THE ROLE OF MATRIX VESICLES IN MINERALIZATION OF BONE
- Review -

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Summary
Matrix vesicles are extracellular round spherical bodies of about 100 nanometres in size which act as initial sites for hydroxyapatite crystal deposition. They were initially described in 1977 and since then a series of research have been performed to determine the process of mineralization. These extracellular organelles are produced by chondrocytes, osteoblasts and odontoblasts under normal physiological conditions. They are located within the calcification areas, i.e. the bone, cartilage and the predentin and they play a pivotal role in the process of mineralization of these calcified tissues. Matrix vesicles originate by the process of polarized budding and pinching off of small vesicles from the plasma membranes of the cells which later disintegrates and degenerates. There are two types of matrix vesicles which contain a variety of molecular components ranging from proteinaceous and non proteinaceous components. The individual particles within the matrix vesicles have a particular role which initiates and progresses the mineralization process. The early stages of mineralization is initiated by the accumulation of calcium and phosphate which provides an optimal environment facilitating the formation of hydroxyapatite. The review discusses in detail the components of matrix vesicles and its role in mineralization process.

Keywords: Matrix vesicle, calcification, mineralization, alkaline phosphatase, calcium, phosphate

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Introduction
Matrix vesicles are oval or round bodies of about 50-200 nanometers in diameter, surrounded by a membrane (Anderson., 1969) They are fundamental in the mineralization of bone and teeth. They are of cellular origin and lie the zone of incipient calcification in all the mineralizing tissues including the cartilage, bone, mantle dentin and many other calcifying extra skeletal tissues (Bernard., 1969). The first deposits of the crystalline hydroxyapatite has been identified on the matrix vesicles (Anderson., 1969). They contain abundant cation binding phosphatidylserine which functions as a calcium trap (Wuthier et al., 1975). The vesicles consist of an amorphous substance and metalloproteinase, pyrophosphatase, Ca-

ATPase (anionic phospholipids), calcium binding lipids, and alkaline phosphatase (Ali et al., 1970). ATPase and phosphatase plays a role in mineralization by increasing local concentration of phosphate concentration and by hydrolyzing calcification inhibitors such as ATP or PP (Robinson., 1923).

History of Matrix Vesicles
The literature of matrix vesicles goes back only as far as 1967 (Bonucci., 1967). In 1967, studies of calcifying cartilage led to discovery of membrane bound electron dense, osmophilic bodies containing inorganic substances. In the same year the other studies identified membrane bound cytoplasmic fragments in hypertrophic cartilage which could be locus of initial mineralization. The matrix
vesicles were later identified in bone, dentin and were found in connection with the cytoplasmic processes of osteoblasts and odontoblasts.

**Types of matrix vesicles**

Two types of mineral-related matrix vesicle have been demonstrated in the mineralization of bone. The type I pattern are round or oval in shape and resemble lysosomes and have been shown to be extruded from the cartilage cells. They contain enzymes like acid phosphatase and aryl sulphatase. The concentrations of proteoglycans and glycosaminoglycans breakdown products drop down as the mineralization proceeds.

Type II matrix vesicles are irregular in shape, bound by a trimellar membrane. They are characterized by presence of ribosomes which appears like detached pieces of cartilage materials within the cytoplasm. They contain alkaline phosphatase and ATPase enzyme characteristic of the membrane of cartilage cells, and lipids chiefly phophatidylserine, a substance previously shown in lipids of calcifying front. The substance has a strong affinity for calcium and it seems that type II vesicles may promote mineralization in several ways involving the entire hypothesis proposed over the last 50 years. The presence of ATPase suggests that the reactions proposed by Cartier may occur in the vesicle (Gerard et al., 2003).

**Biochemical property of matrix vesicle**

The first isolation of matrix vesicles was done in 1970 from cartilage by enzymatic digestion and differential centrifugation. This direct biochemical analysis led to the conclusion that alkaline phosphatase, is the “enzymatic marker” of matrix vesicles. This observation of enzymatic marker was confirmed by electron microscopy. A proteomic analysis of Matrix vesicles and the structure and function of them were studied in detail (Xiao et al., 2007).

**The origin of matrix vesicle:**

The formation of matrix was first hypothesized by Robinson and Anderson in 1973 and suggested 4 different ways in which matrix vesicles may be formed. Though the generation of matrix vesicles is still uncertain, the suggested one is of cellular origin (Anderson et al., 1976). The four ways of formation are (i) Budding from cells and cellular processes. (ii) Extrusion of performed site (iii) Disintegration of degenerated cells and (iv) Extra cellular assembly of secreted subunits. The formation by budding process and disintegration of degenerated cells are supported as they are documented several times by conventional electron microscope. Similarly the origin of matrix vesicles from degenerated disrupted chondrocytes has been amply documented.

