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Review Article

Characterisation of matrix vesicles in skeletal and soft tissue mineralisation

L. Cui *, D.A. Houston, C. Farquharson, V.E. MacRae

The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG, UK

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ABSTRACT

The importance of matrix vesicles (MVs) has been repeatedly highlighted in the formation of cartilage, bone, and dentin since their discovery in 1967. These nano-vesicular structures, which are found in the extracellular matrix, are believed to be one of the sites of mineral nucleation that occurs in the organic matrix of the skeletal tissues. In the more recent years, there have been numerous reports on the observation of MV-like particles in calcified vascular tissues that could be playing a similar role. Therefore, here, we review the characteristics MVs possess that enable them to participate in mineral deposition. Additionally, we outline the content of skeletal tissue- and soft tissue-derived MVs, and discuss their key mineralisation mediators that could be targeted for future therapeutic use.

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Abbreviations: AB, Apoptotic body; ADP, Adenosine diphosphate; Anx, Annexin; ASARM, Acidic serine- and aspartate-rich motif; ATP, Adenosine triphosphate; BMP, Bone morphogenetic protein; CK, Choline kinase; CKD, Chronic kidney disease; ECM, Extracellular matrix; GPI, Glycosylphosphatidylinositol; GRP, Gla-rich protein; HA, Hydroxyapatite; JNK, c-Jun N-terminal kinase; LPS, Lysophosphatidylserine; MEPE, Matrix extracellular phosphoglycoprotein; MGP, Matrix gla protein; miRNA, MicroRNA; MV, Matrix vesicle; NPP1, Ectonucleotide pyrophosphatase; nSMase2, Neutral sphingomyelinase 2; OPG, Osteoprotegerin; OPN, Osteopontin; PC, Phosphotidylcholine; PE, Phosphatidylethanolamine; P_iT, Pituitary-specific transcription factor; PP_i, Pyrophosphate; PS, Phosphatidylserine; PSS, Phosphatidylserine synthase; RANKL, Receptor activator of nuclear factor kappa-B ligand; RGD, Arginine-glycine-aspartic acid; Runx2, Runt-related transcription factor 2; SIBLING, Small integrin-binding ligand N-linked glycoprotein; siRNA, Small interfering RNA; TGM2, Transglutaminase 2; TNAP, Tissue-nonspecific alkaline phosphatase; VDAC1, Voltage-dependent anion channel 1; VIC, Valve interstitial cell; VSMC, Vascular smooth muscle cell.

* Corresponding author.
E-mail address: Cui.Lin@roslin.ed.ac.uk (L. Cui).

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1. Introduction

The skeleton encompasses bone and cartilage. It is a multifunctional and highly specialised system which comprises both mechanical and biochemical properties that provide the basis for its roles in locomotion, growth, and protection [44]. The skeleton also stores 98% and 85% of the total body calcium (Ca^{2+}) and phosphate (P_i), respectively [52,66]. Furthermore, in recent years, research has uncovered the emerging role of bone as an endocrine organ that regulates development and energy homeostasis [74,118]. The development and lifelong maintenance of the skeletal tissues is tightly regulated through the actions of distinct cell types. Hypertrophic chondrocytes in the epiphyseal plate mineralise the extracellular matrix (ECM) through specialised structures named matrix vesicles (MVs). The first hydroxyapatite (HA) depositions are located within the confinement of these nano-spherical bodies. MVs are membrane-bound particles of cellular origin, that range from 100 to 200 nm in diameter [8,45]. The ability for MVs to calcify is dependent on their content. Mineralising MVs typically contain abundant proteins and lipids that are known to chelate P_i and Ca^{2+}. MVs have also been reported in osteoid, mantle dentin, and calcifying tendons [7,10,24,86,89,158,178]. However, the density of these particles appear to decrease with the increasing compactness of collagen fibrils in the mature bone [25]. Therefore, MVs may be attributed a role in the mineralisation of the embryonic bone, rather than the mature lamellar bone [88]. Indeed, mineral nucleation is a complex process, and whilst MVs are important for this process they are unlikely to be the sole mechanism responsible for the first steps of skeletal mineralisation. Throughout the years, there have been many studies conducted with knockout models on various proteins implicated in the initiation of mineralisation, that consistently show different levels of mineralisation [13,66,113,147]. These studies have provided *in vivo* proof that mineralisation can be achieved through various means. Hence multiple rational theories which describe mineral crystallisation exist. One of the most discussed theories is the nucleation of apatite through collagen polypeptide stereochemistry with Ca^{2+} and P_i, where apatite crystals precipitate and propagate from an amorphous phase, in the gap zone of collagen fibrils [49,87,91,117]. In contrast, studies conducted using electron microscopy and X-ray diffraction analysis on human cortical femur bone, revealed that the majority of the mineral is present outside of collagen fibrils and in the interfibrillar compartment in the form of elongated mineral plate structures [102,103,139]. However, the present review focuses on our current knowledge and understanding of the role of MVs in the mineralisation process. During recent decades, the role of MVs in the pathogenesis of vascular mineralisation has become increasingly apparent, with a number of studies reporting the presence of vesicles in vascular tissues that are comparable in both structure and content to skeletal MVs (Table 1). However, the exact mechanisms through which MVs orchestrate the mineralisation process remain unclear. This review presents a summary of our current knowledge to date on the secretion, function, and content of MVs during both physiological and pathological mineralisation.

