



The Biologic Treatment of Osteoarthritis with Mesenchymal Stem Cell Exosomes: The Future is Now

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Abstract

Over the last several years it has become increasingly understood by researchers and clinicians that the clinical efficacy of utilizing Mesenchymal Stem Cells (MSCs) to treat Osteoarthritis (OA) is not dependent on the cells differentiating into articular cartilage but entirely on their paracrine release of Growth Factors (GFs) and exosomes. Living MSCs are not required to accomplish the release of GFs and exosomes into an arthritic joint. The purpose of this paper is to introduce the concept of using acellular MSC exosomes to treat OA and the rationale of why acellular will replace all current cellular therapies both autogenous and allogeneic.

Keywords: Bone marrow concentrate; Cell-based therapy; Exosomes; Mesenchymal stem cells; Stem cells

What Causes the Pain in Osteoarthritis?

In patients with Osteoarthritis (OA), why is the correlation between what a patients imaging studies look like and their report of pain so low? Articular cartilage has no direct nerve or blood supply. Without direct innervation, cartilage is incapable of generating pain [1].

In contrast, the synovium and joint capsule are richly innervated and are likely the primary source of the pain in OA [2]. The synovial reaction in OA includes synovial hyperplasia, fibrosis, thickening of the synovial capsule, activated synoviocytes and in some cases lymphocytic infiltrate (B- and T-cells as well as plasma cells) [3-7]. The synovium is of obvious relevance as one of the most densely

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Citation: Pettine KA, Dordevic M (2019) The Biologic Treatment of Osteoarthritis with Mesenchymal Stem Cell Exosomes: The Future is Now. J Stem Cell Res Dev Ther: S1001.

Received: February 15, 2019; **Accepted:** March 06, 2019; **Published:** March 13, 2019

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innervated structures of the joint. The white adipose tissue of the fat pad can also show evidence of inflammation and act as a rich source of inflammatory adipokines [8]. Synovial causes of pain include irritation of sensory nerve endings within the synovium from osteophytes and synovial inflammation that is due, at least in part, to the release of prostaglandins, leukotrienes, proteinases, neuropeptides and cytokines. Pro-inflammatory examples include Interleukins 1,6 and 8 along with various tumor necrosis factors. A semi-quantitative measure of synovitis from the infrapatellar fat pad is associated with pain severity. Any decrease in synovitis is associated with a reduction in OA pain severity [9].

What is the Future for using MSC Exosomes to Treat OA?

The MSC has always been the primary cell for orthopedics because only it can become a chondroblast, osteoblast or fibroblast. Recently we have begun to appreciate and realize the MSC may be the most critical cell in your body because of what it releases to communicate with other cells. The MSC modulates your immune system to control inflammation by releasing exosomes, secretomes, growth factors, cytokines, and chemokines. These proteins are what is essential in regenerative medicine, NOT the MSC itself. Caplan even suggests changing the name to Medicinal Signaling Cells [10].

Arnold Caplan, the Ph.D. responsible for naming the Mesenchymal Stem Cell (MSC) states, "Now that Mesenchymal Stem Cells (MSCs) have been shown to be perivascular *in vivo*, the existing traditional view that focuses on the multipotent differentiation capacity of these cells should be expanded to include their equally interesting role as cellular modulators that brings them into a broader therapeutic scenario. We discuss existing evidence that leads us to propose that during local injury, MSCs are released from their perivascular location, become activated, and establish a regenerative microenvironment by secreting bioactive molecules and regulating the local immune response. These trophic and immunomodulatory activities suggest that MSCs may serve as site-regulated "drugstores" *in vivo*" [11]. Extensive research has shown Caplan to be correct.

The MSC produces numerous growth factor proteins to treat orthopedic pathology. But the most crucial paracrine method by which the MSC functions may be through the creation of the acellular structure named the exosome [12-15]. The exosome is a tiny 30 to 150 nanometer-sized (1 billionth of a meter) bi-phospholipid membrane-enclosed structure created by the Golgi body or apparatus. An MSC (12 to 18 microns) is 1,000 times larger than an exosome. The diameter of a hair is 80,000 nanometers. Exosomes contain growth factors, signaling lipids and micro, and messenger RNA. The RNA contents in exosomes mediate most of their anti-inflammatory effects. The RNA is placed into an exosome along with numerous peptide growth factors and signaling lipids by the Golgi bodies within the donor MSC. The exact type and amount of growth factor proteins, signaling lipids, and RNA placed into an exosome are dependent on the surrounding microenvironment of the MSC. The exosomes are released into the extracellular matrix and taken up by a receptor cell. The exosome RNA is then taken into the receptor cell ribosome where the RNA

is translated to create numerous anti-inflammatory growth factors, chemokines, cytokines, and secretomes. This process is illustrated in figure 1. Exosomes do not elicit acute immune rejection, and there is no risk for tumor formation. The effect of exosome RNA may last months or longer as the receptor cell ribosomes continue to translate the donor RNA [16-20]. BEES MAKE HONEY AND MSCs MAKE EXOSOMES [21].

