Vesicles of the intracellular and extracellular transport – key structures in the process of tissue differentiation towards bone and cartilage

ABSTRACT

Differentiation of cells of the skeletal tissue, such as osteoblasts and chondrocytes, into mineralization-competent cells is a necessary step of the physiological process of bone and cartilage mineralization. Vascular cell calcification accompanies a pathological process of atherosclerotic plaque formation, which occurs due to trans-differentiation of vascular smooth muscle cells into cells resembling bone mineralization-competent cells. The activity of tissue-nonspecific alkaline phosphatase (TNAP), an enzyme necessary for physiological mineralization, is also induced in vascular cells in response to inflammation. TNAP acquires its mineralizing function when anchored to the plasma membrane (PM) of mineralizing cells and to the surface of vesicles derived from these cells. Numerous important reports indicate that various types of vesicles play a crucial role in initiating cell differentiation. In this review, we would like to highlight various functions of different types of vesicular structures of the cellular transport machinery such as intracellular vesicles (IVs), extracellular vesicles (EVs) or matrix vesicles (MVs) at distinct stages of both physiological and pathological processes of tissue differentiation.

INTRODUCTION

Mineralization is a physiological process by which growth plate chondrocytes and osteoblasts deposit calcium phosphate crystals during endochondral and membranous ossification, respectively. This process is initiated by TNAP activity [Narisawa et al. 2013]. Deficiency of this enzyme in human cells results in a severe disease called hypophosphatasia causing in utero death of fetuses devoid of a mineralized skeleton [Millan & Whyte 2016]. TNAP activity is necessary for physiological bone mineralization, but also for the induction of pathological vascular calcification. Vesicular structures produced by mineralization-competent cells may resemble rafts, that is, membrane microdomains. In this article we would like to consider a possible role of vesicles of the intracellular and extracellular transport in the process of tissue differentiation and to discuss the stimulating effect of their cargo on bone mineralization and vascular calcification processes.

TYPES AND STRUCTURES OF VESICLES

INTRACELLULAR VESICLES

The ability of cells to interact with one another and with their environment relies on a large number of proteins that need to be in the right amount and in the right location. For this reason, eukaryotic cells have evolved complex sorting machineries to ensure a dynamic and strictly controlled flow of proteins between cellular compartments. This machinery exists in both plants and animals, and it functions of different types of vesicles play a crucial role in initiating cell differentiation. In this review, we would like to highlight various functions of different types of vesicular structures of the cellular transport machinery such as intracellular vesicles (IVs), extracellular vesicles (EVs) or matrix vesicles (MVs) at distinct stages of both physiological and pathological processes of tissue differentiation.

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ENs are primarily intracellular sorting organelles which regulate trafficking of proteins and lipids among subcellular compartments of the secretory and endocytic pathway, specifically the plasma membrane (PM) Golgi, trans-Golgi network (TGN) and vacuoles/lysosomes [Marsh 2001; Bottini et al. 2018]. They receive cargo (proteins and lipids) from both the biosynthetic and the endocytic pathways. Most proteins are synthesized at the endoplasmic reticulum, trafficked through the Golgi, sorted at the TGN, and then sent to ENs for their final vacuolar/lysosomal delivery. ENs also receive PM proteins that are internalized by endocytosis and these proteins are either recycled back to PM or are sorted for degradation. The recycling of PM proteins occurs at early and recycling ENs; whereas their degradative sorting is achieved in intermediate/late ENs, which are also called multivesicular bodies/multivesicular endosomes (MVBs/MVENs), 500–5000 nm in size.

