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Characterization and Therapeutic Uses of Exosomes: A New Potential Tool in Orthopedics

[1] Anneliese E Vitha, [1]Austin W Kollefrath, [2] Chun-Yuh Charles Huang, [3] Franklin Garcia-Godoy

[1] Miller School of Medicine, University of Miami, Miami, Florida, USA

[2] Department of Biomedical Engineering, University of Miami, Coral Gables, Florida, USA

[3] Department of Bioscience Research, College of Dentistry, University of Tennessee Health Science Center, Memphis, Tennessee, USA

Corresponding Author

Franklin Garcia-Godoy, DDS, MS, PhD (Biotechnol), PhD (Dent Mater)

Professor, Department of Bioscience Research

Director, Bioscience Research Center

College of Dentistry

University of Tennessee Health Science Center

875 Union Avenue

Memphis, TN 38163

Office.: 901 448-6202

Fax: 901 448-1625

E-mail: fgarciagodoy@gmail.com

and

Senior Clinical Investigator,

The Forsyth Institute,

Cambridge, Massachusetts, USA

Running title: MSC derived exosomes

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Abstract

In recent years, regenerative medicine has directed its interests onto the use of stem cells to heal human tissues. One specific class of cells that has been employed in this field of research is mesenchymal stem cells. Due to difficulties with the usage of whole stem cells, researchers have turned to an alternative, the secretome of the mesenchymal stem cells. In recent years, research has explored numerous aspects of the mesenchymal stem cell secretome, especially the most promising aspect, exosomes. This review explores a variety of interests in exosomes including the classification and molecular composition of exosomes, mechanisms for exosome isolation, and the various biological functions of exosomes. As more is discovered about the exosomes, their different diagnostic and therapeutic uses in the medical field have also been explored. A new field attempting to exploit the exosomes in clinical practice is orthopedics. While a significant deal of research has been carried out, even more is being discovered to allow utilization of the exosomes in clinical practice.

Keywords: Mesenchymal stem cell, Exosomes, Stem cell therapy, Cell-derived vesicles, Orthopedics, Therapeutics

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Introduction

Throughout the past few decades, regenerative medicine has focused its attention on the use of various stem cells to heal human tissues. Mesenchymal stem cells (MSCs) have been the most widely used experimental model for stem cell research; however, the therapeutic results of the clinical trials of MSCs have yet to fulfill their expectation[1]. These trials have shown problems with loss of cells, lack of differentiation and lack of engraftment at target site after MSCs reach their destination. However, numerous early stage clinical trials are still able to achieve primary endpoints[1]. Due to these results and the major risks and limitations of stem cell transplantation, a new avenue of research into the trophic factors from MSCs has begun[1,2]. In more recent years, one alternative that has surfaced is the therapeutic use of the secreted factors or secretome of MSCs[3]. The MSCs secrete numerous substances with immunoregulatory, anti-apoptotic and other properties forming unique MSC secretome that could be exploited for a wide variety of clinical use, both diagnostic and therapeutic[4]. The secretome has two parts to it, the soluble and vesicular aspects[2]. While the soluble part has been studied greatly in prior years, recently there have been interests in the vesicular aspect secreted by cells including extracellular vesicles, exosomes and microvesicles[5]. Of these vesicle types, exosomes have received a large amount of interests in research due to their roles in intercellular communication, immunomodulatory function and their potential for use in identifying and treating diseases[3]. The composition and roles of the exosomes greatly depend on where MSCs are derived from, the physiologic state of MSCs and the medium in which MSCs are grown[5,6]. These various compositions and roles of exosomes show their potential for widespread diagnostic and therapeutic use throughout clinical practice, including applications in orthopedics.

