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MATRIX VESICLES: ARE THEY ANCHORED EXOSOMES?

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Abstract

Numerous studies have documented that matrix vesicles are unique extracellular membrane-bound microparticles that serve as initial sites for mineral formation in the growth plate and most other vertebrate mineralizing tissues. Microparticle generation is not confined to hard tissues, as cells in soft tissues generate similar structures; numerous studies have shown that a common type of extracellular particle, termed an exosome, a product of the endosomal pathway, shares many characteristics of matrix vesicles. Indeed, analyses of size, morphology and lipid and protein content indicate that matrix vesicles and exosomes are homologous structures. Such a possibility impacts our understanding of the biogenesis, processing and function of matrix vesicles (exosomes) in vertebrate hard tissues and explains in part how cells control the earliest stages of mineral deposition. Moreover, since exosomes influence a spectrum of functions, including cell-cell communication, it is suggested that this type of microparticle may provide a mechanism for the transfer of signaling molecules between cells within the growth plate and thereby regulate endochondral bone development and formation.

Keywords

matrix vesicle; mineralization; bone; cartilage; chondrocyte; osteoblast; growth plate; exosome; cell-cell communication

Introduction

Since their discovery in 1967 [1], a great many studies have reported that matrix vesicles are unique extracellular membrane-bound microparticles that provide initial sites for mineral formation in endochondral bone (Figure 1).[2–3] Subsequent investigations have indicated

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The authors dedicate this review to the memory of Ellis E. Golub, Ph.D., a great friend, a brilliant scientist and a pioneer in the field of matrix vesicle research.

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that these microparticles serve as the nidus for apatite generation in many other vertebrate mineralizing tissues (Figure 2) [4, 5] as well as foci for dystrophic mineral accumulation.[6] The presence of matrix vesicle-like microparticles is not confined to mineralizing tissues; it has been known that cells of non-calcifying tissues generate membrane-bound microparticles. [7] These particles have been found in serum, urine, cerebrospinal fluid, tears, milk and saliva and they are involved in a wide spectrum of physiological processes that include intercellular signaling, immune regulation and tissue repair.[8] Despite differences in function, similarities between one form of microparticle (exosome) and matrix vesicles of most vertebrate mineralizing tissues suggest that they are homologous structures. Such a possibility prompts questions beyond that of semantics, impacting our understanding of the biogenesis, processing and function of matrix vesicles in hard tissues.

From the point of view of the endochondral mineralization process and challenging the concept that matrix vesicles are primary sites of mineral deposition, recent publications suggest that mineralization is initiated as an intracellular event. In other words, mineral is first formed within cells, not in extracellular particles (matrix vesicles). More specifically, apatitic salts or possibly pre-nucleation ion clusters are formed initially within a subcellular compartment (endosome); this intracellular mineralization process is regulated by metabolic activities controlling calcium and phosphate homeostasis and the energy landscape.[9] Once released from the cell, the pre-formed mineralized microparticles become embedded or anchored in the dense extracellular tissue as matrix vesicles. In contact with ions of the extracellular fluid, further mineral phase transformations and mineral growth occur. In other words, the secreted exosome (matrix vesicle) assumes an important second role – serving as a locus for mineral ion and crystal accretion in addition to its involvement with critical mineral nucleation activities.

Aside from its role in regulating the endochondral mineralization process, the exosome concept may provide an alternative understanding of how cell-cell communication is achieved within complex tissues like the avascular growth cartilage. Thus, in contrast to current gradient and diffusional concepts that purport to describe regulation of chondrocyte terminal differentiation within the different regions of the growth plate, exosomes transfer informational molecules from one cell to another. From this perspective and in line with current thinking concerning microparticle function,[8] the exosome provides a mechanism for transporting cargo to and from specific regions of the plate, and it ensures docking and uptake of molecules by target cells and tissues.

The goal of this review is to stimulate discussion concerning the possible multiple roles of exosomes and other microparticles in the mineralizing tissues of vertebrates. The following questions are addressed: First, based on what is known of matrix vesicle size and structure, can these microparticles be considered to be exosomal in origin? Second, are there compositional and molecular similarities between matrix vesicles and other microparticles? And third, what are the functional implications of the exosome concept? Through this review, the authors acknowledge that, while the arguments advanced herein may appear to be novel, the relationship between exosome function and matrix vesicle biogenesis has been noted by other investigators, especially in reference to specific diseases.[10–12]

Are Matrix Vesicles Anchored Microparticles?

