Activation of Cyclic Adenosine Monophosphate/Protein Kinase A Signaling Pathway Enhances Osteoblast Cell adhesion on Biomaterial for Bone Tissue Engineering Application

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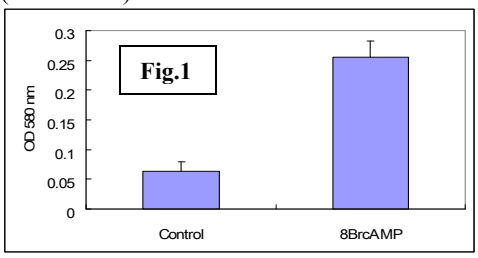
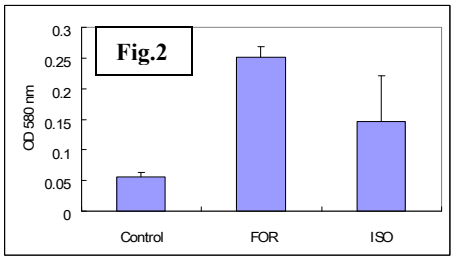
Statement of Purpose: Cell adhesion is an important goal of implants and bone tissue engineering technology. While it is known that integrins are important for osteoblast cell adhesion to various surfaces, not much is known about the regulatory mechanisms involved in integrin mediated osteoblast cell adhesion to biomaterial used for bone tissue engineering. Cyclic adenosine monophosphate (cAMP) signaling pathway is one of the known mechanism required for integrin mediated cell adhesion and has been studied extensively. The cAMP is found ubiquitously in mammalian cells and act as a common second messenger controlling many cellular processes. Protein Kinase A (PKA) was thought to be a general receptor for cAMP, resulting in the phosphorylation of a large variety of downstream target proteins and, in turn, regulating numerous cellular events. Lately, it has been shown that a novel cAMP signaling pathway, namely Exchange Protein Directly Activated by cAMP and Rap1 (Epac-Rap1) which is responsible for cAMP induced integrin mediated cell adhesion in a number of cell types. It is currently unknown whether cell adhesion of osteoblast is mediated by the cAMP molecule. If it does, it is of our great interest to study which signaling pathway of cAMP is involved in osteoblast cell adhesion. In this study, we show that osteoblast cell adhesion is promoted by cAMP via PKA signaling pathway. It implies that cAMP should be introduced in the bone tissue engineering protocol.

Methods: Two-dimensional thin films of PLAGA (85:15) (Lakeshore Biomaterials) were fabricated using a solvent casting method. The polymer was dissolved in methylene chloride (Fisher) and poured into a Teflon-coated dish, and placed at −20°C. Once the solvent evaporated the two-dimensional thin film matrices discs were formed by cutting the polymer sheet into circle films and sterilized by UV light. MC3T3E1 subtype 4 preosteoblast cell line (ATCC) was used for initial cell adhesion assay and cAMP induced signaling pathway studies. Briefly, cells were maintained in tissue culture flask contain minimal essential medium alpha (Invitrogen) supplemented with 10% FBS and 1% of antibiotic at 37°C in a humidified incubator containing 5% carbon dioxide. Cells were treated with cAMP analogs or cAMP generating agents as indicated in the figures for at least 1 hr. Crystal violet stain was carried out to analyze the initial cell adhesion. After an hour incubation, unattached cells were removed by washing with phosphate buffer saline, crystal violet stain solution (Sigma) was then added and incubate for 30 min at room temperature. Stained cells were washed couple times with water and the stain dye in the cells was solubilized with 1% SDS solution. The amount of the dye taken up by the cells was measured in a spectrophotometer.

Results: 100 μM 8-Br-cAMP, a target non-specific cAMP analogue, significantly improved osteoblasts (MC3T3E1) attachment on 2D-PLAGA surface (Fig.1).



Other commonly used cAMP-raising agents, for example 100 μM Forskolin (FOR) and 20 μM Isoprotenenol (ISO), also enhanced cell adhesion on 2D-PLAGA (Fig.2).



To distinguish which of the two independent cAMP signaling pathways (PKA vs Epac-Rap1) is involved in the increased cell adhesion, two target specific cAMP analogs were employed. 8-CPT-2Me-cAMP (8CPT) activates EPAC-RAP1 pathway exclusively while 6-Bnz-cAMP (6Bnz) specifically acts on PKA pathway. The data in figure 3 indicated that 100 μM 6Bnz (3A), not 8CPT (3B), significantly enhanced the cell adhesion. **Conclusion:** These observations suggested that the PKA signaling cascade is involved in the cAMP-induced cell adhesion of MC3T3E1 cells.

Fig. 3A

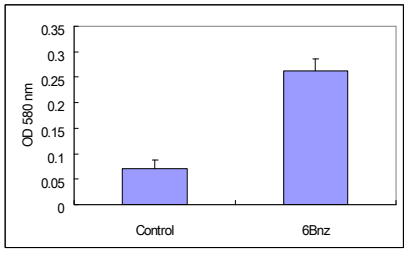


Fig. 3B