Three primary mechanisms of endocytosis may be distinguished [Marsh 2001]. Receptor-mediated endocytosis (RME) is the most specifically-targeted form of the endocytic process. Through RME, active cells are able to take in significant amounts of particular molecules (ligands) that bind to receptor sites extending from the cytoplasmic membrane into the extracellular fluid surrounding the cell. These receptor sites are commonly grouped together along coated pits in the membrane, which are lined on their cytoplasmic surface with a bristle-like collection of coat proteins. The coat proteins are thought to play a role in enlarging the pit and forming a vesicle [Marsh 2001]. As plasma membrane vesicles (PMVs) 50–500 nm in diameter we refer to caveolae and clathrin-coated vesicles or pits (CCVs/CCPs). Clathrin-mediated endocytosis (CME) is the major endocytic pathway in mammalian cells. It is responsible for the uptake of transmembrane receptors and transporters, for remodeling PM composition in response to environmental changes, and for regulating cell surface signaling, CME occurs via the assembly and maturation of CCPs that concentrate cargo as they invaginate and pinch off to form CCVs. In addition to the major coat proteins, clathrin and adaptor protein complexes, CME requires endocytic accessory proteins and phosphatidylinositol lipids. It is regulated at multiple steps (initiation, cargo selection, maturation, and fission) and monitored by an endocytic checkpoint that induces disassembly of defective pits via posttranslational modifications, allosteric conformational changes, isoform and splice-variant differences among components of the CME machinery, including the GTPase dynamin [Mettlen et al. 2018]. Vesicles produced via RME may internalize other molecules in addition to ligands, though the ligands are usually brought into the cell in higher concentration. One of the main factors causing bacterial diarrhea are AB5 enterotoxins. The AB5 subunit has a toxic activity, the B5 subunit contains a catalytic subunit A and pentameric subunit B, which binds to the cell surface within lipid rafts. The Cholera toxin family cause the constitutive activation of Gsα protein, which results in cAMP production, opening of the chloride channels and release of chloride ions into the lumen of the small intestine. In contrast, the Shiga toxin family has a cytotoxic effect on epithelial cells. It can inhibit protein synthesis leading to cell death. Although AB5 has a toxic activity, the B5 subunit has a significant potential as a transporter for proteins with anticancer activity and as a tool for the visualization of lipid rafts and cancer cells (Fig. 1) [Komiazyk et al. 2015].

A less specific mechanism of endocytosis is pinocytosis [Marsh 2001]. By means of pinocytosis, a cell is able to ingest droplets of liquid from the extracellular fluid. All solutes found in the droplets outside of the cell may become encased in the vesicles formed via this process, with those present in the greatest concentration in the extracellular fluid also becoming the most concentrated in the membranous sacs. Pinocytic vesicles tend to be smaller than vesicles produced by other endocytic processes.

The final type of endocytosis, termed phagocytosis [Marsh 2001], is probably the most well-known manner in which cells may import outside materials [Marsh 2001]. By means of phagocytosis, a single-celled organisms engulf by stretching out pseudopodia, encircling of the particles and subsequent packaging of them into vesicles. Phagocytic vesicles are usually large enough to be referred to as vacuoles or phagosomes. Phagocytosis is commonly associated with amoebas, but in multicellular organisms, phagocytic cells function in bodily defense rather than as a means to gain nourishment. For example, leukocytes in the human body often phagocytose protozoa and bacteria or dead cells and cellular components in order to help stave off infections or autophagy problems [Altman & Rathmell 2009].

When both phagocytosis (of particles) and pinocytosis (of solutes) was investigated in macrophages in the absence or presence of metabolic and cytoskeletal inhibitors, Percoll (30 nm diameter) was captured by pinocytosis, whereas polystyrene beads (100, 300, 600, 800 or 1100 nm diameter) were taken

Figure 1. Binding of bacteria enterotoxin to membrane rafts. Monkey African Green kidney Vero cells (ECACC 84113001) were fixed, permeabilized (B, D, F) or non-permeabilized (A, C, E) in 0.08% TX-100 and analyzed by fluorescent microscopy with structured illumination ApoTome. Cholera Toxin B subunit FITC conjugate binds to ganglioside GM1 (GM1) (A, B, green, arrows). Cholesterol (CHOL) was visualized by staining with filipin (C, D, blue, arrowheads). Sites of GM1 and CHOL co-localization are visible in cyan on merge images (E, F), scale bar 10 µm. (MK and JD, unpublished results).
by phagocytosis with increasing Endocytic Index (microliter/10⁶ cells per h) rate. The pattern of inhibition of particles uptake suggests that there is no radical discontinuity between pinocytosis and phagocytosis, but that the contribution of phagocytosis steadily increases with increasing particle diameter [Pratten & Lloyd 1986].

EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) are membrane-delimited subcellular particles, which contain multiple classes of bioactive molecules including markers of the cell from which they are derived. EVs are implicated in an increasing number of physiological and pathological contexts as mediators of local and systemic intercellular communication and detection of specific EVs may be useful in monitoring disease progression [Lynch et al. 2017].

EVs can be released from cells both constitutively and in response to activation or stress. Although there is a lack of consensus on the properties and nomenclature within the EV field, it is generally accepted that there are at least three different types of EVs, which have been classified according to size, biogenesis, or isolation technique, composition and function [Kanada et al. 2016; Lawson et al. 2016; Lynch et al. 2017; Bottini et al. 2018]. Exosomes (EXs) are the smallest category (30–150 nm) formed by inward budding of the endosomal membrane via an endosomal pathway, which involves formation of MVBs that are trafficked to the PM, where they fuse to release their contents by exocytosis. Microvesicles/microparticles (MicroVs/MicroPs) (100–1000 nm) are produced by budding or blebbing directly from the PM. Apoptotic bodies (ABs) are formed in cells undergoing programmed cell death named apoptosis, and apoptotic cell-derived vesicles have very broad size ranges (typically described as 1000–5000 nm). Relatively large ectosomes (ECs), >1 μm in diameter and not apparently associated with apoptosis, have also been described. They contain intact organelles but not nuclear components. EV cargoes are diverse, ranging from genetic material such as mRNA, genomic DNA and microRNA (miRNA) to lipids, and cytosolic or membrane proteins such as growth factors, enzymes and cytokines. Thus, they can be considered as transport vehicles, which may influence on the behavior of distant cell types within the body, as well as may transfer information between individual organisms of the same or separate species [Lawson et al. 2016].

It is widely accepted that EVs contain biomolecules indicative of the cell from which they derive, its state of activation, its metabolic activity, and in some cases its genotype. Since EVs are accessible in various body fluids including plasma, urine, milk, tears, sweat and semen, their cargoes may be useful as non-invasive biomarkers for diagnosis and prognosis of many diseases including cancer, cardiometabolic diseases and diabetes [Lawson et al. 2016]. Recent investigations serve to highlight the association of EVs and their cargos with diverse cancer types. Because of their structure and highly specific cargos, EVs are likely to prove to be most useful targets for biomarker screening in cancer diagnosis, prognosis and therapy, as well as in other disease settings. EVs are involved in the regulation of tumor growth, progression (pre- and post-therapy), and antitumor immunity, in the latter context showing potential application in antitumor vaccination. Lately it has been proposed that the “onco-regenerative niche” (ORN) represents a microenvironmental signaling network driven by apoptosis and involving tumor cells, non-tumor stromal and immune cells, connective tissue, soluble factors, and EVs [Lynch et al. 2016].