Cells generate a wide range of different-sized zeiotic bodies formed from microparticles ranging from exosomes of endosomal origin, blebbing microvesicles or ectosomes, shed membrane fragments, and very small apoptotic particles.[8, 13] Ignoring apoptotic particles, we advance the argument that matrix vesicles generated by resident cells of normal mineralizing cartilage, bone, dentin, cementum and tendon[1–7] originate as exosomes and possibly ectosomes. If this is the case, then this proposition would add considerable strength to recent findings based on proteomics and deletion studies of autophagy genes that indicate that mineralization is cell-mediated and involves the endosomal-autophagic pathway.[14, 15] We examine this possibility by first comparing the size of matrix vesicles with exosomes and then examining their biogenesis.

Size analysis

Chondrocyte-derived matrix vesicles are often described as being 0.1 – 0.2 μm diameter, although most authors would agree that there can be as much as a ten-fold difference in this measure. Indeed, in the hypertrophic region of the growth plate, in the calcified cartilage zone of articular cartilage, and in newly forming bone, pre-dentin and the mineralizing entesis, matrix vesicles are reported as a heterogenous population of nanosize particles.[16] Similarly, in matrix vesicles formed in vitro, great variations in diameter are apparent (0.02 – 2.0 μm).[17] Like matrix vesicles, conventional exosomes vary in diameter from 0.04 – 1.0 μm and ectosomes range from 0.15 – 1.0 μm . [8] While the diameters of matrix vesicles and exosomes and ectosomes are of comparable magnitudes, size alone is insufficient to conclude that matrix vesicles belong to a specific group of microparticles.

Biogenic analysis

Matrix vesicles and microparticles (exosomes and ectosomes) are very similar in that they are all spherical in shape and constrained by a bilaminar membrane that encloses a central cavity containing cytosolic components of the parent cell. A singular notable difference between the two types of particles is that, despite sharing a number of outer leaflet proteins, exosomes are non-adherent structures; matrix vesicles are always anchored to protein components of the surrounding extracellular matrix.[18]

Morphological studies suggest that matrix vesicles are generated by budding of the cell plasma membrane. Blebs are thought to originate at surface microvilli; retraction of the supporting microfilament network, working against matrix attachment forces, promotes their release from the cell.[13] Early electron microscopic studies of cartilage confirm that vesicles are formed by polarized budding from cell protrusions of the chondrocyte membrane.[19–21] In osteoblast-like cells in culture, Thouverey et al.[23] noted that matrix vesicles were formed at the apical membrane. While the location of the bleb has been described, conditions that facilitate blebbing are poorly understood. Current hypotheses posit that a rise in intracellular calcium levels leads to changes in the localization of membrane phospholipids and proteins (annexins), thus initiating vesiculation.

While most studies support the view that matrix vesicles are formed through membrane blebbing, Akisaka et al.[19] reported that some vesicles appeared to pass through the intact plasma membrane and, related to this observation, Thyberg et al.[24] regarded vesicles as extrusions of lysosomal dense bodies. Adding to this literature discussion, Xiao et al.[25] examined osteoblast vesicle biogenesis and reported that budding of aggregate clusters from polarized regions of the plasma membrane resulted in the concerted release of matrix vesicles from the cell surface. They noted that the aggregates were contained in a membranous structure which is lost when the individual vesicles are released from the cell (Figure 3).[25] It was not determined whether such a membrane was the same as that evident in multivesicular bodies, structures that enclose exosomes.

A study of matrix vesicles in zebrafish bone showed the presence of multivesicular structures, bodies which contained vesicles of about 100 nm diameter; isolated matrix vesicles with diameters of 100–550 nm were also found. X-ray spectroscopy indicated that both types of vesicles mineralized.[26] This discrepancy concerning vesicle biogenesis (membrane blebbing versus multivesicular aggregates) can be explained if it is assumed that, in common with many other cells, chondrocytes, osteoblasts, odontoblasts, cementoblasts and tenocytes generate a number of different types of particles that are involved in initiating mineral deposition. From this perspective, it would not be unreasonable to consider that matrix vesicles are one type of exosome and that its biogenesis would be influenced by the maturation state of the mother cell, its redox status and the chemical and osmotic characteristics of the anchoring extracellular matrix.