Classification/Molecular Composition

The current classification of extracellular vesicles is based on size, cellular origin and their subsequent biological function[7]. Microvesicles originate from the plasma membrane and range in diameter from 100-1000 nm[6]. Exosomes originate from intracellular compartments called multivesicular bodies (MVBs), organelles which functions in the

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endocytic and secretory pathways of cells[3,8]. These MVBs bud into exosomes within the cytoplasm of the cell and the exosomes are then released from the cell[3]. They can range in diameter from 30-100 nm due to the lipid bilayer restricting the exosomes from being smaller and the MVB origin preventing a larger diameter[9]. Due to a small diameter, the exosomes can hold only 20-90 nm across of total cargo, which translates into approximately ≤ 100 proteins and ≤ 10,000 net nucleotides of nucleic acid[5].

Aside from this characteristic structure and carrying capacity of exosomes, they may also have a unique protein, lipid and carbohydrate composition. Over 4,563 human exosomal proteins exist with a wide range of functions and origins[10,11]. Since extracellular vesicles contain tetraspanin-enriched membrane microdomains, tetraspanins (CD9, CD63, CD81, CD82) as well as other membrane transport and fusion proteins (flotilin, Ras-related protein Rab-5B) and proteins involved in multivesicular body biogenesis (Alix, Tumor susceptibility gene 101 protein) are specifically used as positive markers to confirm exosomal presence[5]. Some other common proteins within exosomes include fusion and transport proteins, heat shock proteins, adhesion molecules, cytoskeletal proteins and metabolic enzymes[8,12,13]. However, the proteins within each exosome greatly depend on the cell type it originates from[5]. While proteins make up a large part of exosomes, they also contain over 194 lipid structures[11]. The lipid composition of exosomes varies greatly from parental cells containing higher amounts of certain phospholipids and neutral lipids and lower concentrations of phosphatidylcholine[14]. Exosomes contain a lipid bilayer membrane, bioactive lipids, and mostly saturated fatty acids as well as eicosanoids and prostaglandins that are not only transported, but also produced within the exosomes[14]. The membrane lipids function in the formation, release and endocytosis of exosomes[14]. Along with the membrane lipids, carbohydrates also have specific roles in the transport and function of the exosomes[15]. The carbohydrate composition of the exosome membranes varies from parental cells with higher mannose, polylactosamin, α-2,6 sialic acid and complex N-linked glycans, however, no terminal blood group A and B antigens[15]. This composition indicates the carbohydrate function in membrane protein sorting and exosome formation[15].

Aside from proteins, lipids and carbohydrates, exosomes contain significant amounts of RNAs in the form of mRNA, microRNA, and other non-coding RNAs[16]. Some of the RNAs found in exosomes have been shown to produce proteins through an in-vitro translation system[16]. The composition of RNAs in exosomes, just like the protein, lipid and carbohydrate composition, varies greatly between donor cells[17]. In exosomes released from cancer cells, the miRNA content in exosomes is similar to the cancer cell source, alluding to the potential use of miRNA levels in exosomes as a diagnostic marker for cancer and other diseases[18,19]. The discovery of exosomal RNA shows not only the potential for biomarkers as diagnostic tools, but also as vectors for gene therapy[18,19].

Isolation

In order to exploit the use of exosomes in diagnostic and therapeutic applications, it is necessary to establish an efficient and effective way to isolate exosomes with minimal alteration to their structure and contents. Several methods have been developed to isolate exosomes (Table 1) depending on which trait of the exosome is most vital in isolation [20].

The currently accepted gold standard protocol for isolation of exosomes involves differential ultracentrifugation[21,22]. This method applies sequentially increasing centrifugal forces to a solution with exosomes to separate them from cells, large debris, and organelles[23]. This ultracentrifugation typically is often used in combination with sucrose density gradients or sucrose cushions so that the contaminants with densities different from exosomes can be removed[21,23]. The drawbacks of this protocol are that it requires extensive training, expensive laboratory equipment and is highly labor-intensive and extremely time consuming[24]. Some other problems include contamination, difficulty with standardization and exosome loss from heterogeneity and overlapping sizes of extracellular vesicles[20,25]. However, ultracentrifugation produces high purity exosomal isolates and has been estimated to be used in 56% of all exosome isolation techniques used in exosome research[20,24]. Numerous ultracentrifugation techniques including analytical and preparative (differential, density gradient) ultracentrifugation exist to improve yield of pure exosomes that can be further utilized and analyzed in research[20].

