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Matrix Vesicle Mediated Mineralization - A Review

Devika. B[1], K. R. Don[2], V. Vishnu Priya[3],

[1]Research Associate, Saveetha Dental College & Hospital, [2]Reader, Department of Oral and Maxillofacial Pathology, [3]Professor, Department of Biochemistry, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS) Saveetha University, Chennai

Abstract

Teeth are composed of the mineralized tissues dentine and cementum, and a mineralized structure, the dental enamel. The pulp may also mineralize in response to aging processes and/or pathologic aggressions. The process of mineralization is specific for each dental tissue. Matrix vesicles have been implicated in the mineralisation of calcified cartilage, bone and dentin. It is an orchestrated sequence of ultrastructural and biochemical events that lead to crystal nucleation and growth. Matrix vesicles appear to mineralize by concentrating calcium and phosphate at a protected site close to the inner leaflet of the vesicle membrane. Calcium may be attracted by its affinity for acidic phospholipids of the vesicle membrane, and phosphate may be concentrated by the action of transmembrane phosphatases of the matrix vesicle membrane. Evidence is accumulating to suggest that alkaline phosphatase of the matrix vesicle membrane functions as a phosphotransferase or phosphate vector, transporting PO_4 across the vesicle membrane. 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 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. Their exact role, if any, in the nucleation of hydroxyapatite mineral, and its association with collagen fibres The organic Matrix has been debated and is controversial. Several hypotheses have been recently introduced to explain in greater detail how Matrix vesicles function in biomineralization. This review will summarise recent advances, and ultra-structural and biochemical aspects on Matrix vesicles - mediated mineralization.

Keywords: *Matrix vesicle, mineralisation, TNAP, ENPP1, PHOSPHO1, mineralised nodule.*

Introduction

Matrix vesicles refer to small spherical bodies, around 20 to 200 nm observed in pre-mineralized Matrix of dentin, cartilage and bone. They are bounded by lipid bilayer, and are often associated with small crystals of calcium phosphate mineral [1] . Mineralised bone matrix consists of abundant calcium phosphate, type I collagen and non - collagenous organic materials[2]. When the least matrix vesicle contains abundant protein and lipids that are known to chelate inorganic phosphatase (Pi) and Ca^{2+} [3]. These vesicles are often reported to be found in osteoid, mental dentin and calcifying tendons [4]. The density of these particles appear to decrease with the increasing compactness of collagen fibrils in mature bone [5]. Multiple rational theories which describe mineral crystallisation exist. One of the most discussed theories is the new creation of apatite through collagen polypeptide stereochemistry with Ca^{2+} and inorganic phosphate (Pi), where apatite crystals precipitate and propagate from an amorphous phase, in the Gap zone of collagen fibres [6]

Corresponding Author:
K. R. Don,
Reader, Department of Oral and maxillofacial pathology, Saveetha Dental College & Hospital
Saveetha Institute of Medical and Technical Sciences (SIMATS) Saveetha University, Chennai - 600 077
Email Id - donkr.sdc@saveetha.com

Bone mineralisation is divided into two ultra-structural phases. They are primary and secondary mineralisation. Primary mineralisation is biologically orchestrated by osteoblast and osteoclast during bone modelling and remodelling. It is achieved by fine – tuning synthesis of organic materials and subsequent apposition of calcium phosphates. Secondary mineralisation is a phenomenon by which bone mineral density elevates it after primary mineralisation. This is achieved physicochemically, through crystal maturation and by osteocyte network. The primary mineralisation has two distinct phases: matrix vesicle mediated mineralization and collagen mineralisation [2].

Previously, our team had conducted numerous original studies [7-13] and surveys [14-21] over the past 5 years. Now we are focussing on applying this knowledge to write the review on new recent advancements in various fields. In this review, the ultra structural and biochemical evidence of matrix vesicle mediated mineralization is introduced.

Ultrastructural Evidences of Matrix Vesicle - Mediated Mineralization In Bone

In matrix vesicle mediated mineralisation, osteoblast secrete matrix vesicles on which the membrane transporters and enzymes involved in mineralisation are equipped [22]. Before the introduction of matrix vesicle mediated mineralisation theory, alkaline phosphatase theory was followed. According to alkaline phosphatase theory, alkaline phosphatase supplies more phosphate ion by hydrolyzing phosphate substrates. Then accelerate to form crystalline calcium phosphate [23].