Are There Compositional and Molecular Similarities between Matrix Vesicles and Other Microparticles?

There is good morphological evidence indicating that at least some matrix vesicles are formed by polarized blebbing of the membranes of chondrocytes and osteoblasts. Analysis of lipids and proteins and measurement of enzymatic activity point to considerable similarities between the mother cell and specific matrix vesicle components. The question raised herein is whether the lipid and protein composition of matrix vesicles is comparable to those of exosomes. In addition, since exosomes originate from raft regions of the plasma membrane, a secondary question is whether raft components are present in matrix vesicles. In general, these analyses lend sound support for the notion that matrix vesicles and exosomes share a considerable number of important molecules and point to a role of the endosomal pathway in the biogenesis of these microparticles.

Lipid composition

In terms of total composition, Wuthier [27, 28] showed that cartilage matrix vesicles contained elevated levels of neutral lipids compared with the plasma membrane of the parent chondrocyte; this finding suggested that molecular sorting had occurred during vesicle biogenesis. [29] The phospholipid composition of the vesicle is also different from that of the chondrocyte membrane. The acidic phosphatide, phosphatidylserine, evidenced a three- to four-fold increase whereas sphingomyelin was elevated over two-fold. In contrast to the acidic phosphatides, phosphatidylcholine (a neutral phospholipid) and some

lysophospholipids were consistently depleted in matrix vesicle preparations when compared with the membrane lipids of the parent chondrocyte.[27, 28] The similarities and differences in the lipid composition of matrix vesicles and chondrocytes are not unlike those seen in exosomes isolated from mast and dendritic cells. In both cases, the proportion of sphingomyelin was elevated compared to cell membranes whereas the proportion of phosphatidylcholine was decreased. Although there are differences in neutral lipid composition, the similarities in the phospholipid profiles of matrix vesicles and exosomes, suggests that the two microparticles originate from similar subcellular compartments; indeed, the differences may reflect specialization in chondrocyte exosome biogenesis.

The changes in the lipid composition of matrix vesicles have been attributed to the activity of flippases, scramblases, floppases and phospholipases during biogenesis. Laulagnier et al. [30] reported that, while exosome membranes are stable, there was a high rate of flip-flop activity. Other workers have reported the presence of GPI-anchored myristoylated and palmitoylated proteins in matrix vesicles derived from SAOS-2 cell cultures.[14] Lipid raft proteins annexin A6 (AnxA6), AnxA2, carboxypeptidase M, H1-ATPase, G proteins, tissue non-specific alkaline phosphatase (TNAP), actin and integrins have also been identified in matrix vesicles.[31] These observations are of some importance as they suggest that vesicles are formed at membrane rafts [32], implying that the endosomal pathway is involved with vesicle biogenesis in chondrocytes, osteoblasts and other cells of vertebrate mineralizing tissues.

Protein composition

Enzymatic as well as proteomic analysis has identified more than 280 proteins in matrix vesicles. These include TNAP; annexins A1, A2, A4, A5, A6, and A11; phospho-1, nucleotide triphosphate pyrophosphatase, and phosphodiesterase (NPP1 or glycoprotein-1, PC-1), as well as extracellular matrix metalloprotease degrading enzymes, MMP-2, MMP-3, MMP-9, and MMP-13 [12, 13, 31, 33, 34, 35] The role of each of these proteins has been closely linked to matrix vesicle function in mediating matrix turnover and mineralization.

Proteomic analysis indicates that both matrix vesicles and exosomes derived from the endosomal pathway share a large number of proteins (See Table 1 for comparison of exosomal proteins of matrix vesicles with proteins commonly found in exosomes [36]). As an aside it should be recognized that this type of generic exosome analysis is needed as a osteoblast-like cell exosome-like preparation has been reported in only one instance [37]. In terms of the endosomal origin of vesicles, it should be acknowledged that AnxA2 is required for the formation of multivesicular endosomes, fusion of endosomal contents with the plasma membrane, and release of the intraluminal vesicles (endosomes) into the extracellular environment. Annexins are major protein components of chondrocyte and osteoblast derived matrix vesicles. With respect to small GTPase Ras-associated binding proteins (Rab) Rab4, Rab5, Rab11, Rab14, Rab18, and Rab21 have been reported present in matrix vesicles isolated from SAOS osteoblast-like cells.[12] This family of proteins regulate a wide range of activities that include vesicle formation and fusion to cognate membrane. [38, 39] The SNARE protein (Soluble NSF Attachment Protein REceptor) α is also present in these vesicles together with an ATPase N-ethylmaleimide-sensitive fusion