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Another method to isolate exosomes is size-based isolation techniques such as ultrafiltration and size exclusion chromatography[20]. Ultrafiltration employs membrane filters to separate exosomes based on size and molecular weight[20]. This process is less time consuming and does not require the special equipment needed for ultracentrifugation[24]. The major limitation of ultrafiltration is that only exosome enriched samples are obtained, not pure exosomes, due to a large number of nanoparticles in the sample with similar sizes to exosomes[24]. The nanoparticles may be removed; however, the means of removal have potential to damage the structure and integrity of exosomes[26]. Another disadvantage of ultrafiltration is that exosomes used in ultrafiltration may adhere to membranes and be lost for analysis[26].

Size exclusion chromatography[27] is another size-based technique used to isolate exosomes using a porous stationary phase consisting of heteroporous cross-linked polymeric gels or beads [20,26]. Size exclusion chromatography has been used together with other techniques to improve purity of isolated exosomes[20]. The main advantage is its ability to maintain the structure and integrity of exosomes and its scalability and reproducibility[26]. Its widespread use is limited by the need for dedicated equipment and long running time[20,24,26].

A newer method used to isolate exosomes is field-flow fractionation which uses a porous rectangular channel and crossflow to isolate exosomes by hydrodynamic diameter[28]. A simple approach recently used to isolate exosomes in large sample volumes is hydrostatic filtration dialysis [29]. Low hydrostatic pressure forces a sample through a dialysis tube and retains exosomes while solvent and solutes are filtered [29]. Hydrostatic filtration dialysis may serve as an initial isolation technique due to the lower labor demand and cost[29].

Due to the abundance of proteins and receptors on exosomal membrane, techniques have been developed based on the affinity of these structures to antibodies and receptors[5]. Specifically, using antibodies to CD63, CD81, and other cell surface markers, either by themselves or in combination, could isolate exosomes and be used to characterize them[5]. This method relies on an antibody bound to a substrate, microbeads or other

structure which then binds the antigen expressed on the exosome[30]. Microplate-based enzyme-linked immunosorbent assay (ELISA) employs this immunoaffinity technique to isolate exosomes and has shown comparable results to ultracentrifugation even with lower sample volumes[20]. The advantages of this technique include its rapid, easy to use approach that requires only routine bench equipment, the ability to differentiate subclasses of exosomes, and the capability to obtain highly pure exosome yields[20,30]. However, immunoaffinity based techniques may require additional size-based isolation due to the expression of some antigens on cells and other membrane vesicles. It may show difficulties when used in tumor studies due to tumor heterogeneity and/or masking of antigen expression on tumor cells surface and the exosomes released by the cells[20].

One method to improve immunoaffinity is magneto-immunocapture[20]. This method uses antibody coated magnetic particles to locate and isolate exosomes, resulting in yields on a par with ultracentrifugation[31]. This magneto-immunocapture has been improved further with the interaction with Tim4 protein on exosomes[32]. Tim4 protein exhibits Ca^{2+} dependent binding to phosphatidylserine, allowing researchers to use Tim4 protein on a magnetic bead to capture exosomes by binding phosphatidylserine on exosome surface [32]. The magnetic bead is then easily separated from exosomes by removing Ca^{2+} with a complexing agent[32]. This method has been shown to have better quality and purity of exosomes than all other techniques[32]. Immunoaffinity capture has also been enhanced by coupling it to mass spectrometric immunoassay and using it in combination with other isolation techniques[18,20].

Similar to the immunoaffinity approach, an affinity capture method using lectins could be used to isolate exosomes via specific saccharide residues present on the surface of exosomes. Lectins function in sugar-binding and cell-agglutination[20]. They have been shown to have a high affinity for surface saccharide residues on urinary exosomes and have been used for isolation of exosomes from urinary samples[33].

