

# THE INFLUENCE OF APIGENIN ON FORMATION OF THE BONE MINERALS BY MINERALIZATION-COMPETENT CELLS: LIGHT, FLUORESCENT AND ELECTRON MICROSCOPE ANALYSES

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**ABSTRACT:** Osteoblasts are the bone cells competent in mineralization, participating in the initial steps of mineral formation. Mineralization is initiated inside matrix vesicles (MVs), which are secreted from osteoblasts. MVs, containing relatively high concentrations of calcium ions ( $\text{Ca}^{2+}$ ) and inorganic phosphate ( $\text{P}_i$ ), create an optimal environment to induce the formation of hydroxyapatite (HA). Mineralization carried out by MVs is a complex process requiring the involvement of low molecular weight (LMW) compounds and various proteins, among them annexins (Anx) and tissue-nonspecific alkaline phosphatases (TNAPs). TNAP hydrolyzes  $\text{PP}_i$ , and AnxA6 can form ion channels across the membrane, being responsible for  $\text{P}_i$  and  $\text{Ca}^{2+}$  uptake into MVs [1]. An important role in mineralization is also played by LMW compounds. Recently published results suggested the effects of flavonoids on mineralization. These compounds can modulate the activity of TNAP, as well as cell signaling through interaction with cell-surface receptors or extracellular matrix proteins. Flavonoids can also enter cells and affect the expression of genes important in the mineralization process [2]. One of the promising and frequently studied flavonoids is apigenin. The aim of this study was to analyze the potency of apigenin to affect mineral formation by two mineralization-competent human cell lines: osteoblastic hFOB 1.19 and osteosarcoma Saos-2. For this purpose, cell lines were cultured for 7 days under resting conditions or after stimulation with ascorbic acid and  $\beta$ -glycerophosphate in the presence of apigenin. Ability of cells to mineralize was confirmed by staining with AR-S observed under LM and determination of the TNAP activity. Results showed that apigenin affects the mineral formation, making minerals more compact. The quality of minerals in MVs produced by both cell lines was checked using TEM-EDX microanalysis. Addition of proteoliposomes (LUV containing AnxA6-FITC) to bone cell cultures modulated their mineralization competence which was prevented by apigenin. Moreover, this flavonoid could disturb the intracellular distribution of AnxA6 and TNAP, especially blocking TNAP attachment to the membrane, as examined by FM analysis. Summarizing, the obtained results may help to understand the apigenin mechanisms of action, and to develop novel therapies of bone cancer treatment.

## REFERENCES:

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