(NSF) attachment protein receptor. The latter protein may drive the fusion of many of the endosomal membrane-trafficking events.[40] Other proteins linked to membrane/vesicle protein trafficking include milk fat globule-EGF factor 8, Niemann-Pick C1, copine III, and low-density lipoprotein receptor-related protein 1.[41]

Integrin receptors are present on both matrix vesicles and exosomes. These receptors mediate interactions with collagens, fibronectin, and laminin, as well as the cell adhesion molecule, VCAM-1 (through integrin $\alpha 4 \beta 1$ or VLA-4).[42] Zhou et al.[43] reported that matrix vesicles derived from SAOS cells display key exosome surface receptors; these included CD9, CD63 and Hsp70. CD9 is a member of the transmembrane-4 tetraspanin superfamily and a phosphatidylserine binding protein. CD 63 is another member of the tetraspanin family and associated with intracellular endocytic vesicles and the formation of membrane rafts. Hsp70 mediates interactions between endosomal cargo selection and the endosomal membrane and can be released into the extracellular milieu in a membrane associated form.[41] It is present in osteoblasts and in MV [31]; in chondrocytes, Otsuka and colleagues reported that its expression promoted hypertrophy and calcification [44]

To summarize, it is clear that the membranes of matrix vesicle contain a large number of proteins that are consistent with those found in exosomes. These proteins include Rab, SNAREs, NSF attachment protein receptor, annexins, integrins and cell surface antigens. Their presence lends strength to the argument that vesicle biogenesis is linked to endosomal activity and exosome formation. In addition, the lipid profile of matrix vesicles is not dissimilar to that of exosomes. Finally, lipid and protein components suggest that vesicles exit chondrocytes, osteoblasts and presumably other cells of normally mineralizing vertebrate tissues at membrane raft sites as do exosomes formed in the endosomal pathway.

What are the Functional Implications of the Exosome and Microvesicle Concept?

From a historical point of view, the earliest work on matrix vesicle biogenesis focused on particles that were seen to bud from the plasma membrane. While these studies were progressing, parallel investigations revealed that a host of different types of vesicles are released from all living cells and tissues.[reviewed in 8] It is not possible to comment with certainty on which of these cell-derived particles are equivalent to matrix vesicles, although it is likely that some are exosomes generated through the endosomal pathway. Regulated by ubiquitin molecules and the Endosomal Sorting Complex Required for Transport (ESCRT), this pathway provides a mechanism by which vesicles with their internalized cargo of proteins, nucleic acids and lipids can be collected and passaged to specific intracellular sites (see Figure 4).[8] Loaded with cellular miRNA, mRNAs and cytosolic proteins, these vesicles are released from the cell as exosomes. Figure 4 also shows that the endosomal pathway and the autophagic pathways are linked and that cargo that is not secreted from the cell may undergo proteolysis in autophagolysosomes. Remarkably, exosomes regulate a number of physiological processes, including intercellular exchange of proteins and RNA (mRNA, miRNA and other non-coding RNAs), antigen presentation, induction of angiogenesis, storage vesicles and immune regulation.[8] The functional importance of these vesicles in cartilage, bone and skeletal biology is discussed below.

Microparticles and cell-cell communication

More important than microparticle size and cargo release characteristics is the observation that matrix vesicles contain a variety of intracellular components that mirror the composition of the mother cell. These components include enzymes normally resident in the cytosol, as well as membrane proteins and lipids, and even ribosomes. This variety of molecules would be expected to influence not only the mineralization properties of the matrix, but also cell-cell communication. Indeed, since the release of small particles as mediators of cell communication appears to be an evolutionarily conserved process, it is plausible to consider that this type of communication may also exist in the growth plate and at other mineralizing sites.[45, 46]

With respect to targeting exosomes, as has been discussed earlier, recognition molecules such as heparan sulfate proteoglycans and collagen X are present in the extracellular matrix and, as co-receptors; these proteins would serve to anchor the vesicle to selected sites in the matrix. It is likely that exosomes specifically bind receptors on the target cell and are internalized by phagocytosis, endocytosis or fusion with the plasma membrane. [45] For example, it has been shown that phosphatidylserine (PS) binds PS receptors (Tim1 and 4 and BA11). [47, 48] By triggering membrane ruffling, the PS activates macropinocytosis promoting tyrosine kinase receptor activity. Not surprisingly, if vesicle PS binding is blocked with annexin V, then there is a loss of exosome activity.[49]