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**Figure 2.** Localization of calcium and phosphate ions within matrix vesicles during bone mineralization. Matrix vesicles (MVs) were isolated from 17-day old chicken embryo leg bones, digested with collagenase type-I (200 U/g) at 37°C for 3h in synthetic cartilage lymph (SCL), centrifuged at 13 000 x g for 20 min at 4°C followed by 80 000 x g for 60 min at 4°C. They were subsequently incubated in SCL with 2 mM Ca²⁺ at 37°C for 24 h, negatively stained and analyzed by transmission electron microscopy with energy dispersive X-ray microanalysis (TEM-EDX). A – TEM image of MVs with minerals (arrows) and attached to collagen fibers (arrowheads), scale bar 1 µm. B, C – Ion maps for Ca (B) and P (C) measured by TEM-EDX. Sites of Ca and P co-localization are visible in yellow on merge images (D, E). F – Ca/P ratio in empty MVs (MV) or in MVs containing minerals (MV + mineral) calculated from TEM-EDX spectra; n=4 ± s.d. (ASK and LB, unpublished results).
The putative network engenders pro-repair and regenerative responses that promote tumor cell proliferation, angiogenesis, and invasiveness while at the same time suppressing antitumor immunity. EVs, whose membranes protect their cargo from degradation, are being explored for their ability to deliver targeted molecules to specific cell types for directed therapy [Kanada et al. 2016].

**MATRIX VESICLES**

Matrix vesicles (MVs) are released by budding from PM of hypertrophic chondrocytes and mature osteoblasts, that is cells responsible for endochondral and membranous ossification (Fig. 2) [Anderson 2003, 2007; Thouverey et al. 2009, 2011]. In addition, MVs from odontoblasts contribute to the onset of mineralization of mantle dentin during its genesis [Goldberg et al. 2011]. Under pathological conditions, MVs can also be released from non-skeletal tissues for example from vascular smooth muscle cells (VSMCs) (Fig. 3) [Fakhry et al. 2011]. MV release from apical membrane microvilli is triggered by depolymerization of the actin cytoskeleton [Thouverey et al. 2009]. MVs are 100-500 nm in diameter, able to bind to collagen in vitro and to form apatite within 1-3 h in a medium containing 2 mM calcium and 1-2 mM phosphate (equivalent to a Ca/P molar ratio around 1.5) (Fig. 2) [Buchet et al. 2013; Bottini et al. 2018].

Three phosphatases have been implicated in the concerted regulation of inorganic pyrophosphate (PPi): orphan phosphatase 1 (PHOSPHO1) present in the lumen of MVs, nucleotide pyrophosphatase/phosphodiesterase (NPP1) and tissue-nonspecific alkaline phosphatase (TNAP). The latter two act on the outer surface of MVs to regulate the extracellular Ca2+ and in the biophysical process that initiates mineralization in the MV lumen [Wang et al. 2003]. Several members of the vertebrate annexin (AnxA) family (AnxA1, AnxA2, AnxA5, AnxA6 and AnxA7) are present in MVs [Balcerzak et al. 2008; Thouverey et al. 2011; Cmoch et al. 2011, Roszkowska et al. 2018]. They may be found in the lumen, on the inner leaflet of the bilayer in contact with phosphatidylserine (PS), or on the outer surface of MVs. AnxA5 [Kirsch 2012; Bolean et al. 2015], AnxA6 or an unidentified calcium channel (UCC) may function as calcium carriers [Bottini et al. 2018]. Annexins (AnxA2, AnxA5 and AnxA6) and TNAP also have collagen-binding capacity [Kirsch 2012], a property that may help align MVs along collagen fibers to promote propagation of mineralization onto the extracellular matrix (ECM) scaffold.

Fetuin A and osteopontin are inhibitors of apatite formation and can restrict further propagation of mineralization within the ECM [Jahnen-Dechent et al. 2008]. MVs are enriched in lipid markers of lipid rafts such as cholesterol (CHOL), sphingomyelin (SM) and PS as well as in protein markers of lipid rafts such as AnxA6, H+ ATPase, G-proteins and glycosylphosphatidylinositol (GPI)-anchored TNAP (Fig. 3) [Balcerzak et al. 2008; Wuthier & Lipsoncomb 2011; Roszkowska et al. 2016, 2018]. MVs can be considered as a model of membrane microdomains due to their selective lipid and protein recruitments, with the additional advantage that they can be extracted without detergent [Bottini et al. 2018].