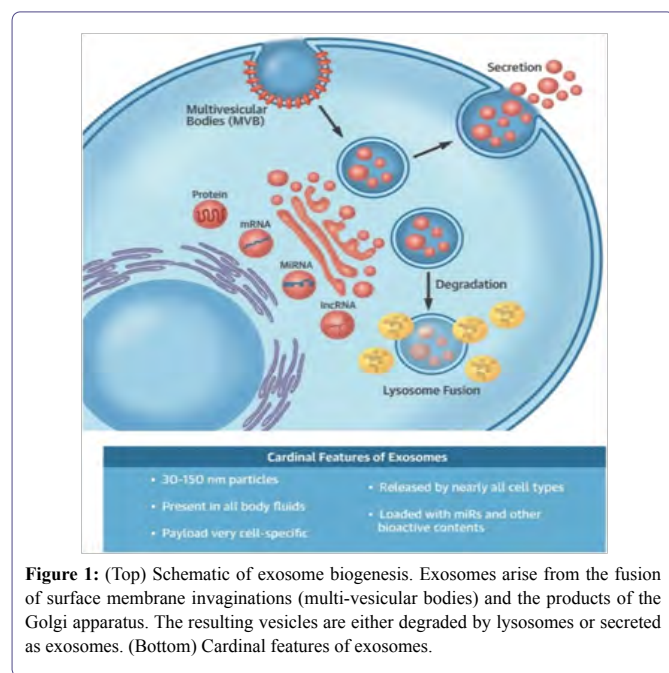


Figure 1: (Top) Schematic of exosome biogenesis. Exosomes arise from the fusion of surface membrane invaginations (multi-vesicular bodies) and the products of the Golgi apparatus. The resulting vesicles are either degraded by lysosomes or secreted as exosomes. (Bottom) Cardinal features of exosomes.

The amount of scientific research on exosomes has dramatically increased since 2008 when it was discovered exosomes contain DNA. There were 28 scientific citations on exosomes in 1996 and 24,765 in 2016 [22]. Exosome research has created a renaissance in our understanding of cellular communication. Cells communicate near and far by a dynamic of exosome secretion and uptake. Extensive research is ongoing for utilizing exosomes for the treatment of various autoimmune conditions, the diagnosis and cure for various cancers and in treating cardiac diseases.

Phinney recently published an excellent concise-review on MSC-derived exosomes for cell-free therapy and stated: “The majority of the published MSC exosome literature recapitulates in large part the nature and scope of that previously devoted to the study of MSC action in animal models of disease. For example, various groups have confirmed that MSC-derived exosomes exhibit cardio and renal-protective activity, are efficacious in animal models of myocardial infarction, stroke, perinatal hypoxic-ischemic brain injury, and hind-limb ischemia. The MSC-derived exosomes also ameliorated carbon tetrachloride-induced liver fibrosis and conferred cytoprotective effects in models of necrotizing enterocolitis. In lung studies, the mouse MSC exosomes were effective in improving pulmonary hypertension, silicosis and human MSC-exosomes improved endotoxin-induced pulmonary edema and cleared alveolar fluid from human lungs. Other studies have shown that MSC-derived exosomes also promoted re-epithelialization of cutaneous wounds by inducing epithelial cell proliferation and angiogenesis, activated collagen and elastin secretion by fibroblasts and prevented myofibroblast formation thereby reducing

scarring. The MSC-derived exosomes also promoted muscle regeneration, protected against experimental colitis and exhibited potent neuroprotective activities in neurons and in models of traumatic brain injury. MSC-derived exosomes are also immunologically active based on evidence that they suppressed proliferation and IFN- γ secretion by T cells stimulated with anti-CD3 and anti-CD28 antibodies and also enhanced the survival of allogeneic skin grafts in mice by enhancing T cell polarization to a regulatory phenotype. A growing number of studies suggest that MSC-derived exosomes mimic the ability of MSCs to influence the activity of immune effector cells including B, T, NK, dendritic cells, and macrophages although not all studies show positive effects. Collectively, these studies readily demonstrate that MSC-derived exosomes recapitulate to a large extent the immensely broad therapeutic effects previously attributed to MSCs” [23].