Most relevant to chondrocytes of the growth plate are studies of Wnt trafficking. Wnt, a hydrophobic protein critical for embryonic development and cell migration, is a key regulator of chondrocyte maturation and mineralization. [50] How this secreted molecule travels among cells of the growth plate has been a conundrum as it is post-translationally modified with fatty acids to generate a hydrophobic environment that would delay or block Wnt gradient formation. [51] This problem was addressed by Koles and Budnik [52] and Gross et al. [53] who showed that Wnt intracellular transport was mediated by exosomes; Wnt-containing exosomes are generated by membrane budding in multivesicular bodies and the Wnt protein is located on the exosome surface. Promotion of Wnt-related changes in phenotype and function is mediated through the frizzled family of receptors and co-receptors on target cells. As was indicated above, while the interaction of PS or Wnt has been clarified in non-skeletal systems, more work needs to be done to determine if similar interactions occur between exosomes and chondrocytes of the growth plate

Also from the perspective of the cartilaginous growth plate, and even more intriguing, by transporting and tracking parathyroid hormone-related protein (PTHrP), exosomal particles could control both chondrocyte proliferation and terminal differentiation. Indeed, Ruchon et al.[54] reported that neprilysin, a protein present in bone matrix vesicles, is required for the processing of PTHrP; these workers also showed that neprilysin activated calcitonin gene-related peptide (CGRP), a molecule required for chondrocyte proliferation.[42]

Aside from protein regulators of cell function, control of gene expression is achieved through the activities of small non-coding RNAs (miRNAs), polynucleotides that are present in exosomes.[55] Although as yet the miRNA composition of matrix vesicles has not been delineated, many of these RNA molecules play important functional roles in chondrocytes of

articular cartilage and the growth plate. In this context, Papaioannou and co-workers[56] showed that chondrocyte proliferation is regulated by let-7 family miRNAs and overexpression of the let-7 inhibitor Lin28a slowed chondrocyte proliferation and influenced bone growth. Another critical miRNA, miR140, influenced bone morphogenetic protein (BMP) signaling and endochondral bone development.[57]

Based on the studies of the growth plate and the cells and exosomes from other tissues, it is likely that matrix vesicle miRNAs regulate post-transcriptional gene expression of differentiating chondrocytes. Such control would impact mesenchymal cell-chondrocyte transitions and possibly influence downstream events involved with chondrocyte-osteoblast interactions and bone formation. Finally in a recent study, Kato et al.[11] demonstrated that catabolic changes in chondrocytes were mediated by exosomes released from synovial fibroblasts. Relevant to the growth plate, this study showed that exosomes are taken up by chondrocytes and regulate chondrocyte function. Based on all of these reports, it is clear that, whether generated through endo or exocytic pathways, the exciting possibility is that these microparticles can influence development, growth and disease processes in cartilage and bone.

Microparticles and mineralization

A defining characteristic of the matrix vesicle is that, at sites of provisional mineralization, many of these membrane-invested particles contain calcium phosphate that is undergoing an apparent amorphous-to-crystalline transition.[58, 59] Morphological studies of many types of mineralizing cells and tissues suggest that the mineral is formed in the vesicles after they are released from the cell and when attached firmly to the extracellular matrix through critical protein residues (Figure 1).[60] Initial mineral foci appear to radiate out from the vesicle membrane, an observation suggesting that the lipid bilayer encloses a chemical environment that catalyzes mineral deposition, possibly by promoting calcium and phosphate ion accumulation by membrane-associated pumps and gates.[61] Within the vesicle interior, a calcium-phosphate nucleating complex is thought to facilitate nucleation of a mineral phase and its transitions, ultimately leading to apatite formation.[62] If, as has been suggested by Habraken et al. 2013 [63] and Nudelman [64] the extracellular matrix contains prenucleation clusters of calcium phosphate nanoparticles, these would most likely be concentrated at charged sites (given that the clusters are themselves charged). Such sites could include the negatively charged groups on proteoglycans or anionic or cationic side chain residues on collagen fibrillar surfaces. The same sites would also serve to attract and anchor exosomes, as mentioned previously. Prenucleation clusters together with the presence of mineral ions from exosomal sources would increase the local ion activity product and lead to apatite formation.