**BIOGENESIS AND PROPERTIES OF EXTRACELLULAR VESICLES**

**SEPARATION AND PURIFICATION OF EXTRACELLULAR VESICLES**

The size range of different vesicles may overlap, and the markers which are displayed on the outer leaflet of the surrounding membrane may be common to both types of vesicles: intracellular (IVs) and extracellular (EVs). Thus, most isolation methods do not guarantee a pure population of vesicles. With this in mind, and based on the most common protocols involving sequential/differential ultracentrifugation steps, some researchers now refer to the speed at which each vesicle population is pelleted, rather than to vesicle type [Lawson et al. 2016; Bottini et al. 2018].

There are guidelines available for the collection of blood in particular [Lacroix et al. 2012] and several protocols have been published for the sequential isolation of different populations of EVs via ultracentrifugation. In the first step, bodily fluid or culture medium is centrifuged at low speed (1500-3000×g (times gravity)) to remove cellular material and debris. The supernatant from this step can then be further centrifuged at 10000–17000×g to pellet MicroVs, with a final centrifugation

![Figure 3. Localization of tissue non-specific alkaline phosphate and cholesterol during vascular calcification. Mouse vascular smooth muscle MOVAS cells (ATCC CRL-2797) were trans-differentiated (B, D, F) or non-differentiated (A, C, E) in 50 μg/ml ascorbic acid (AA) and 10 mM β-glycerophosphate (β-GP) for 7 days, fixed and analyzed by fluorescent microscopy with structured illumination ApoTome. Tissue non-specific alkaline phosphate (TNAP) was immunostained with anti-TNAP primary antibody conjugated with Alexa Fluor 594 secondary antibody (A, B, red, arrows). Cholesterol (CHOL) was visualized with staining with filipin (C, D, blue, arrowheads). Sites of TNAP and CHOL co-localization are visible in magenta on merge images (E, F), scale bar 10 μm. (MK and JG, unpublished results).](image-url)
at 100000×g to pellet EXs. In addition to EXs this population is likely to contain small MicroVs and possibly some lipoproteins. Density gradient ultracentrifugation may be further employed for a purer exosome population [Thery et al. 2006]. There is still no perfect method to isolate MVs [Wuthier & Lipscomb 2011]. One of the most challenging aspects of sample preparation is establishing approaches that faithfully preserve biological material in situ in a fully hydrated state. During extraction and purification of chondrocyte-released MVs without collagenase, usually two bands of membranous layers are visible on Percoll gradients, while four bands of membranous layers are found in sucrose gradients [Balcerzak et al. 2007]. The less dense membrane fraction with the highest lipid-to-protein ratio (around 3 mg/mg), the highest specific TNAP activity and the highest ability to form apatite, corresponds to MVs and the addition of collagenase increases the yield of released MVs [Cmoch et al. 2011; Bottini et al. 2018; Roszkowska et al. 2018].

In addition, commercially available kits utilize size exclusion chromatography and magnetic separation based on CD9 or CD63, cell surface markers which are exposed on exosomes but not thought to be expressed on MicroVs. EXs can be directly precipitated from plasma or culture supernatant using these approaches [Lawson et al. 2016]. However, there is no clear consensus as to the efficacy of these methods in the published literature. For these reasons, it is imperative to characterize the vesicle population of interest as fully as possible.

