# In vitro and in vivo biocompatibilities of the granule type porous –TCP bone graft substitutes fabricated by fibrous monolithic process

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**Introduction:** Bone defects caused by bone tumors and trauma should be repaired to the original form through bone graft. Calcium phosphate ceramics such as -tricalcium phosphate ( -TCP) have been used as a bone graft biomaterials because of their good biocompatibility and similarity of chemical composition to natural bones. -TCP nano powders were synthesized by microwave-assisted synthesis. To increase the mechanical and osteoconductive properties, the granule type porous –TCP bone graft substitute was prepared by fibrous monolithic process [1]. Their biocompatibilities were evaluated through in vitro and in vivo study.

**Methods:**

**In vitro cytotoxicity by extract dilution method**: The extract solution was prepared through the -TCP granules with culture medium placed in a shaking incubator for 72 hrs at 37•. Fibroblast L-929 cells ($2.0 \times 10^3$/well) were seeded on 24-well plate containing serially diluted extraction solutions and cultured in an incubator containing 5% $CO_2$ at 37• for 3 days. The cellular toxicity was evaluated by MTT assay. The growth behavior of osteoblast-like MG-63 cells cultured on the porous –TCP bone graft substitute was observed.

**Gene expression by microarray**: Differentially expressed genes of osteoblast treated with -TCP extract solution were analyzed by microarray. The cDNA microarray containing a set of 17,448 sequence-verified human cDNA clones was provided by GenomicTree Inc. (Korea). The synthesis of target cDNA probes and hybridization were performed according to manufacture's direction [2].

**Animal study:** The granule type porous –TCP bone graft substitutes were implanted into distal femur of four adult rabbits. The osteoconduction and biodegradation of bone graft substitutes were evaluated by follow-up X-ray, micro-CT and histological finding.

**Results and Discussion:** The average size of a -TCP nano powder was 70~100 nm in diameter. The diameter of porous granule body was 800 um and it contains seven pores (Fig. 2. a, b). XRD profiles of -TCP granules showed the -TCP phase peaks only. Fibroblast L-929 cells cultured with diluted extract media showed similar viability compared with that of the control by MTT assay. Osteoblast-like MG-63 cells grown on the top surfaces of the pore was spindle-shape with circular condense growth from the margin (Fig. 2. c). On day 7, the top of pore was fully covered with osteoblasts (Fig. 2. d).

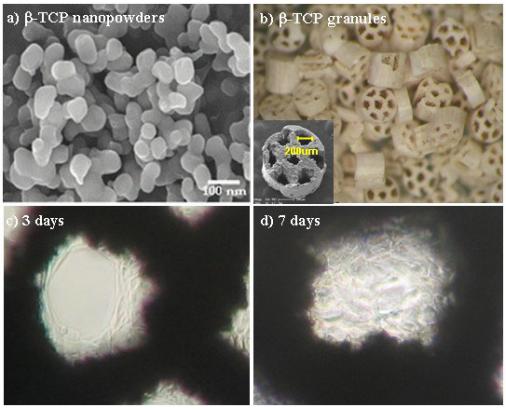


Fig. 1. Photographs of –TCP nano powders (a) and granule type –TCP bone graft substitutes (b). Light microscopic images of osteoblast grown on the top surface of porous granule body on day 3 (c) and 7 (d).

**Gene expression:** Over 2-fold expression of genes between experiment and control groups were subjected to a scatter-plot analysis. 12 up-regulated and 25 down-regulated genes compared to the untreated-cell were confirmed. These genes are involved in cell cycle regulation, cell growth and metabolism.

**Animal study:** Six months after implantation, the rabbits were sacrificed and both femurs of rabbits were isolated from the body. Active new bone formation induced by –TCP bone graft substitutes was observed (Fig. 2b). By micro-CT analysis, bone volume was increased over 3 times in the trabecular bone implanted with -TCP granules.

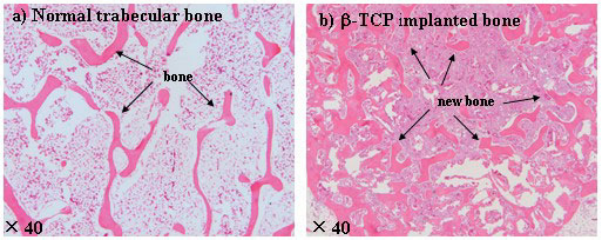


Fig. 2. Histological findings of distal femur of rabbits.

**Conclusions:** The granule type porous –TCP bone graft substitute fabricated by fibrous monolithic process showed good biocompatibility and osteoconductivity.

**Reference**

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