**Components of matrix vesicle:**

**Lipids:**

The isolated matrix vesicle constitute a lipid bilayer consist of an outer plasma membrane. Lipid analysis demonstrates these to be enriched in plasma membrane phospholipids like cholesterol and sphingomyelin (Peress et al., 1974) They contain of acidic phospholipids which may serve as a non-energy-requiring calcium trap during mineralization process (Wuthier., 1975)

**Alkaline phosphatase:**

The enzyme alkaline phosphatase was first identified by Robison in 1923, and he was the first to suggest that activation of ALP activity in bone stimulates CaPO$_4$ mineral deposition (Ali et al., 1970). Alkaline phosphatase also known as "tissue non-specific alkaline phosphatase" (TNAP), is an enzyme that is most concentrated in bone, liver and kidney, and is enriched in Matrix Vesicles, more than 10 folds in that of the normal tissues (Harrison et al., 1995). The enzyme is concentrated at the outer surfaces of the matrix vesicle where it is anchored to glycosyl phosphatidylinositol (GPI).
enables calcium deposition by isolated matrix vesicles (Hsu et al., 1993). The activity of enzyme alkaline phosphatase is higher at the mineralization front and release of this enzyme leaves the MV membranes intact, results in decrease in the ability of isolated MVs to deposit CaPO$_4$ (Hsu et al., 1993).

**Adenosine monophosphoesterase:**

Adenosine monophosphoesterase is a non-ALP phosphatase that is enriched in MVs (Harrison et al., 1995). It plays a major role in augmenting the calcium phosphate deposition and supports the crystalline particles of calcium phosphate (Matsuzawa et al., 1971) ALP and AMPase hydrolyzes AMP, releasing inorganic phosphate for incorporation into nascent CaPO$_4$ mineral (Hsu et al., 1993).

**Inorganic pyrophosphatase:**

Inorganic pyrophosphatase (PPiase) have been shown to be present in Matrix vesicles (Ali et al.,1970). They hydrolyze the inorganic pyrophosphate and neutralizes the inhibitory effect of PPi on hydroxyapatite mineral crystal formation. It also hydrolyses PPi, yielding two Pi molecules that gets incorporated into the nascent CaPO$_4$ mineral. Thus PPiase plays a role in promoting Matrix vesicle mineralization (Kirsch et al., 1997).

**ATPase :**

Matrix vesicles are enriched in ATPases including Ca$^{2+}$ ATPase (Ali et al.,1970). It has been demonstrated by invitro experiments that hydrolysis of ATP, by the ATPases in isolated MVs, significantly enhances CaPO$_4$ deposition (Hsu et al., 1993). However this hypothesis is still questionable because of 2 reasons (Fleisch et al., 1966). (i) ATPase activity in isolated MVs in significant proportion in the form of ATP triphosphate pyrophospho hydrolase. The major hydrolytic product of ATP hydrolysis product is PPi which in excess inhibit crystalline CaPO$_4$ mineral deposition (Hsu et al., 1996).(ii) The addition of AMP is more effective than adding ATP to promote the calcification of matrix vesicles (Anderson et al.,2004) (iii) When ATP is used as a substrate against AMP, the mineral deposited by isolated MVs lacks apatite crystallinity (Garimella et al., 2006). Thus ATPase does not play a significant role in regulating mineral initiation. However it plays a major role in providing PPi for hydrolysis by ALP, PPiase, etc., thus yielding Pi for incorporation into nascent mineral (Derfus et al.,1996).

**Phospho1:**

This is a phosphate protein concentrated in areas where mineralization begins in MV. The presence of matrix vesicles in intense localization zones suggests that Phospho1 activity releases Pi for incorporation into nascent CaPO$_4$ mineral. The Phospho1 and ALP activities are also synergistic in generating Pi (Houston et al., 2004).

**Annexin:**

Annexin V has a high concentration near the Matrix vesicles and it functions in such a way that the Ca$^{2+}$ channel is directed inwards .This transport of calcium inside the matrix vesicles raise the intravesicular (Ca$^{2+}$) x (PO$_4$$^{3-}$) ion product, promoting initial mineral formation (Kirsch et al., 1997).