Yet another way to isolate exosomes is by precipitating exosomes from samples[24]. Volume-excluding polymers, such as polyethylene glycol[34], are used to precipitate exosomes from culture media[24]. The volume-excluding polymers hold up water

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molecules, allowing less soluble components such as exosomes to precipitate out and then be collected by low speed centrifugation[24]. Numerous commercial exosome precipitation kits are available for isolating exosomes from serum, plasma, ascites, urine, cerebrospinal fluid and culture medium using volume-excluding polymers suck as ExoQuick. The advantages of isolation kits are their speed, straightforward protocol and maintained integrity of exosomes[5]. However, most of these kits still have a lack of specificity towards exosomes with problems of co-precipitation with non-exosomal contaminants, requiring pre and post isolation steps to aid in prevention of contamination[31]. This also yields less pure exosomes and shows difficulty in the standardization of protocol[20].

A newly developing isolation technique is microfluidics-based. Microfluidics-based devices quickly isolate exosomes using size, density and immunoaffinity as well as novel manipulations including acoustic, electrophoretic and electromagnetic isolation techniques[35,36]. This technique shows major advantages due to the benefits of a reduction in sample volume, reagent consumption and isolation time[20] This technique is also able to employ immunoaffinity to identify specific subtypes of exosomes and identify which exosomes contain intact RNA for microRNA profiling[37]. Numerous immunochips have been developed using this novel idea and show potential for a more effective and efficient isolation technique[35,36]. This method has been unable to be translated yet into clinical trials due to problems in scalability, validation and standardization[20].

Biological Functions

The discovery of exosomes came from two studies showing the release of small vesicles from reticulocytes into the extracellular space[38,39]. Since those initial studies of reticulocytes, numerous cell lines have been shown to release exosomes in vitro, including hematopoietic cells, adipocytes, neuronal cells, intestinal epithelial cells, fibroblasts and tumor cell lines[5,13]. Exosomes have also been found in synovial fluid, amniotic fluid, breast milk, saliva, urine, ascites and blood[40,41]. The wide array of locations and variety of exosome compositions has led to an abundance of interests in exosomal function in a variety of clinical research fields. Exosomes have been shown to function in cell to cell

communication during development, immune responses, tumor progression and metastasis, spread of infection, cell death, angiogenesis and coagulation[40,42-45].

Excretion was the first documented function of exosomes[46]. The initial exosomes found were released from reticulocytes to remove certain plasma membrane proteins that need to diminish and disappear during the reticulocyte maturation[46]. These exosomes serve as a mechanism for cells to dispose of unnecessary proteins and RNA. This is useful for cells that are unable to degrade waste by lysosomal and other means or are located close to a drainage system (tubules of kidney, intestinal epithelium) and able to release them into waste products[46,47].

The major role of exosomes with clinical implications is their function in intercellular communication. Exosomes carry proteins, RNA, and lipids that have the ability to influence the functions of recipient cells in several ways. Exosomes mediate cell to cell communication both locally and over long distances and can specifically target certain locations and cell types[5,48]. The methods of communication vary greatly due to the different origin cell and content of exosomes. Exosomes can alter the target cell actions by acting as a signaling complex, transferring proteins, including surface receptors, transferring bioactive lipids, and transferring genetic material in the form of RNA[48,49].

Exosomes have been shown to play a role in the spread of certain viral, bacterial, protozoal and fungal infections. The exosomal transfer of viral proteins and RNA from virally-infected cells to uninfected cells lead to spread of the infection and modulation of the immune responses to these infections[50,51]. Human immunodeficiency virus-1(HIV-1) uses exosomes to transfer RNA to uninfected cells and both infects and elicits the expression of pro-inflammatory cytokines in the target cell[52]. Aside from HIV-1, several RNA viruses including hepatitis C virus (HCV), human T-cell lymphotropic virus and dengue virus have shown exosomal transport of viral proteins and RNA from infected cells to neighboring cells[51]. The alteration of exosomal function may also play a role in the development of tumors from certain classes of viruses, including EBV[53]. For these reasons, exosomes have been promising in the development of antiviral and vaccine treatments[51]. Aside from viruses, exosomes are also able to transfer pathogen-associated molecular patterns

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(PAMPS) from bacteria, protozoa and fungi[50]. Some parasites, as well as gram negative bacteria and fungi, may use their own exosomes to gain access into cells. For example, *Leishmania* exosomes are taken up by macrophages leading to immune response suppression and subsequent infection[54]. While both host-derived and pathogen-derived exosomes are used by organisms to spread infection, the same exosomes play a role in promoting host immunity to these pathogens.