Over the past decades, information has been accruing that the mineralization process is initiated in cells prior to the development of mineral foci in matrix vesicles or in collagen of the extracellular matrix of vertebrate mineralizing tissues.[9, 65–68 and reviewed in 69]. The mechanism by which cells can accumulate high levels of mineral ions without damage to underlying structures is not clear. Nevertheless, Rohde and Mayer[66] report that in differentiating bone marrow cells, amorphous calcium phosphate deposits are evident in the

cytoplasm and vacuoles which are “most probably (released) by membrane fusion to the outside of the cells...and secreted via an exocytic process.” In a very recent study of bone cells, Nollet and colleagues[15] provided evidence that the vacuoles are autophagosomes that contain apatitic crystallites; the mineral is present in the osteoblasts prior to the induction of extracellular mineralization. Noteworthy, the presence of mineral was identified using transmission electron microscopy and hence there is the possibility that the “crystal-like structures” that the authors describe represent “the artifactual crystallization of calcium phosphate” due to chemical fixation. They comment “we cannot completely rule out the possibility that the mineral is in fact present under an amorphous state or as nonstoichiometric microcrystals”. In a related study, Dai et al.[70] showed that modulation of autophagic activity influenced both matrix vesicle release and mineralization in vascular smooth muscle cells. These findings beg the question as to whether there is a relationship between autophagy and exosome formation and release.

It should be recalled that while autophagy is conventionally considered to be a route to promote cell survival, there is now evidence to indicate that the pathway has many other functions ranging from antigen presentation to unconventional protein secretion.[71, 72] New findings point to interactions between exosome formation through the endosomal pathway and terminal events in the autophagic pathway. Accordingly, a branch point must exist that specifies cargo fate, either fusion with lysosomes for cell survival activities or following incorporation into multivesicular bodies, secreted as exosomes (Figure 4). The presence of the alternate routes has been further established using agents that control autophagy and influence both formation of multivesicular bodies and exosome secretion. [73] Based on these findings, which together confirm that there is cellular control of the mineralization process, it can be argued that at least some of the microparticles found in cartilage, bone and other mineralizing vertebrate tissues are derived from intracellular vacuoles. In this case, it must be concluded that the cell directly controls the initial formation of mineral as well as its export to the extracellular matrix.

Summary and Conclusions

There can be little doubt that matrix vesicles are present at some point in the development of almost every vertebrate mineralizing tissue. While many very talented investigators have shown that the singular activity orchestrated by these microparticles is mineral formation, other workers have demonstrated that vesiculation is a generalized property of almost every cell type. The goal of this review is to reconcile the generalized biological observation with the special role of matrix vesicles in the mineralization of cartilage and bone and other vertebrate tissues. The argument is made that matrix vesicles are generated in cells as microparticles (endosomes) and that they are subsequently released from the cell as exosomes. The exosomes anchor to the extracellular matrix and adopt the morphological appearance and functional activities of matrix vesicles. Because they are passaged through the cell in endocytic vesicles, their biogenesis is regulated by the metabolic status and phenotype of chondrocytes, osteoblasts or cells of other vertebrate mineralizing tissues. Against this background, there is new information that links endosomal processing with autophagic activity and suggests that mineralization is initiated within vacuoles prior to their export from the cell. Thus, the critical stages of the mineralization process, nucleation and

crystal growth, are cell-regulated. Finally, studies in many systems point to these microparticles as mediating intracellular communication within a tissue. From this perspective, exosomes may influence the activities of neighboring as well as distant cells. Thus, pharmacological targeting of pathways for exosome formation may provide new avenues for regulation of endochondral bone development and formation, tooth mineralization, fracture repair and limb growth.