CHARACTERIZATION AND IMAGING OF EXTRACELLULAR VESICLES

A selection of methods have been utilized for characterization of EVs. Transmission electron microscopy (TEM) is considered the gold standard of imaging techniques, as it can give accurate information about the sizes of all classes of vesicles. However, as it is not a quantitative technique and requires specialized expertise and equipment, TEM may be limited in appeal. Energy dispersive X-ray microanalysis (EDX) spectra quantitation and ion mapping give quantitative and qualitative results of ion ratios and replacements in MVs or apatite crystals [Strzelecka-Kiliszek et al. 2017a]. The overall goal of using TEM-EDX was to find a simple method for imaging and quantifying the distribution of multiple ions in various minerals from different types of cells during distinct stages of bone mineralization [Bozycki et al. 2018] or vascular calcification [Roszkowska et al. 2018] processes (Fig. 2). Mineralization profiles may also be analyzed by different optical methods: Alizarin Red-S (AR-S) staining or von Kossa silver nitrate or lactate staining and light microscopy with RGB filters may be used for imaging of cells and calcium-phosphate minerals [Strzelecka-Kiliszek & Bozycki 2017b] while ultraviolet (UV) light facilitates visualization of cell lysates or apatite powders [Strzelecka-Kiliszek et al. 2017c, Bozycki et al. 2018]. The formed minerals may also be analyzed by Fourier transform infrared spectroscopy (FTIR). It was proved that the infrared spectrum of the mineral deposit formed by MVs of mineralization-competent cells was identical to the spectrum of the HA standard [Thouvery et al. 2009, 2011]. The mineralization process may also be imaged using different macro- and microscopy techniques. This can be achieved by clearing organs such as leg bone or AS-R staining and macrophotography. In addition, the process can be observed by staining of crucial cellular enzymes such as Src kinases or Rho small GTPases and Rho-associated coiled-coil kinase (ROCK) [Strzelecka-Kiliszek & Bozycki 2017b] or ganglioside GM1, TNAP and CHOL (Fig. 1 and 3) with fluorophores and imaging by fluorescent microscopy. Some advanced microscopy techniques like scanning probe microscopy (SPM), contact mode and dynamic mode of atomic force microscopy (AFM), scanning tunneling microscopy and scanning near-field optical microscopy have been recently proposed to study proteoliposomes, as models of biological membranes. Analysis of proteoliposomes facilitates the understanding of lipid-protein interactions with emphasis on the properties of physicochemical and biochemical reactions during the biomineralization process [Bolean et al. 2017, Bottini et al. 2018]. Proteoliposomes provide a means of reconstituting lipid vesicles that function like MVs, making these structures an advantageous and convenient experimental model to understand MV-mediated mineralization. They may be constituted of a single type of lipid or a mixture of lipids, with proteins and/or electrolytes. Comparisons of proteoliposomes and osteoblast-derived MVs or MVs deficient in TNAP, NPP1 or PHOSPHOI, using natural substrates such as ATP, adenosine 5′-diphosphate (ADP) and PP_i, confirmed the validity of proteoliposome models [Yadav et al. 2011]. The ultimate goal of the in vitro MVs biominetic models is to replicate in vitro the key events leading to the domain-induced MV budding, and to initiation of apatite crystal formation in chondrocyte- and osteoblast-derived MVs. Once the proteoliposomes are obtained and characterized, they can be added to fixed amounts of MVs, either WT or deficient in specific enzymes, to modulate their in vitro mineralization properties [Bottini et al. 2018].

Several other non-optical methods have also been utilized but are limited [Lawson et al. 2016]. Thus, many researchers have opted for one of a range of optical techniques, of which flow cytometry (FCM) is the most widely reported for MV detection. FCM enables EVs phenotyping using fluorochrome conjugated antibodies to determine the parental cell type, and is also quantitative. Small particle size at the limit of detection may be an issue; however, there are a number of sophisticated protocols to eliminate background noise. Newer instruments have lower detection limits and image stream technology (IST) is able to further differentiate between different types of particles in whole blood and plasma [Headland et al. 2014]. Accurate detection of EXs using FCM should be carried out with caution, as their size is below the limit of detection of many instruments. However, alternative methods are available including dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and tunable resistive pulse sensing (TRPS). These techniques enable quantitation of EXs and small MicroVs, but they have limited capabilities for phenotyping and may require additional time for purification of vesicles before measurement [van der Pol et al. 2010]. Raman spectroscopy, which is a highly sensitive quantitative technique for analysis of the biochemical composition of vesicles without labelling, has also been used to define vesicular populations [Lawson et al. 2016].