During an HIV-1 infection, exosomes from CD8+ T cells have shown suppression of HIV-1 transcription in infected cells leading to the ability to inhibit HIV replication[55,56]. While HCV has shown viral spread through exosomes, the HCV RNAs present in exosomes also acts to activate IFN-α release from uninfected plasmacytoid dendritic cells leading to activation of innate immune response[57]. While pathogens can employ exosomes to evade immune response and spread infection, the host may employ similar exosomal methods to activate immune response and ward off infection[44]. These exosomal functions may be exploited in the future for therapeutic treatments of severe and difficult to treat infections.

Aside from functioning in the immune response to infection, other immunological functions of exosomes include modulation of antigen presentation, immune activation, suppression and surveillance as well as intercellular communication relating to immunity[42]. Antigen presentation of exosomes was established when Raposo et al (1996) portrayed the release of exosomes displaying MHC-II from B lymphocytes[9]. These exosomes present the peptide-MHC II complexes and can activate T cell responses[9]. Dendritic cells and macrophages have also been shown to produce exosomes which are capable of stimulating T cells[58,59]. However, exosomes have difficulty activating naïve T cells unless they also receive a maturation stimulus from dendritic cells[58,60]. Exosomes have also been shown to be more effective when acting on the plasma membrane of antigen presenting cells that can then display the antigen to T cell [61].

These immune responses of exosomes have been of interest in the fields both of tumor pathogenesis and immunity. Tumor cells must communicate with their environment and neighboring cells. Both tumor cells and immune cells use exosomes for communication to

promote tumor progression and immunity[43]. One proposed mechanism of tumor formation is through tumor-derived exosomes regulating synthesis of cell-independent microRNA and through transfer of oncoproteins promoting proliferation[62]. Aside from direct cell to cell communication to form tumors, exosomes may also play a role in the modulation of tumor microenvironment aiding in tumor invasion and metastasis by altering the factors released from immune cells, inducing differentiation and apoptosis of cells, and encouraging formation of tumor blood vessels due to actions on endothelial cells[63-67].

Along with tumor formation, a large area of interest is exosome function in tumor suppression of immune responses. Exosomes released from tumor cells have been shown to alter the functions of dendritic cells, T cells, macrophages, and natural killer cells[43]. Exosomes have been shown to inhibit the differentiation and maturation of dendritic cells through the proteins transported by them including IL-6, TGF- β, and prostaglandin E2[68]. This exosomal function in immune suppression may provide a means to alter immune response to tumors and prevent tumor progression.

Although exosomes play an important role in pro-tumor mechanisms including the modulation of the microenvironment and the suppression of the immune response, they also are a major factor in antitumor functions. Exosomes released from dendritic cells can activate T cells leading to inhibition of tumor growth[69]. This role of exosomes in eradicating or slowing the growth of tumors has provided means for clinical trials to employ them in difficult cancers[70-72] (Table 2).

Diagnostic Uses

During normal cell function and when pathological injury strikes a cell, numerous components are released from the cell including exosomes[7]. These exosomes carry proteins and nucleic acid with information on the condition of the cell and may be utilized for the diagnosis of various disease states and stages[22]. The exosomes which are released into body fluids contain disease associated markers and are easily accessible in blood, urine and/or saliva making them a very appealing diagnostic marker[22,73]. These

diagnostic markers are currently being researched for use in cancers, cardiovascular disease, kidney disease, and numerous others.

