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Evaluation of new porous β-tri-calcium phosphate ceramic as bone substitute in goat model

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Abstract

The present study was carried out to evaluate the porous β-tri-calcium phosphate (TCP) (prepared by aqueous solution combustion technique) as bone substitute and compared with normal healing in 12 adult Black Bengal goats on the basis of clinical and radiographic findings, histological studies, oxytetracycline labeling, angiography studies (on day 90). Bone defects created in the diaphysis of radius were left unfilled in control animals (group I); while in treated (group II) animals the defects were filled with porous TCP blocks. The three months study showed no marked acute inflammatory reactions in all animals, wound healing was uneventful and the implants were clinically stable in the bone. Radiological studies showed presence of unabsorbed implants which acted as a scaffold for new bone growth across the defect whereas in control animals the defect was more or less same except that the newly formed bony tissue was less organized. Histological section showed moderately differentiated lamellar bone in the cortical part with presence of woven bone at peripheral cortex whereas control animals showed moderate fibro-collagenisation and good amount of marrow material, fat cells and blood vessels. Oxytetracycline labeling study showed crossing over of new bony trabeculae along with presence of resorption cavities within the new osteoid tissues whereas in group I, the process of new bone formation was active from both the ends; the defect site appeared as a homogenous non-fluorescent area. Angiogram of the animals in control showed uniform angiogenesis in the defect site with establishment of trans transplant angiogenesis, whereas in group II there was complete trans transplant shunting of blood vessels communication. The results of this study pointed out that the porous TCP promoted extensive bone formation over the entire extension of the defect in comparison to control group, thus conforming their biological osteoconductive property.

Keywords: β-Tricalcium phosphate; Bone defects; Radiography; Histology; Oxytetracycline labeling; Angiography

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1. Introduction

Bone grafts are often necessary to provide support, fill voids, and enhance biologic repair of osseous defect. Although autogenous bone is considered the gold standard, to which other methods are compared, autograft has significant limitations, including donor site morbidity, inadequate availability in inappropriate form (Summers and Eisenstein, 1989; Banwart et al., 1995). Allograft bone has been widely used, which of course have several problems encountered when used, including immunogenicity and disease transmission such as hepatitis and HIV (Friedlander, 1983), loss of biologic and mechanical properties secondary to its processing and increased cost. Consequently significant strides have been made to search ideal bone graft substitutes.

Various synthetic materials have been developed as bone substitute which include marine coral, hydroxyapatite (HAp), bioactive glasses and synthetic polymers with chemical composition and crystallinity equivalent to that of the mineral phase of natural bone (Heise et al., 1990; Peltola, 2001). Tri-calcium phosphate offers excellent bioresorbable and biocompatible properties. The small particle size and interconnected microporosity are believed to improve osteoconductive properties of the material and promote timely resorption concomitant with the process of remodeling (Erbe et al., 2001). The porous structure of bioactive materials support tissue in/on-growth and are generally affected for supplementing the implant stability by biological fixation (Engh et al., 1995). However, depending on the preparation technique the material exhibits grossly different powder characteristics, microstructure and associated mechanical and biological properties.

A biomaterial made of β-tri-calcium phosphate has been prepared by a novel aqueous solution combustion technique using calcium nitrate tetrahydrate and di-ammonium hydrogen orthophosphate as the starting raw materials and glycine/urea as the fuel. The aim of this present study was to evaluate the porous β-TCP, having an interconnecting porosity (apparent porosity of ∼37%) and regular pore shape and size as bone substitute in segmental bone healing.

2. Materials and methods

2.1. Preparation of β-TCP powder by aqueous solution combustion technique

In this study, calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) (A.R. grade, S.D. Fine-Chem. Ltd., India), and di-ammonium hydrogen *ortho*-phosphate (DAP, $(NH_4)_2HPO_4$) (A.R. grade, S.D. Fine-Chem. Ltd., India) were used as the starting raw materials. Glycine ($C_2H_5NO_2$) (Glaxo, Qualigens, India) was used as fuel. Aqueous stock solutions of calcium nitrate tetrahydrate (2.72 M) and DAP (2.20 M) were mixed in the ratio of 1.67 and 1.50, which are required for the formation of calcium phosphates. A few drops of concentrated nitric acid (Merck, India) were added to dissolve the resulting white precipitate to make a clear homogeneous solution. A predetermined amount of fuel was added to the clear solution which was homogenized by stirring with a magnetic stirrer for 30 min at room temperature. A glass–ceramic coated mild steel wide container containing the solution was introduced into a muffle furnace preheated to the desired temperature. A stainless steel wire mesh was put on the reaction container to reduce particle loss through aerosol formation and helped in attaining uniformity of temperature. When placed in the furnace, the mixed solution soon started to boil, underwent dehydration, decomposition and a large volume of gases containing oxides of nitrogen and traces of ammonia were evolved. The mass then frothed and swelled to yield foam, where a flame appeared and produced incandescence. The entire process required less than 10 min with flame duration of nearly 1 min. The as-synthesized products were fluffy foam-like mass that occupied a large volume. The resulting soft-agglomerated powders were readily ground manually in an agate mortar/pestle into fine powder and were thoroughly characterized for its phase composition, crystallinity, etc.

2.2. Fabrication of porous β-TCP

In the present study, porous (35–40% by volume) β-TCP was fabricated by using β-naphthalene and polyvinyl alcohol (both from S.D. Fine-Chem. Ltd., India) as combustible organic materials. TCP powder was milled separately with oleic acid surfactant and pre-calculated amount of β-naphthalene. Rectangular shaped ($12 \times 5 \times 3\,mm^3$) blocks were uniaxially cold compacted with low pressure, which were subsequently cold iso-statically pressed at 100 MPa for homogeneous densification. All specimens were slowly dried at 80 °C for 3 days. Finally, β-TCP specimen was sintered at 1050 °C with holding time of 2 h at that temperature. Archimedes' principle using water as the immersing medium was used to calculate the density and apparent porosity of sintered specimens. The porous struts were initially pasteurized using distilled water and subsequently autoclaved at 121 °C for 30 min before implantation. The physical properties thus calculated are shown in Table 1.

2.3. Animal experimentation

In the present study, 12 Black Bengal goats of either sex, weighing 10–12 kg were randomly distributed into two groups of six animals each: control group I (in which the created defects were left as such) and the test animals, group II (in which porous β-TCP blocks were inserted within the created defects). The animals were group housed indoor in standard conditions, given water *ad libitum* and were without restriction of movement according to the guidelines of Institutional