FUNCTIONS OF VESICLES IN THE PROCESS OF TISSUE DIFFERENTIATION

Vesicles derived from normal cells and those from diseased and malignant cells exhibit unique ‘molecular signatures’ that differentially modulate cellular functions in re-
recipient cells [Kanada et al. 2016]. Intracellular vesicles (IVs), produced in different cellular compartments or extracellular vesicles (EVs), released by different cell types may participate in physiological and pathological processes [Lener et al. 2015; Malda et al. 2016; Karpman et al. 2017, Silva et al. 2017]. They mediate intracellular or intercellular communication and transmit specific information from their cell of origin to their target. As a result of these properties, vesicles of defined cell types may serve as novel tools for various therapeutic approaches, including (a) anti-tumor therapy, (b) anti-pathogen vaccination, (c) immune-modulatory and regenerative therapies and (d) drug delivery.

The translation of vesicles into clinical therapies requires the categorization of vesicle-based therapeutics in compliance with existing regulatory frameworks. As the classification defines subsequent requirements for manufacturing, quality control and clinical investigation, it is of major importance to define whether vesicles are considered the active drug components or primarily serve as drug delivery vehicles. For an effective and, particularly, safe translation of vesicle-based therapies into clinical practice, a high level of cooperation between researchers, clinicians and competent authorities is essential.

The main function of MVs is the initiation of apatite formation in bone, cartilage and dentin due to their high TNAP activity [Bottini et al. 2018]. Pathological mineralization concerns not only skeletal but also non skeletal tissues [Bottini et al. 2018]. It is manifested by apatite deposition, often mediated by MVs, in soft tissues of tendons and/or ligaments (calcific tendinitis and ankylosing spondylitis), in arterial media, in atherosclerosis, in articular cartilage, and during vascular mineralization induced by chronic kidney disease or by type 2 non-insulin-dependent diabetes mellitus, in atherosclerosis, and in articular cartilage.

It was recently speculated that MVs might mediate cell signaling by bone morphogenetic proteins (BMP-2 and BMP-4) and serve as carriers of morphogenetic information to nearby chondrocytes and osteoblasts [Nahar et al. 2008]. The presence of microRNA (miRNAs) in MVs [Lin et al. 2016] suggests that MVs can function as signalosomes in cell-cell communication during cartilage and bone development via transfer of specific miRNAs. However, it remains to be determined whether such cell-cell communication occurs in vivo, because the average life span of MVs as determined in vitro is 1–3 h. Once apatite forms, MV membranes break, releasing their lumen content, and possibly also signaling molecules. Secondly, MVs bind to collagen produced by the cells from which they originate, which prevents long-distance displacement. Thirdly, MVs are probably surrounded by many dying cells from which they were released during late stages of differentiation. Taken together, these findings suggest that cell-cell communication mediated by MVs, if it exists, would be of a limited occurrence and of a short distance, rather autocrine or paracrine in nature [Bottini et al. 2018].

Some recent reports of basic and clinical scientists, members of the International Society for Extracellular Vesicles (ISeV) and of the European Cooperation in Science and Technology (COST) program of the European Union, namely European Network on Microvesicles and Exosomes in Health and Disease (ME-HaD), summarize recent developments and the current knowledge of vesicle-based therapies [Lener et al. 2015; Malda et al. 2016; Karpman et al. 2017, Silva et al. 2017; Bottini et al. 2018]. They highlight many aspects of safety and regulatory requirements that must be considered for pharmaceutical manufacturing and clinical application. They discuss the production and quality control processes and address the strategies to promote the therapeutic im-

Table 1. Main vesicles involved in tissue differentiation.