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Glossary

Microparticle	The generic name for fragments of plasma membrane ranging from 100 nm to 1000 nm in diameter, shed by all cells types. Included in the definition of microparticles are exosomes, ectosomes, matrix vesicles and remnants of apoptotic bodies.
Matrix vesicle	A cell-derived particle present in the extracellular matrix of most vertebrate mineralizing tissues that functions to promote mineral formation
Exosome	A cell-derived particle formed in the endosomal system and packaged in multivesicular bodies. Following fusion of the bodies with the plasma membrane, the microparticles are released from cells as exosomes.
Ectosome	A cell-derived particle released directly from blebbing cells. Its relationship to the endosomal system has not been established.
Endosomal pathway	A membrane-bounded intracellular pathway which is used to recycle membrane components and transport and degrade macromolecules. Products of the endosomal pathway are released from the cell as exosomes.
Autophagic pathway	An intracellular survival pathway in which cell organelles, unfolded proteins and other molecules are degraded to generate energy and nutrients.

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Highlights

- Matrix vesicles are unique extracellular membrane-bound microparticles that serve as initial sites for mineralization.
- Exosomes, a product of the endosomal pathway, share many characteristics of matrix vesicles.
- Analyses of size, morphology and lipid and protein content indicate that matrix vesicles and exosomes are homologous structures.
- Processing and function of exosomes in vertebrate hard tissues explain in part how cells control the earliest stages of mineral deposition.
- The exosome concept suggests a new mechanism for the transfer of signaling molecules between cells within the growth plate.

Figure 1. Transmission electron micrographs of matrix vesicles in the rat growth plate. Early mineralization of a vesicle is observed as an accumulation of electron dense material in the vesicle membrane (A, arrowheads) and in the vesicle interior (B). Scale bar = 50 nm. Images used with permission from Amizuka et al. [74].

Figure 2.

Transmission electron micrograph of the leg tendon from a normal 15-week-old turkey. Tissue was fixed in 2.5% glutaraldehyde-1% paraformaldehyde overnight at room temperature, embedded in epoxy, sectioned and stained with uranyl acetate-lead citrate. Matrix vesicles of various sizes are found disposed between parallel arrays of type I collagen fibrils. Vesicles, stained for alkaline phosphatase, exhibit an enhanced electron density. Scale bar = 1 μ m.

Figure 3.

Transmission electron micrographs of osteoblast-like cells showing aggregated vesicles close to the plasma membrane and enclosed in sacs (a–c). Images used with permission from Xiao et al. [75].

Figure 4.

Schematic diagram of pathways for formation of mineralizing exosomes (matrix vesicles). The classical endosomal pathway exhibited by almost all cells of the body requires that protein molecules (blue) are taken into target cells by receptor-mediated endocytosis and form early endosomes. In the presence of ubiquitin (light blue crosses), ESCRT proteins (dark blue shafts), and early endosomes, multivesicular bodies are formed. The multivesicular bodies can also receive molecular cargo from autophagosomes. Multivesicular bodies may fuse with regions of the plasma membrane rich in raft lipids and proteins and release their accumulated microparticles to the outside of the cell as mineralizing exosomes. Very recent studies suggest that autophagosomes may contain mineral nuclei (white particles in the autophagosome). Autophagosomes may transport such nuclei to the plasma membrane and release them from the cell as mineralizing exosomes or blebs of mineralizing ectosomes. Multivesicular bodies and early endosomes can form amphisomes which can fuse with lysosomes to form autophagolysosomes. Multivesicular

bodies and late endosomes may also form autophagolysosomes. Autophagolysosomes can degrade their cargo which is then recycled.

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Table 1

Proteins commonly found in exosomes (adapted from Simpson et al. 2008) and in matrix vesicles isolated from cells of bone, mineralizing cartilage and articular cartilage.

Protein category vesicle	Protein symbol	Matrix
(1) Antigen-presentation		
HLA class I histocompatibility antigen	HLA	n/a
(2) Cell adhesion		
Lactadherin *	MFGE8	3
Thrombospondin-1 **	THBS1	3,4
Integrins 1,2,3,4	ITG	
Claudin-1	CLDN1	n/a
(3) Cell structure and motility		
Actins 1,2,3,4	ACT	
α -Actinin-4	ACTN	1,2,3
Cofilin-1	CFL1	1,3
Ezrin	VIL2	4
Moesin	MSN	1,3,4
Myosin	MYH and MYL	1,3
Radixin	RDX	1,2,4
Tublins	TUB	1,2,4
(4) Heat shock proteins and chaperones		
Heat shock cognate 71 kDa protein 1,2,3,4	HSPA8	
Heat shock protein hsp90b	SP90AB1	1
T-complex protein 1	CCT	1
(5) Metabolic enzymes		
α -Enolase	ALDOA	1,2,4
Fatty acid synthase	FASN	1
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1,2,4
Phosphoglycerate kinase 1	PGK1	1,2,4
1,3Phosphoglycerate mutase 1	PGAM1	1,3,4
Pyruvate kinase isozymes M1/M2 ***	PKM2	1,2,4
(6) Exosome biogenesis		
Alix	PDCD6IP	n/a
ESCRT I complex		
Tumor susceptibility gene 101 protein	TSG101	n/a
Vacuolar sorting protein 28	VPS28	1
Vacuolar protein sorting protein 37 ****	VPS37	1
ESCRT II complex		
Vacuolar protein-sorting protein 25	VPS25	n/a
Vacuolar protein-sorting protein 36	VPS36	n/a
Vacuolar-sorting protein SNF8	SNF8	n/a
ESCRT III complex		