Cancer cell derived exosomes have many components that are currently being explored as diagnostic markers. In ovarian tumor patients, certain exosomal tumor progression related proteins, L1 cell adhesion molecules (CAMs), CD24 and extracellular matrix (ECM) metalloproteinase inducer, were found in higher levels in malignant ascites derived fluid and serum than in healthy patients[74]. Similarly, prostate tumor biomarkers such as Prostate cancer antigen 3 (PCA3) and TMPRSS2: ERG (Transmembrane protease, serine 2:ETS-related gene) were found in exosomes isolated from the urine of prostate cancer patients[75]. In melanoma patients, CD63 and CAV1+ plasma exosomal proteins were also found to be significantly higher in tumor patients when compared to healthy donors[73]. These protein markers may allow for the development of screening tests for clinical biomarkers of not only tumor discovery, but also staging, progression, and even recurrence[73]. Aside from protein markers, microRNAs that are contained within exosomes also have potential diagnostic use in ovarian, lung, colon, prostate and breast cancer[25,76-80].

While tumors secrete exosomes of varying content that can be used to detect their presence, healthy and diseased cardiomyocytes also release exosomal proteins and RNAs that may aid in their detection[81,82]. Specifically, adult myocytes under hypoxia were shown to have increased levels of HSP60[82]. Acute myocardial infarcts have been shown to release microRNAs specifically miR-1 and miR-133a[83]. These microRNAs, especially miR-133a is detectable at significantly elevated levels in the serum of cardiovascular disease patients[84]. Some of these heart-specific exosomal miRNAs, especially miR-1, have also been shown to enter urine and been detected in significantly elevated levels in patients with acute myocardial infarcts[85] These findings in both serum and urine may provide means to detect injured myocardial, perhaps even before the elevation of serum creatinine phosphokinase or cardiac troponins[83].

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Like cardiovascular disease, exosomes may also be a potential biomarker for acute kidney injury (AKI) and renal disease. Both fetuin-A and AQP1 have been shown to be potential biomarkers in urinary exosomes after AKI[86]. AQP1 is decreased significantly in AKI events due to renal ischemia-reperfusion episodes[87]. Fetuin-A was increased after AKI and was seen to be elevated prior to the elevation of serum creatinine[88]. Since AKI shows varying levels of exosomal content, renal fibrosis and end stage renal disease have shown alterations, both increases and decreases, in various levels of exosomal content indicating specific disease state [86]. These urinary levels of exosomal contents may allow for non-invasive testing and diagnosis of a variety of renal diseases in the near future.

While the exosomal content appears to show great promise as a diagnostic biomarker in the diseases mentioned above and numerous others (Table 3), it is still not being utilized in clinical practice[84]. Exosome based diagnostic tests have been and are being developed currently. These tests have been used successfully in ovarian cancer, lupus nephritis and after kidney transplant to predict outcomes, but not yet diagnosis[89-91]. Exosomes as biomarkers may provide a future avenue for the diagnosis, staging and outcomes of a variety of human diseases after more insight and large scale clinical studies are conducted. While research into the use of exosomes as diagnostic markers is greatly expanding as a young field, the discoveries, especially of protective exosomes, show potential not only in diagnostics, but also in therapeutic treatments.

Therapeutic Uses

The expanding knowledge of the roles of exosomes in physiologic and pathologic processes has led to a promising interest in their use as therapeutic vehicles[92]. The role of exosomes in cell to cell communication, immune modulation and progression of neurodegenerative diseases and cancer has provided numerous dimensions for prospective therapeutic applications[92]. Aside from their physiologic and pathologic function, exosomes may also allow for a channel for the delivery of therapeutic agents including drugs and biomolecules[93,94]. They do not require direct use of cells which has posed numerous difficulties, they can cross the blood-brain barrier and may be targeted to specific tissues using surface receptors[2,95]. Along with utilizing exosomes for treatment,

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stopping exosomes that lead to disease progression is another potential avenue of therapeutic use[92]. These numerous factors have greatly advanced research into exosomes functioning as therapeutic vehicles that can aid and interfere with disease and infectious processes[96]. The therapeutic use of exosomes is currently being explored in cancers, CNS diseases, immune mediated conditions, and a variety of other fields including specific roles in drug and biomolecule delivery[23,94,96] (Table 2).