This is illustrated in figure 2. “Think of the extracellular space as a sea containing trillions of messages in a bottle, quickly read and answered, always turning over, and you begin to get a sense of what is going on inside us every moment of every day” [24].

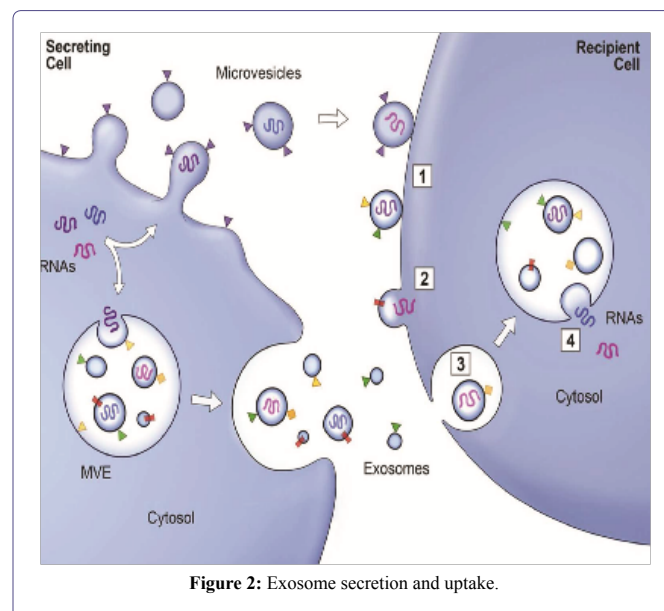


Figure 2: Exosome secretion and uptake.

What is the Future for Using MSC EXOSOMES to Treat OA?

MSCs direct the anti-inflammatory function of other cells by releasing exosomes into the ECM. The future of using MSCs to treat OA will be to expand the MSCs in a defined growth media. The cells are then subjected to 48 hrs. of “stress” conditions of hypoxia, low glucose and low pH to maximize their release of anti-inflammatory exosomes. The growth media is collected, and the exosomes separated and stored for future use [24]. The future of regenerative medicine is the use of ACELLULAR vs. cellular products. Acellular MSC derived exosomes can provide a consistent product that can have proteomic analysis and RNA sequencing. Every growth factor can be identified and quantified. Every micro and messenger RNA can be characterized. Think of acellular MSC exosomes as a bio-pharmaceutical quality product that can be standardized and tested regarding dose and biological activity.

None of this is possible with a cellular product. Perhaps most important is that an acellular product will not introduce extensive foreign DNA into the recipient patient that an allogeneic cellular source does. No one knows the long-term effects of having foreign DNA. Is it carcinogenic? Replacing the administration of live cells with acellular exosomes will mitigate the safety concerns and limitations associated with the transplantation of viable replicating cells [25].

MSC derived exosomes do not have any of the immunogenic concerns related to the administration of allogeneic cellular products. Acellular exosome ‘off-the-shelf’ products have no immunogenicity [26]. There have been measured to be 10^{10} exosomes per cc of conditioned growth media [27]. Exosomes can be stored at room temperature for up to three years with no loss to their biological activity [28,29]. In contrast to cell-based therapy, MSC derived exosomes provide an ‘off-the-shelf’ therapeutic product that has safety and may have clinical efficacy superior to any allogeneic MSC treatment for orthopedics and spine [23,30].

The Acellular MSC Paracrine Treatment for OA

All di-artrodial joints have a synovial lining and a joint capsule. The synovial capsules contain numerous synovial MSCs (more than found in bone marrow or adipose). These MSCs have more chondrogenic potential than bone or adipose MSCs [6,7]. During the development of OA, pro-inflammatory growth factors are produced by these synovial MSCs. This creates a chronically inflamed painful joint environment. Bone Marrow Concentrate (BMC) contains on average only about 2,500 MSCs per cc [27]. Despite the incredibly small number of MSCs found in BMC; there is extensive literature reporting clinical efficacy in animals and humans using BMC for the treatment of OA. This effect cannot be dependent upon BMC/MS cell survival or differentiation. The efficacious effect must be from the release of acellular paracrine factors. The future of the biologic treatment of OA will be the utilization of acellular MSC derived growth factors, secretomes, chemokines, cytokines and especially exosomes. These paracrine factors can be placed into the knee joint in concentrations of 100,000 or more times that of any cellular MSC treatment. These proteins and exosomes will function in a paracrine fashion to, directly and indirectly, alter the inflammatory environment of any painful arthritic joint to a normal non-painful physiologic environment. Zhang et al., [31] investigated the cellular processes modulated by MSC exosomes and the mechanism of action underlying the exosome-mediated responses in cartilage repair. They observed that exosome-mediated repair of osteochondral defects was characterized by increased cellular proliferation and infiltration, enhanced matrix synthesis and a regenerative immune phenotype. Using chondrocyte cultures, they could attribute the rapid cellular proliferation and infiltration during exosome-mediated cartilage repair to exosomal CD73-mediated adenosine activation of AKT and ERK signaling. Inhibitors of AKT or ERK phosphorylation suppressed an exosome-mediated increase in cell proliferation and migration but not matrix synthesis. The role of exosomal CD73 was confirmed by the attenuation of AKT and ERK signaling by AMPCP, a CD73 inhibitor, and theophylline, an adenosine receptor antagonist. Exosome-treated defects also displayed a regenerative immune phenotype characterized by a higher infiltration of CD163+ regenerative M2 macrophages over CD86+ M1 macrophages, with a concomitant reduction in pro-inflammatory synovial cytokines IL-1 β and TNF- α . Together, these observations demonstrated that the efficient osteochondral regeneration by MSC exosomes

