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Review Article

Ultrastructural and biochemical aspects of matrix vesicle-mediated mineralization

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Summary Matrix vesicle-mediated mineralization is an orchestrated sequence of ultrastructural and biochemical events that lead to crystal nucleation and growth. The influx of phosphate ions into the matrix vesicle is mediated by several proteins such as TNAP, ENPP1, Pit1, annexin and so forth. The catalytic activity of ENPP1 generates pyrophosphate (PPi) using extracellular ATPs as a substrate, and the resultant PPi prevents crystal overgrowth. However, TNAP hydrolyzes PPi into phosphate ion monomers, which are then transported into the matrix

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vesicle through Pit1. Accumulation of Ca^{2+} and PO_4^{3-} inside matrix vesicles then induces crystalline nucleation, with calcium phosphate crystals budding off radially, puncturing the matrix vesicle's membrane and finally growing out of it to form mineralized nodules.

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1. Introduction

Bone is a living mineralized tissue composed of calcium phosphates and a variety of organic materials, with collagen being the most abundant. Bone mineralization has two phases: primary and secondary. Primary mineralization is orchestrated by osteoblasts; osteoblasts secret a large amount of collagen fibrils, non-collagenous proteins, and matrix vesicles, which are extracellular vesicles that trigger mineralization *via* membrane transporters and enzymes. The degree of the primary mineralization is controlled by fine-tuning bone formation and mineral apposition rates. In contrast, secondary mineralization is a phenomenon whereby there is a gain in bone mineral density after primary mineralization. It is hypothesized that secondary mineralization is regulated physicochemically, *i.e.*, through crystal maturation, and by the osteocytic network inside the mineralized bone matrix. Still, the mechanisms behind secondary mineralization are relatively untapped.

In bone, primary mineralization can be divided into two phases: first, matrix vesicle-mediated mineralization, and second, collagen mineralization. During the process of the matrix vesicle-mediated mineralization, osteoblasts regulate vesicle synthesis as well as the activity of membrane transporters and enzymes with which matrix vesicles are equipped. Discovery of matrix vesicles was a breakthrough in the field of bone mineralization [1–7]. Long before that, it was proposed that alkaline phosphatase may supply phosphates by hydrolyzing phosphate substrates and then help forming crystalline calcium phosphates [8]. However, this theory is based on the physicochemical regulation of bone mineralization. In contrast, the theory behind matrix vesicle-mediated mineralization sustains that the processes are mainly under the control of osteoblasts through the regulation of membrane transporters/enzymes and surrounding extracellular organic materials. Knowledge of the ultrastructure and biological activities of membrane transporters/enzymes may clarify the cellular mechanisms of matrix vesicle-mediated mineralization and explain, for instance, why mineral appositional rate is higher in regions of accelerated bone turnover.

In this review, we will present the ultrastructural and biochemical aspects of matrix vesicle-mediated mineralization in bone.

2. Ultrastructural aspects of matrix vesicle-mediated mineralization in bone

Matrix vesicles are extracellular vesicles enveloped by a plasma membrane (ranging 30–1000 nm in diameter) secreted by osteoblasts [2] (Fig. 1). Matrix vesicles bear several membrane transporters and enzymes on their membranes and in their interior, providing a nurturing microenvironment for calcium phosphate nucleation and subsequent crystal growth. Mineralization begins when a crystalline calcium phosphate, *i.e.*, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], appears inside the matrix vesicles, growing and eventually breaking through the vesicle's membrane to form mineralized nodules—also known as calcifying globules (Figs. 1 & 2). Under transmission electron microscopy (TEM), each hydroxyapatite crystal has a small, ribbon-like structure profile approximately 25 nm wide, 10 nm high and 50 nm long [9,10].

2.1. Ultrastructural properties of the matrix vesicles under electron microscopy

Some of these incipient mineral crystals were initially found associated with the inner leaflet of the matrix vesicle membranes, and it seems plausible that crystal nucleation would begin at that specific site. Matrix vesicle membranes are

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