Bone mineralization initiates inside the matrix vesicle. Matrix vesicles are small extracellular vesicles enveloped by a plasma membrane secreted by osteoblast [24]. Chondrocytes and pre-osteoblast differentiate into hypertrophic chondrocytes and osteoblast respectively [25]. These two cell types are mineralisation – competent cells that regulate Matrix calcification by modification of extracellular matrix composition and releasing matrix vesicles [26].

The initial step of extracellular matrix calcification by promoting the deposition of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ in their lumen [27]. Ion channels and transporters present in matrix vesicle membranes act for Ca^{2+} [28] and inorganic phosphate uptakes into these organelles [29]. By accumulating Ca^{2+} and inorganic phosphate, matrix vesicles create an optimal environment to induce the formation of hydroxyapatite [30]. Then, the breakdown of matrix vesicle membrane releases hydroxyapatite crystals into the extracellular where the mineralization is propagated [3]. These crystals serve as a template for the formation of crystalline arrays, leading to tissue calcification [30]. The extracellular matrix contains sufficiently high concentration of Ca^{2+} And inorganic phosphate concentration to propagate the mineralisation. Negatively charged proteins of the matrix interact with minerals and thus, control their growth, orientation and size [31].

Ultrastructural and Biochemical Function Of Enzymes And Membrane Transporters For Matrix Vesicle - Mediated Mineralization

Abundant Ca^{2+} was evenly distributed in the peripheral region of the matrix vesicle, while PO_4^{3-} was predominantly associated with organic material such as collagen fibrils [32]. Inside the matrix vesicle, "needle-shaped" crystalline calcium phosphates form a stellate assembly, grow in all direction, and then, come out of the vesicle penetrating the plasma membrane to form mineralised nodules, also referred to as calcifying globules [33]. Several enzymes and membrane transporters found in the Matrixx by cycle are involved in metallisation. Among these enzymes and transporters, TNAP, ENPP1, ANK, PHOSPHO1, PIT1, appear to play pivotal roles in phosphate transport on matrix vesicle mediated mineralisation [2].

Tissue Nonspecific Alkaline Phosphatase (Tnap)

One of the most important enzymes that initiate mineralisation inborn must be TNAP, a glycosylphosphatidylinositol anchor enzyme associated with cell membrane. TNAP is responsible for the production of inorganic phosphate. Hence it is called a potent inducer of mineralisation [34]. The distribution of TNAP on cell membranes is not uniform in osteoblast, which are polar cells with distinct basolateral and secretary domains [35].

The ratio of inorganic pyrophosphate (PPi) to inorganic phosphate (Pi) is important in the promotion or restriction of minerals in physiological tissue. TNAP

is one of the regulators of the extracellular PPi/Pi ratio. TNAP, encoded by Alpl, is abundant on the surface of matrix vesicles derived from osteoblast, hypertrophic chondrocytes and odontoblast [36]. TNAP produces an environment surrounding matrix vesicles conducive to mineralization not only through regulation of PPi/Pi Ratio but also through modulating the phosphorylation status of osteopontin. Osteopontin inhibits nucleation and growth of hydroxyapatite [37]. Hypophosphatasia, is a clinically heterogeneous heritable disorder characterised by defective bone mineralization which is caused due to impaired TNAP activity due to mutation of TNAP gene [38]. The phosphatase associated with the osteoidal aspect of TNSALP, which is restricted to the basolateral domains of osteoblasts [39].

Ecto-nucleotide Pyrophosphatase/ Phosphodiesterase (ENNP1)

ENPP1 he is a member of the family ENPP family of proteins. It is composed of 2 N-terminal somatomedin B (SMB) - like domain, a catalytic domain and nuclease like domain. The catalytic and nuclease like domains for bone mineralisation and SMB like domains for insulin signalling respectively [40]. ENPP1 is one of the regulators of the extracellular PPi/Pi ratio [41]. ENNP1, that's not have a major PPi generating role, but rather can act as a 'backup' phosphatase in the absence of TNAP. This role as a 'Plan B' phosphatase is proposed as the reason why the PHOSPHO1 , mineralisation of the axial skeleton can occur occasionally [42].