<table>
<thead>
<tr>
<th>Type</th>
<th>Biogenesis</th>
<th>Reference</th>
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<tbody>
<tr>
<td>IVs</td>
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<td></td>
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<tr>
<td>ENs</td>
<td></td>
<td></td>
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<tr>
<td>MVBs</td>
<td>Marsh 2001; Otegui et al. 2010; Bottini et al. 2018</td>
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<tr>
<td>PMVs</td>
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<td>OMFs</td>
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<td>ECs</td>
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<tr>
<td>EVs</td>
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<tr>
<td>ABs</td>
<td>Lawson et al. 2016; Kanada et al. 2016; Lynch et al. 2017; Bottini et al. 2018</td>
<td></td>
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<tr>
<td>MicroVs</td>
<td></td>
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<tr>
<td>EXs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen-free</td>
<td>Wuthier &amp; Lipscomb 2011; Roszkowska et al. 2018; Bottini et al. 2018</td>
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<tr>
<td>Collagen-attached</td>
<td>Balcerzak et al. 2008; Thouverey et al. 2009, 2011; Cmoch et al. 2011; Buchet et al. 2013; Fakhry et al. 2017; Roszkowska et al. 2018; Bottini et al. 2018</td>
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Table 1. Main vesicles involved in tissue differentiation.
plementation of vesicles in future clinical studies. Finally, what is most important, these reports consider how transport of vesicles and proteoliposomes can be easily detected and blocked. However, there is still much left to understand regarding the mechanisms of vesicles formation and release, cargo loading and their specific targeting to a particular tissue.

CONCLUDING REMARKS

There has been a growing interest in using vesicles of the intracellular and extracellular transport as carriers of biomarkers of disease, especially since they carry markers of their cell of origin and may represent the pathophysiological status of the cells. With increasing evidence for the importance of them as mediators of signal transduction, their potential as biosensors and/or delivery vehicles for signaling molecules to specific target cells, interest in their measurement and isolation has grown exponentially over the last decade. This has led to the availability of an ever-increasing range of instruments and isolation kits, but more established techniques of separation, characterization and imaging, should be accessible to individuals wishing to start exploring this exciting new field to better understand mechanisms of the process of tissue differentiation.

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STRESZCZEŃE

Różnica między komórką tkanki kostnej i chrzęstnej

Pęcherzyki transportu wewnątrzkomórkowego i zewnątrzkomórkowego – kluczowe struktury w procesie różnicowania tkanki kostnej i chrzęstnej

Pęcherzyki transportu wewnątrzkomórkowego i zewnątrzkomórkowego – kluczowe struktury w procesie różnicowania tkanki kostnej i chrzęstnej

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Słowa kluczowe: różnicowanie tkanki, pęcherzyki wewnątrzkomórkowe, pęcherzyki zewnątrzkomórkowe, pęcherzyki macierzy pozakomórkowej, transport

STRESZCZENIE

Różnica między komórką tkanki kostnej i chrzęstnej, takich jak osteoblasty i chondrocyty, do komórek kompetencyjnych w procesie mineralizacji jest ważnym etapem fazylogeniczego procesu mineralizacji kości i tkanki chrzęstnej. Wapnienie komórek naczyń krwionośnych towarzyszy także patologicznemu procesowi powstawania płytki miażdżycowej, która jest wynikiem różnicowania komórek mięśni gladkich ściany naczyń krwionośnych oraz komórek przypominających komórkę tkanki kostnej. Dla komórek mięśni gladkich i komórek przypominających komórkę tkanki kostnej, różnica między zespołami farmakologicznymi i zewnątrzkomórkowymi jest istotna. W kontekście różnicowania tkanki kostnej i chrzęstnej, pęcherzyki transportu wewnątrzkomórkowego i zewnątrzkomórkowego pełnią kluczową rolę w procesie mineralizacji tkanki kostnej i chrzęstnej.