was effected through a coordinated mobilization of multiple cell types and activation of several cellular processes.

The future acellular treatment for OA will involve a two-front attack. First, highly concentrated anti-inflammatory MSC derived growth factors are injected into the arthritic joint. These growth factors will enter the nucleus of the recipient synovial MSC. The donor growth factors will stimulate DNA transcription of mRNA containing instructions for the production of continuous anti-inflammatory secretomes, chemokines, and cytokines. These will be released from the recipient synovial MSC into the synovial fluid. Second, the highly concentrated donor exosomes will enter recipient synovial MSCs to deliver their mRNA. This delivered mRNA will directly undergo translation in the recipient synovial MSC ribosomes to produce anti-inflammatory secretomes, cytokines, and chemokines. These salubrious effects could last months or years. This acellular biologic treatment can all be achieved with a single arthritic joint injection, not requiring the morbidity and cost of obtaining autogenous MSCs. The future of regenerative medicine in orthopedics and spine may well be the utilization of highly concentrated acellular MSC derived growth factors and especially EXOSOMES [32-34]. Figure 3 illustrates some of the mechanisms on how a single injection of bone marrow-derived MSC exosomes can have a salubrious effect on returning the inflamed OA joint to a normal physiologic state.

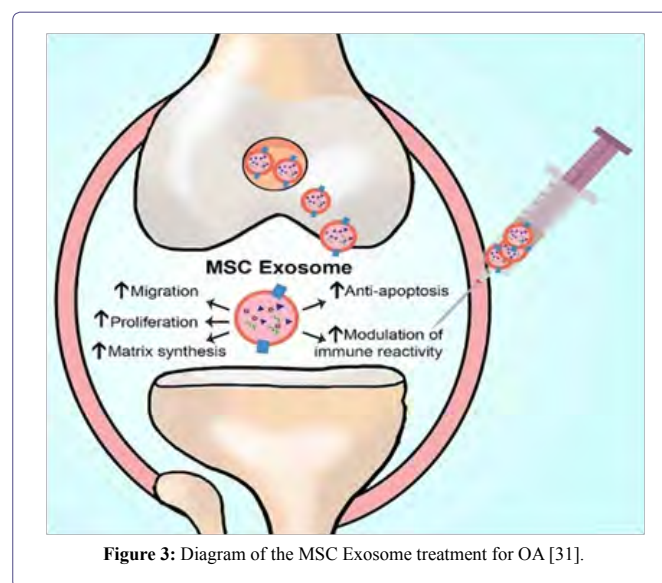


Figure 3: Diagram of the MSC Exosome treatment for OA [31].

Conclusion

The pain of osteoarthritis is mostly the result of inflammation of the synovial capsule. This synovitis is perpetuated by the intra-articular synovial mesenchymal stem cells. Bone marrow MSCs create intracellular exosomes that are filled with hundreds of various anti-inflammatory growth factors along with micro and messenger RNA. The anti-inflammatory growth factors and RNA redirect the synovial MSC's from inflammation to a physiologic state. Autogenous cellular treatments have no consistency between donors. Allogeneic cellular products introduce large amounts of foreign DNA and after cell death, unwanted cellular debris including membranes, mitochondria, Golgi bodies, cytoplasm, etc., all within the joint

inflammation. Acellular products can be produced to have a consistent known quantity of GFs and exosomes along with proteomic analysis of the GF proteins and a genetic analysis of the RNA. This represents a bio-pharmacological quality product. Acellular MSC exosomes deliver the positive aspects of cellular therapy without all the negative aspects of cellular therapy.

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