In cancer as discussed previously, exosomes play a role not only in the invasion, metastasis, and angiogenesis of tumors, but on the other side they also function in the immune reaction against tumors[92]. These roles may allow numerous therapeutics options to be developed both utilizing exosomes to fight tumors and ridding the body of them to stop tumor progression. The latter may be possible by halting exosome formation, release and uptake[92]. Aethlon Medical Inc. (San Diego, CA, USA) has designed a plasmapheresis platform which decreases the systemic secretion of HER2-positive exosomes and inhibits progression of HER2-positive breast tumors[97]. While the elimination of exosomes has shown useful in some cancers, trials using dendritic cell-derived exosomes for their immunologic antitumor properties has been successful in combination with other cancer treatments in other cancers[98]. Aside from utilizing or fighting the inherent properties of exosomes, cancers and other diseases may be effectively treated using exosomes as drug delivery vehicles.

Exosomes may be made as drug delivery vehicles in three distinct ways: 1) exosomes may be isolated from donor cells and then loaded, 2) donor cells can be loaded with therapeutic agents and the exosomes released can be captured, and 3) donor cells can be infected with DNA encoding therapeutic compounds or specific surface proteins and the exosomes released can be captured[23,99]. These three means provide numerous avenues depending on the therapeutic agents used, diseases being addressed and type of exosomes needed[23]. Exosomes as drug delivery systems have numerous advantages including their ability to target specific tissues, ability to cross numerous barriers, including the blood-brain barrier, and their tolerance within body fluids[92]. These properties may allow enhanced delivery of drugs to locations where they are needed and avoidance of unnecessary side effects when drugs are distributed throughout the body[92]. Exosomes

can carry numerous compounds including proteins and nucleic acids and even anti-inflammatory and anticancer agents[92]. All these properties may allow exosomes to be brought into clinical practice in a variety of disease fields[92]. These methods have allowed clinical trials to be initiated within numerous cancer fields including metastatic melanoma, non-small cell lung cancer and colorectal cancer[70-72].

A promising field in exosomal therapeutics is the use of stem cell exosomes for the regeneration of damaged tissue in a variety of organs and diseases. Exosome based therapies are currently proving to be more advantageous then stem cells alone due to greater stability, quicker incorporation into host with no issues of cell loss and the ability to alter exosomal content for targeted therapies[100]. Exosomes from human cardiac stem cells showed enhanced angiogenesis and promoted regeneration of infarcted human heart[101,102]. In murine models, human MSC exosomes showed decreased inflammation and reduced collagen deposition in carbon tetrachloride induced fibrotic liver disease[103]. Along similar lines, stem cell derived exosomes enhanced lung tissue recovery in hypoxia induced pulmonary hypertension and enhanced neural cell growth and recovery after stroke[104,105]. The main mechanism discovered in these experiments were through the action of exosomal contents, specifically miRNAs leading to inhibition of inflammation and activation of proliferation and regenerative responses[101,102,106]. These stem cell exosomal therapies acknowledge the key role of exosomes in the regeneration of damaged tissues. This may hold the key to the utility of exosomes as cell-free therapeutic candidates in a variety of disorders.

Future Exosome Uses as Orthopedics Therapy

One particularly interesting field of research involving stem cell exosomes is potential use in the treatment of orthopedics disorders. Numerous orthopedic diseases could be treated with exosomes including bone fractures, rheumatoid arthritis (RA) and osteoarthritis. While this is a young field, a wealth of research is underway exploring avenues to employ exosomes to halt and alter the course of these disorders.

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A potential use of exosomes in this field is packaging miRNAs within exosomes and delivering this into joints to prevent inflammation and tissue damage[7]. One study performed in rats with mono-iodoacetate induced arthritis found that miRNA-101 prevents cartilage degradation[107]. In other experiments, miRNAs were involved in endochondral ossification and cartilage development in rat models[108]. These functions of miRNA may lead to their potential use in prevention and treatment of osteoarthritis. Exosomal miRNA-494 also functions in angiogenesis and myogenesis which can be utilized in orthopedics to aid in quicker recovery times.