Protein category vesicle	Protein symbol	Matrix
Charged MVB proteins	CHMP	n/a
(7) Signaling proteins		
14-3-3 Proteins	YWHA	1,2,3
GTPase HRas	HRAS	3
Rho GDP-dissociation inhibitor 1 [#]	ARHGDI	1,3
Rho-related GTP-binding protein RhoC precursor	RHOC	n/a
Ras-related protein Rap-1b ^{##}	RAP1B	1,2,4
Ras-related protein Rap-2b ^{##}	RAP2B	1
Ras-related protein R-Ras2	RRAS2	1
Ras GTPase-activating-like protein IQGAP1	IQGAP1	n/a
Syntenin-1	SDCBP	1,2,4
Transforming protein RhoA	RHOA	n/a
(8) Guanine nucleotide-binding protein (G proteins)	GNB	3,4
(9) Tetraspanins		
CD9 antigen	CD9	4
CD63 antigen	CD63	n/a
CD81 antigen	CD81	1
CD82 antigen	CD82	n/a
(10) Transcription and protein synthesis		
Histones	HIST	1, 3
Ribosomal proteins	RPS	1
Ubiquitin	RPS27A	1,3,4
Elongation factor 1- α 1	EEF1A1	1,3,4
(11) Trafficking and membrane fusion		
Annexins 1,2,3,4	ANXA	
ADP-ribosylation factor	ARF	n/a
AP-2 complex subunit α -1	AP2A1	n/a
AP-2 complex subunit β -1	AP2B1	n/a
Clathrin heavy chain 1	CLTC	1
Rab GDP dissociation inhibitor β	GDI2	2,
Ras-related protein rab ^{###}	RAB	1,4
Synaptosomal-associated protein 23 ^{####}	SNAP23	n/a
Syntaxin-3 ^{#####}	STX3	1

Listing of common protein fragments present in many different types of exosomes (modified from Simpson et al [40]). The first column shows proteins classified according to function, the second column provides the protein symbol, the third column shows the presence or absence of peptides isolated from matrix vesicles from mineralizing osteoblast-like (Saos-2) cells (1; [14]); from chick embryo osteoblasts and chondrocytes (2; [31]); from murine calvaria-derived MC3T3-E1 cells (3; [25]); from human articular cartilage (4; [12]). The vesicles were isolated by enzymatic digestion of tissue (1,2,3,4) or released without digestion (3) and collected by ultracentrifugation. Methods used to characterize the mineralizing vesicles include morphologic appearance, FTIR analysis of mineral, ultracentrifugation characteristics and measurement of alkaline phosphatase activity. For studies of articular cartilage vesicles, morphologic appearance and ultracentrifugation characteristics were used. It should be noted that only a single study has attempted to distinguish between non-anchored matrix vesicles (exosomes) and collagenase-released vesicles (3). While these two particles shared a considerable number of proteins significant differences were apparent [3] [25]. The individual proteins are listed with no indication given for the number of peptide hits.

For details of molecular weight, score numbers and peptide sequence coverage, the reader should consult the original publications. In some cases, the specific protein was not identified and is shown here replaced by one of similar structure/function:

* lactadherin precursor;

** Thrombospondin-2 and Thrombospondin precursor;

*** pyruvate kinase 3;

**** Vacuolar protein-sorting-associated protein 35;

Rho GDP-dissociation inhibitor 2;

Ras-related protein Rap-1A;

Ras-related protein Rab-precursor;

Synaptosomal-associated protein-1;

Syntaxin-4; n/a =not available.