An Emerging consensus now emphasises the central role of the matrix vesicle enzymes TNAP and NPP1, in conjunction with the cell associated and ankylosis protein (ANK), in regulating the onset of calcification [43]. NPP1 along with ANK function to suppress mineralization by increasing the extracellular concentration of the calcification inhibitor, PPi (pyrophosphate) [44]. In human infants, severe ENPP1 deficient state's were recently linked to a syndrome of spontaneous infantile arterial and periarticular mineralization [45].

Ankylosis (Ank)

Ankylosis is an non-enzymatic plasma membrane pyrophosphate (PPi) channel. It is encoded by mouse progressive ankylosis (Ank) gene [46]. Ank Gene appears to regulate both intra and extracellular levels of an important inhibitor of hydroxyapatite crystal formation [47]. The infant carrying Ank mutation gene caused a 3-to-5 fold decrease in extracellular level of inorganic pyrophosphate in contrast to stimulatory effect on intracellular inorganic pyrophosphate. With the loss of ANK activity, the extracellular inorganic pyrophosphate levels attenuate, the intracellular inorganic pyrophosphate levels rise, and unregulated mineralisation begins in joints and tissues [2]. Extracellular inorganic pyrophosphate is formed from extracellular nucleoside triphosphate (NTP) by NPP1 and exported from cells is through the action of ANK. It is hydrolysed to inorganic phosphate by TNAP [1].

PHOSPHO 1

PHOSPHO 1 is Enzyme highly expressed in minimising cells like in bone and cartilage, with systematic name phosphoethanolamine phosphohydrolase [48]. PHOSPHO 1 is a Phosphate and a member of the halo acid dehalogenase superfamily. It is essential for the initiation of skeletal mineralisation [41]. PHOSPHO1 appears to serve for initial mineralisation inside matrix vesicles [49]. PHOSPHO1 appears to serve for initial mineralization inside matrix vesicle [2].

FORMATION AND DEVELOPMENT OF MINERALIZED NODULES

Mineralised nodules is a globular assembly of numerous needle shaped mineral crystals that has been exposed to an extracellular environment for matrix vesicles [2]. Osteopontin is suited to the task of regulating mineralisation because it effectively inhibits apatite formation and growth [50]. Osteocalcin may play an important role in the globular assembly of needle shaped mineral crystals, probably binding together the organic component of the crystal [51]. Zinc (Zn^{2+}) has long been known to play important roles in mineralization and ossification of skeletal tissues, but the mechanisms of Zn^{2+} action are not well understood. the effects of Zn^{2+} on mineralization in a cell culture system in which terminal differentiation and mineralization of hypertrophic growth plate chondrocytes was induced by retinoic acid (RA) treatment. Addition of Zn^{2+} to RA□treated cultures decreased mineralization in a dose□dependent manner without affecting alkaline phosphatase (APase) activity. Characterization of matrix vesicles, particles that initiate the mineralization process, revealed that vesicles

isolated from RA-treated and RA/Zn^{2+}-treated cultures showed similar APase activity, but vesicles from RA/Zn^{2+}-treated cultures contained significantly less Ca^{2+} and Pi. MVs isolated from RA-treated cultures were able to take up Ca^{2+} and mineralize in vitro, whereas vesicles isolated from RA/Zn^{2+}-treated cultures were not able to do so [52].

ULTRASTRUCTURE OF COLLAGEN MINERALIZATION

After the beginning of matrix vesicle mediated mineralization, mineralized nodules would contact the surrounding collagen fibrils. There are two theories explaining collagen mineralisation: Hole zone theory and one supporting that mineralization. Initial mineralization begins in the collagen fibrils, 'holds'. While decorin/biglycan-double knockout mice revealed osteopenia as a result of impaired GAG - linking to decorin and biglycan cone protein, mineralization was not stimulated [53].

Conclusion

Our current knowledge of mineral vesicles is undoubtedly building towards the foundation in understanding the complex mechanism of the development of matrix mineralisation. It causes a series of orchestrated ultrastructural and biochemical events in bone to achieve proper mineralisation, a variety of membrane transporters and enzymes are put to work.

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