Another potential therapeutic avenue is through the blockage of naturally occurring exosomes which function in bone related complications and osteoarthritis. Exosomes released from multiple myeloma have been proven to cause osteoclast differentiation and result in the osteolytic lesions, bone pain and bone degradation that occurs with this disorder[109]. Inflammatory cytokines and matrix metalloproteinases involved in osteoarthritis could be products of exosomes[7]. If the exosomes involved in multiple myeloma or osteoarthritis can be identified and made non-functioning, exosomes targeted therapy may be a key component in treatment if these diseases. *In mouse models, exosomes from synovial mesenchymal stem cells promote chondrocyte proliferation and migration[110]. In rat models, the MSC-derived exosomes promote cartilage regeneration even with a full-thickness cartilage defect[111]. These factors may make exosomes a viable therapy for osteoarthritis and fractures [110].*

Another major disease with a promising future for exosomal treatment is RA, an autoimmune disorder leading to degradation of joints. Exosomes from synovial fibroblasts of individuals with RA have a membrane bound TNF-*α that allows T cells to be resistant to apoptosis[112]. Another substance believed to be a major inducer of RA is citrullinated proteins, which have been detected in exosomes from synovial fluid[113]. If these exosomes were restrained, the progression of RA could be halted. Rat models with collagen induced arthritis treated with dendritic cell exosomes have shown anti-inflammatory effects, as well as the ability to suppress arthritis onset and reduction in the severity of arthritis[114,115]. These avenues hold great promise within the field of orthopedics. However, significantly more research and clinical trials are necessary to discover the*

mechanisms and therapy opportunities available to employ therapeutic functions of exosomes to orthopedics.

Conclusions

Recently there has been an exponential increase in research discovering the structure and makeup of exosomes as well their physiologic and pathologic functions. These newly developing insights have provided avenues for exosomes to be utilized within numerous aspects of clinical medicine. Exosomes have shown potential use in diagnostic and therapeutic applications in various fields of medicine with even more being discovered as the knowledge of exosomes expands. While a great deal of information about exosomes has recently been discovered, there is still a great deal more to learn, understand and begin to utilize. This is an exciting time in the field of exosomal research and the knowledge and functional use of exosomes only continue to expand and improve, becoming more efficient to study and more effective in diagnostic and therapeutic applications.

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Table 1: Various isolation methods available for exosomes [20].

Isolation Methods	
Differential centrifugation	Magneto-Immunocapture
Ultrafiltration	Lectin Affinity Capture
Size Exclusion Chromatography	Volume Excluding Polymers and Centrifugation
Field-Flow Fractionation	Microfluidics-based
Microplate-based ELISA	

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Characterization and Therapeutic Uses of Exosomes: A New Potential Tool in Orthopedics (DOI: 10.1089/scd.2018.0205)
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Table 2: Clinical trials with exosomal interventions (clinicaltrials.gov).

List of Diseases with Active Clinical Trials Involving Exosomal Therapies	
Acute Ischemic Stroke	**Melanoma**
Breast Cancer	**Osteosarcoma**
Cholangiocarcinoma	**Pancreatic Cancer**
Colon Cancer	**Parkinson's Disease**
Gastric Cancer	**Prostate Cancer**
Head and Neck Cancer	**Sepsis**
Lung Cancer	**Type I Diabetes**
Macular Damage	**Wound Healing**

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Table 3: List of exosomal markers with potential use as diagnostic markers [73-75,82,84,85,88].

Potential Diagnostic Uses of Exosomes	
Exosomal Marker	**Diagnosis**
L1 CAMs, CD24 and ECM metalloproteinase inducer	Ovarian cancer
PCA3 and TMPRSS2: ERG	Prostate cancer
CD63 and CAV1+	Melanoma
HSP60	Hypoxic myocytes
miR-1 and miR-133a	Acute Myocardial Infarction
fetuin-A and AQP1	Acute Kidney Injury

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