2. Bone formation

Bones develop through two different mechanisms. Mesenchymal stem cells can directly differentiate into osteoblasts through intramembranous ossification. This process is responsible for the formation of flat bones such as the cranium, sternum, and rib cage. Alternatively, the mesenchymal stem cells may differentiate into chondrocytes, which serve as templates for bone formation by endochondral ossification that leads to the development of long bones [119]. Endochondral ossification begins with a primary centre in the diaphysis consisting of a cartilage model, hypertrophic chondrocytes and vascular invasion. This is followed by the extension into secondary centres in the epiphyseal plate, which are responsible for longitudinal growth. Concomitant invasion of the cartilaginous scaffold occurs accompanied by haematopoetically derived bone resorbing cells, known as osteoclasts. The latter resorb the mineralised chondrocyte remnants and much of the cartilaginous matrix [41]. Furthermore, mesenchymal cells in the perichondrium begin to differentiate into osteoblasts, directed by the expression of the transcription factors, Runt-related transcription factor 2 (Runx2) and osterix [83]. These bone forming cells deposit a bone-specific matrix, rich in type I collagen, on remnants of chondrocyte ECM and in the perichondrium, which are subsequently mineralised [125]. Throughout lifetime, synchronised actions of osteoblasts and osteoclasts continue to remodel the bone, allowing growth and adaptation in response to mechanical loading. The most abundant cellular component of mature bone are the terminally differentiated osteoblasts, known as osteocytes [82], which reside deep within the bone matrix. The osteocytes orchestrate the actions of the osteoblasts and osteoclasts through relaying of external mechanical signals, to trigger deposition or resorption of bone possibly *via* the expression osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-B ligand (RANKL) [23,111,152,173].

The intricate process of skeletogenesis can be clearly observed in the formation of the appendicular skeleton, which proceeds *via* a cartilage primordium [84]. Under the influence of the transcription factor Sox9, mesenchymal stem cells differentiate into chondrocytes that proliferate and generate type II collagen and a proteoglycan-rich ECM [125]. The chondrocytes within the prospective bone progress through morphologically distinct zones, co-ordinated by sequential expression of transcription and growth factors [101]. Chondrocytes in the most advanced region of the epiphyseal plate exit the cell cycle and become hypertrophic. The hypertrophic chondrocytes,

Table 1
Some of the common proteins identified in MVs derived from VSMCs, mineralising osteoblasts, and femurs of chicken embryo [18,71,172].

Protein type/family	Protein name/family member
Calcium-binding proteins	Anx A1
	Anx A2
	Anx A5
	Anx A6
	Voltage-dependent anion channel-1 (VDAC1)
Phosphate transporter	5′-Nucleotidase
Oxidative stress	Peroxiredoxin 1
	Peroxiredoxin 2
Extracellular matrix	Collagen type VI, a1
Cytoskeletal and surface proteins	Actin-B
	Moesin
	Integrin, a3 isoform A
	Integrin, a5
	Integrin, b1 isoform 1A
	Sodium-potassium adenosine triphosphatase, a1
Chaperones	Calreticulin