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실크 피브로인의 파골세포 형성 억제 효과

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Conditioned Medium of Silk Fibroin-Treated Osteoblasts inhibits the RANKL-induced Osteoclastogenesis in RAW264.7 cells

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Objectives

We investigated the indirect effect of silk-fibroin on osteoclastic differentiation of RAW264.7 cells.

Materials and Methods

Materials and methods are showed in figure 1.

Results

The conditioned medium were collected from MC3T3-E1 osbeoblasts treated with 0.001 mg/ml - 0.1 mg/ml silk fibroin for 6 days, mixed in 1:1 ratio with osteoclast medium, and then added into RAW264.7 cells with receptor activator of nuclear factor kappa B ligand (RANKL), a differentiation inducer for 3 days. Of osteoclastic cytokines in the conditioned medium, the protein expression of osteoprotegerin (OPG) with silk-fibroin was not significantly different. However, the protein expression of Interleukin(IL)-1β was specifically lower in a dose dependent manner. In RAW264.7 cells, the conditioned medium with silk-fibroin inhibited RANKL induced osteoclastic differentiation as total number of multinucleated tartrate-resistant alkaline phosphatase (TRAP)-positive osteoclasts in dose dependent manner. Taken together, we demonstrate that the conditioned medium of silk-fibroin treated osteoblasts inhibits RANKL induced differentiation of osteoclasts with inhibiting selective expression of IL-1β.

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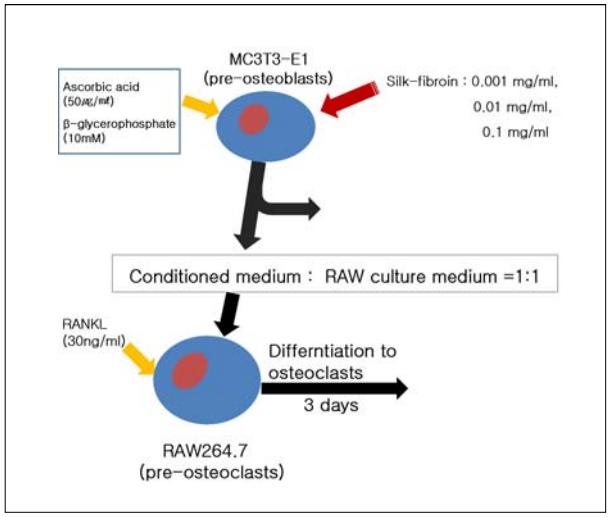

Fig. 1. Materials and methods

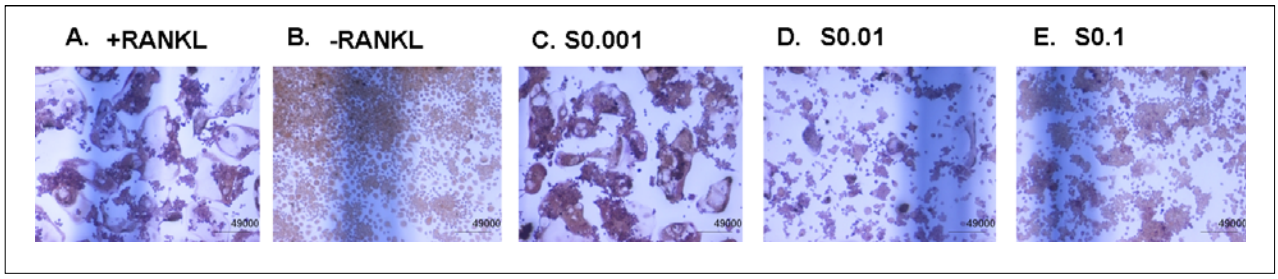

Fig. 2. The cells treated with silk-fibroin with or without 30 ng/mL RANKL were plated at 1×104 cells/well into 96-well culture dish. After 3 days of culture, the cells from RANKL (+RANKL, -RANKL: with or without RANKL, A, B) or silk-fibroin (S0.001～S0.1 mg/mL, C～E) were fixed and stained for TRAP.

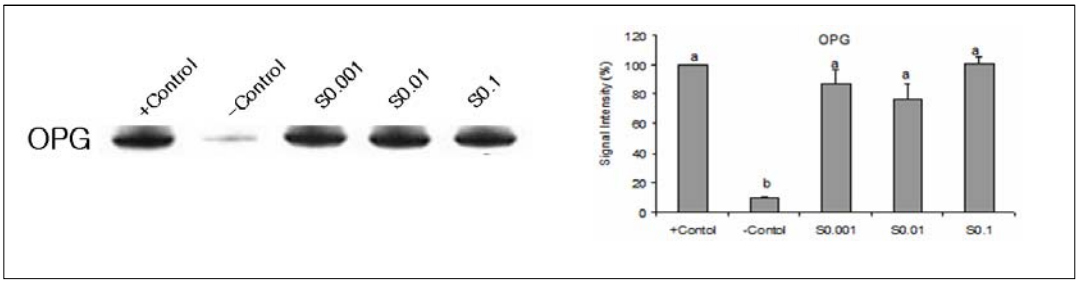

Fig. 3. The protein expression of osteoprotegerin (OPG) in conditioned medium of MC3T3-E1 cells. Protein extracts(25ug/lane) from control(+control, -Control : with or without differentiation medium, lanes 1-2), Silk-fibroin (0.001 mg/ml - 0.1 mg/ml, lanes 3 - 5) were subjected to SDS-PAGE and immunoblotting with OPG specific antibodies (abcam Inc.)