiO2, the soluble rate of these biomaterials and their crystalline tendency were better controlled [13–16].

Based on its excellent mechanical properties, Zirconia based ceramic materials have received extensive interest in the past decade [17]. Tetragonal zirconia has not only high strength and fracture toughness but also a long term biocompatibility and reduced wear rates. This makes it a major benefit for use in prosthetic hips, knee bearings, and as a superior dental material for crowns and bridges [18,19]. Zirconia is a great biomedical success, and has a negligible failure rate of less than 0.01%, has been reported in two particular series of femoral heads for total hip replacement [20]. Yttria-stabilized zirconia (Y-TZP)–TiO_2 composites were attempted in another study [21], but it was limited to the high zirconia contents and had the problem of the formation of zirconium titanate compounds [22].

In the present study, several sol gel and reinforcement materials, such as CaO, P_2O_5, KH_2PO_4, Na_2CO_3, Yttria, Ag and Zr were added into hydroxyapatite and their effects on the properties of HA based grafts were investigated. The morphological and chemical properties of grafted regions were determined by SEM and XRD and the mechanical properties of biografts were investigated by comparing them with a control/commercial and a non-defective control group. Furthermore, the effects of Zr, Yt and Ag – added into biografts in *in vitro* tests–on cell viabilities of biografts were determined and compared with

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Table 1
Chemical compositions of H40ZrYt and H50Yt10 biografts.

Biografts	HA (%)	KH_2PO_4 (%)	Na_2CO_3 (%)	P_2O_5 (%)	CaO (%)	$AgNO_3$ (%)	Zr (%)	Yttria (%)	PEG (%)
H40ZrYt	40	10	10	10	5	5	10	10	–
H50Yt10	50	10	10	–	–	5	–	10	15

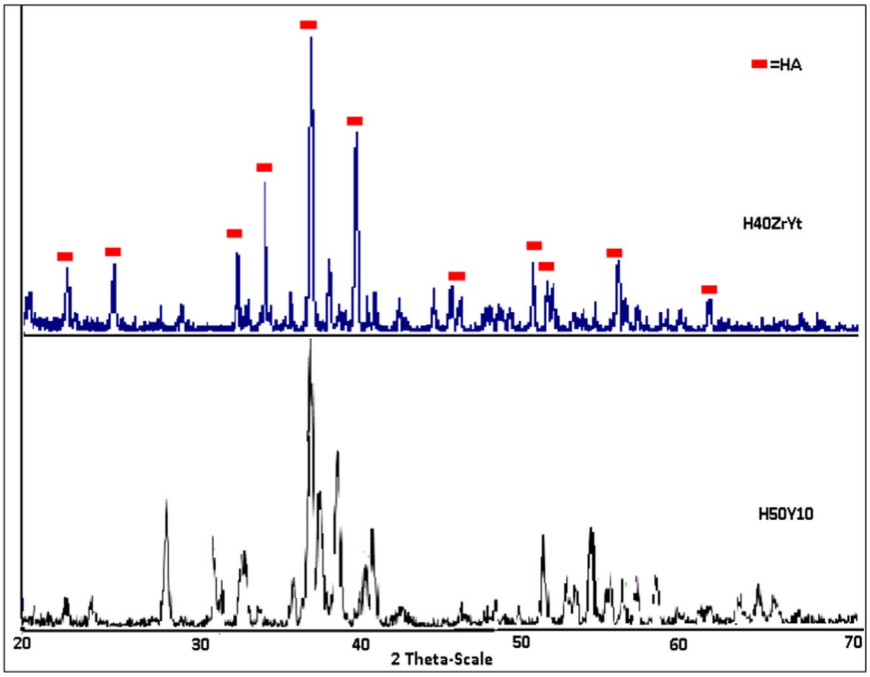

Fig. 1. XRD spectrums of H40ZrYt and H50Yt10 biografts.

control/commercial grafts and non-defective control groups.

2. Materials and method

2.1. Materials and sol gel process

Hydroxyapatite based biografts were doped with Zr and Yt (H40ZrYt) and the 50%HA based biograft was doped with Yt (H50Yt10) then they were synthesized using the sol-gel method. The mechanical properties of hydroxyapatite supplemented with KH_2PO_4 and Na_2CO_3, P_20_5, $AgNO_3$, Zr and Y_2O_3 were tested. Chemical composition and pH values of the synthesized biografts are given in Table 1. During the synthesizing, first; HA, P_20_5, KH_2PO_4, Na2CO3, Zr and Y_2O_3 were stirred at room temperature for 30 min in an ethanol/distilled water mixture using a magnetic stirrer until a gel-like structure was obtained. A homogenous gel structure was then further obtained for the mixture in a homogenizer. The grafts were aged at 120 °C for 3 h and then dried at room temperature for 24 h. Then the H40ZrYt and H50Yt10 were isostatically pressed into a cylindrical disc mold (10 mm diameter and 2 mm in thickness) under 24 N pressure and sintered in vacuum atmosphere at 1180 °C for 2.5 h.

2.2. XRD and SEM analysis

The morphological formation of fabricated biografts was characterized by SEM (JSM-7001F) and XRD analyses. The pulverized X-ray diffraction patterns of the produced biografts were recorded using a brand diffractometer (Bruker D8 Advance, λ=1.5406 Å). Spectrums were obtained by conducting measurements recorded in the range of 2q=3–70° at a scanning rate of 2°/min and 1 s constant time gap.

2.3. Mechanical test

After sacrificing, bending tests were applied after a six-month period with a 5 mm/min rate to the defective regions of the H40ZrYt, H50Yt10 and control/commercial (*Onspine*) grafts (Shimadzu, 5 kN) which had been embedded into holes with a 3.2 mm diameter in non-defective tibias. Flexural tests were applied to the defective and grafted regions of the tibial bones and loading was continued until the fracture of bones in the defective regions. Flexural bending strength and displacements(vertical) after fracture were recorded by the test machine during the 3PB tests.

2.4. In vivo experimentation and in vitro Cell viability

The fabricated biografts H40ZrYt, H50Yt10 and the control/commercial grafts were implanted and tested in rabbits via twelve weeks in vivo experiments. *In vivo* animal studies were performed after approval by *Animal Studies Ethics Committee* with 24.03.2012/7-29 approval number. 15 New Zealand rabbit tibias were used having 3 groups and 5 animals in each group. The average age of the rabbits was 6.5 months (5–8 months) and weight was 3100 g (min 2900- max 3400 g). After the *in vivo* trial, the samples were taken from the drilled holes (3.2 mm diameter). The graft filled zones were decalcified with formaldehyde, water and a nitric oxide solution in order to conduct the histopathology analysis. Then, the samples were tagged, kept in liquid paraffin and then frozen. The paraffin was then cleared and stained. Hematoxylin-eosin (H & E) staining for histological observation and Solochrome Cyanine staining for demonstration of myelin were used. Sections were stained with Solochrome Cyanine solution (Eriochrome cyanine RC, Sigma E-2502; Sigma-Aldrich Corp., St. Louis, MO, USA) for 13 min.

Cell viability tests for H50Yt10, H40ZrYt and commercial biografts