**Bone sialoprotein (BSP), osteonectin (ON) and osteocalcin (OC)**

They are Ca-binding non-collagenous matrix proteins of bone, concentrated in Matrix vesicles (Missana et al., 1998). BSP plays a role in promoting bone mineralization (Hunter et al., 1993) whereas Osteonectin and osteocalcin has a mineral inhibiting function. Thus, only BSP appear to play a positive role in mineral initiation by MVs (Boskey et al., 2003)

**Sodium-dependent phosphate transporter:**
The sodium-dependent phosphate transporter promotes ion accumulation in MVs in early phases of mineralization by ingress of Pi into MVs at early phases of mineral initiation (Montessuit *et al.*, 1995)

**Mechanism of matrix vesicle mineralization**

The mechanism of mineralization of matrix vesicles involves a series of events in 2 cascades.

There is initial Calcium loading and unloading of mitochondria. This is followed by transport of nascent calcium phosphatase precipitates into the cytosol. Later the primed matrix vesicle containing calcium phosphate buds off from the cellular membrane. The vesicles then leave the cell and lie in matrix. The Vesicles then lose water through their membrane; also there is also an accumulation of inorganic ions by intra-vesicle diffusion. This increases the concentration of ions within the vesicle. The nascent calcium phosphate precipitates are then converted into hydroxyapatite (needle shaped) crystals. The vesicle membrane then Break down and the crystals in the organic matrix are deposited. The growth of crystal then occurs by seeding process and the clusters of crystals then coalesce until the whole matrix is mineralized.

**Cascade 1 in matrix vesicle calcification: Crystal formation**

The phase starts with incorporation of Ca\(^{2+}\) into the MV membranes and MV sap, due to the presence of Ca-binding phospholipids and proteins in MVs and inward Ca\(^{2+}\) transporting activity of Annexin V. This is accompanied with PO\(_4\)\(^{3-}\) accumulation augmented by PO\(_4\)\(^{-}\)-concentrating activities of the Na-P0\(_4\) transporter (Sauer *et al.*, 1988). Enzymatic activity of Phospho1, and Alkaline phosphatase activity play a an early role in PO\(_4\)\(^{-}\) concentration (Houston *et al.*, 2004). When sufficient Ca\(^{2+}\) and PO\(_4\)\(^{3-}\) have been accumulated, the precipitation of CaPO\(_4\) begins. The first CaPO\(_4\) deposited is an amorphous CaPO\(_4\) (ACP) which converts to octacalcium phosphate, and further into highly insoluble hydroxyapatite (Dean *et al.*, 1992). Hydroxyapatite crystals then penetrate the MV membrane and get exposed to the extracellular fluid to initiate phase 2. The breakdown of vesicles is assisted by the hydrolytic action of phospholipases and proteases. Thus Phase 1 of mineral initiation reflects a cascade of multiple molecular interactions that occur within the three dimensional membrane structure of matrix vesicles.

**Cascade 2: (Crustal penetration of Matrix vesicles):**

The preformed mineral crystals in matrix vesicles are exposed to the extracellular fluid rich in Ca\(^{2+}\) and PO\(_4\)\(^{3-}\) which then regulates the rate of mineral crystal propagation (Chen *et al.*, 1984). The extra vesicular mineralization-regulating molecules are type I and II collagen and Ca-binding matrix (Landis *et al.*, 1989) proteins including BSP which promotes mineralization, ON and OC may retard mineralization, acidic Ca-binding proteoglycans which inhibits mineral propagation and local concentration of extravesicular phosphoester substrates for MV phosphatases, including ATP, AMP and PPI (Arsenault *et al.*, 1988).

Phase 2 mineral propagation continues with accumulation of hydroxyapatite crystals in the form of new self-nucleated crystals to form spherical clusters. The proliferating extra-vesicular crystals then comes into contact with collagen fibrils which then initiates nucleation and orientation of newly formed apatite crystals(Arsenault *et al.*, 1988). The matrix vesicles then provide a molecular bridge between MVs and collagen type fibrils. Crystals generated at the surfaces of collagen fibrils form in co-alignment with the typical 64 nanometer axial periodicity. Thus MVs and type I or II collagen fibrils work synergistically to complete the full mineralization (John *et al.*, 1987).
Matrix vesicles are active in epiphyseal cartilage, in mantle dentin and in embryonic and other types of bone. On the other hand, they are not seen in circumpulpal dentin and enamel, and few or very few of them have been observed in compact bone. The mechanism of matrix vesicle calcification and spread of calcification process from matrix vesicles into surrounding matrix is a complex process requiring more documentation.

The presence of matrix vesicles varies in calcifying tissues. Few areas contain plenty of and others very few or sometimes it is not present. The matrix vesicles needed for calcification occurs slowly, in compact bone, in which case cells may have enough time to regulate matrix calcification through their cytoplasmic processes (Ali et al., 1970).

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