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Use of Extracellular Vesicles for Cell-Free Regenerative Medicine in Osteochondral and Bone-Related Therapies

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Abbreviations: C28/I2: Human Primary Chondrocytes Cell Line; FLS: Fibroblast Like Synoviocyte; HASC: Human adipose stem cells; hBM-MSC: Human Bone Marrow Mesenchymal Stem Cells; hESC: Human Embryonic Stem Cells; hiPS-MSC: Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells; hMSC: Primary Human Marrow Derived Stromal Cells; HUVEC: Human Umbilical Vein Endothelial Cells; mBM-MSC: Murine Bone Marrow Mesenchymal Stem Cells; MC3T3-E1: Murinepreosteoblastic Cell Line; RA: Rheumatoid Arthritis; SMSCS: Synovial Mesenchymal Stem Cells

Introduction

The tissue engineering paradigm considers cells, signals and scaffolds as the major elements of tissue engineering approaches to repair and/or regenerate tissues [1]. Unexpectedly, a paradigm shift is taking place in this field by the only use of extracellular vesicles (EVs) to deliver the right signals to the damaged tissue. In recent years, EVs role in intercellular signalling has begun to emerge [2]. EVs range in size from 30 to 1000 nm and can be derived from the endosomal system (exosomes, 70-150 nm) or produced by outward budding of the plasma membrane (micro vesicles, 100-1000 nm) [3,4]. All EVs are enriched in proteins, lipids, and nucleic acids (DNA, mRNA, miRNA, tRNA) that can be delivered to recipient cells for cell-to-cell communication [5]. In fact, EVs have recently evolved to be vital components of cell-based therapies based on the observations that the beneficial effects of cell therapies could not be attributed to cell survival and differentiation, leading to the thought that cell therapies act in a paracrine rather than in a cellular manner [6]. This shift was based on *in vivo* data showing that stem cell engraftment and differentiation at injury sites was very low and transient [7-11]. And on the observation that conditioned media from cultured stem cells reproduces some of the beneficial effects of intact cells [12,13]. This paracrine effect exerted by stem cells would depend on their capacity to secrete soluble factors [14], but also, by the release of EVs [15]. In particular, preclinical models studying graft versus host disease, acute kidney failure and ischemic stroke suggest that EVs exert the stem cells' therapeutic effects [16-18].

These findings lead to the hypothesis that EVs could be a cell-free alternative for regenerative medicine [19]. EVs therapy presents several benefits over cell-based therapies, such as the possibility to sterilize the EVs by filtration, their off-the-shelf use, and the variety of available storage buffers or the simple requests on storage conditions [20]. Different cell sources for EVs isolation are being investigated for treatment of a wide variety of diseases and the evaluation of all recent work on EVs for regenerative medicine is beyond the scope of this mini-review and has extensively been reviewed by others [21-23]. Here, we focus on the use of EVs as a cell-free treatment in osteochondral and bone-related diseases (Table 1). For osteochondral diseases, mainly Mesenchymal Stem Cells (MSC) — Synovial Stem Cells (SMSCS) [24], human Bone Marrow Stem Cells (hBM-MSC) [25], Human Embryonic Stem Cells (hESC) [26] or Murine Bone Marrow Mesenchymal Stem Cells (mBM-MSC) [27] — but also neutrophils or synovial fluid [28,29] have been used as source of exosomes and microvesicles. The osteochondrogenic effect of these EVs has been reported using *in vitro, in vivo* or even ex vivo models, showing a better fracture healing and tissue repair when wounds in cartilage are treated with exosomes [25,26] and a modulation of inflammation when wounds are treated with microvesicles derived from neutrophils and synovial fluids.

For bone-related diseases, exosomes derived from MSC or from induced Pluripotent Stem Cells (iPSC) have been used, reporting osteogenic effects in vitro [30–32] and /or in vivo [25,30–33], using different models. Nevertheless, exosomes from other sources are also being studied, such as Adipose Stem Cells (HASC) [34], mineralizing preosteoblasts [35], osteosarcoma cells [25], monocytes [36] or dendritic cells [37]. Two important examples of lack of osteogenic activity are described for exosomes derived from osteosarcoma and dendritic cells exosomes derived. Nevertheless,

dendritic cells show an important role in MSC recruitment despite this lack of osteogenic nor chondrogenic activity [37]. Nevertheless, dendritic cells show an important role in MSC recruitment despite this lack of osteogenic nor chondrogenic activity [37]. What is more, for treatment of bone related diseases, the most recent advances result from the combination of exosomes with already existing biomaterials, such as collagen membranes [32], tricalcium phosphate [30,33] or polymeric scaffolds [34], pointing out that further studies formulating new biomaterials with exosomes will play a key role in the field of bone regenerative therapies in the next years.

In conclusion, the use of extracellular vesicles is emerging as a shift in the basic paradigm in tissue engineering and regenerative medicine as a strategy for cell-free therapies.

Table 1: EVs sources and therapeutic targets for cell-free regenerative medicine in osteochondral and bone-related therapies.

Source	Therapeutic Target	EV subtype	Study model	References
SMSCS	Osteoarthritis	Exosomes	*In vivo* (rat)	[24]
hBM-MSC & HOS	Endochondral ossification in bone fracture	Exosomes	*In vivo* (mouse)	[25]
hESC	Osteochondral defects	Exosomes	*In vivo* (rat)	[26]
mBM-MSC	Protection from cartilage and bone degradation	Exosomes and microvesicles	*In vitro* (mouse)	[27]
Neutrophils (from control and arthritis patients)	Inflammation in the synovia	Microvesicles	*In vitro* (Macrophage-FLS Co-Cultures) *In vivo* (mouse)	[28]
Human RA Synovial Fluid & Neutrophils	Rheumatoid arthritis	Microvesicles	*In vitro* (C28/I2 cell line) *In vivo* (mouse) *Ex vivo* (rat cartilage explants)	[29]
hiPSC-MSC	Osteoporosis	Exosomes	*In vitro* (osteopenic rat's BMSC) *In vivo* (osteopenic rat)	[30]
Mutant BMSC	Femoral head necrosis	Exosomes	*In vitro* (2D and 3D HUVEC) *In vivo* (rabbit)	[31]
hMSCs	Bone regeneration model	Exosomes	*In vitro* (2D and 3D hMSC) *In vitro* (mouse)	[32]
hiPS-MSC	Bone defects	Exosomes	*In vivo* (rats)	[33]
HASC	Bone defects	Exosomes	*In vitro* (hBMSC) *In vivo* (mouse)	[34]
Mineralizing MC3T3-E1	Bone regeneration model	Exosomes	*In vitro* (ST2 mouse BMSC) *In vivo* (mouse)	[35]
Human monocytes	Bone regeneration model	Exosomes	*In vitro* (hMSC)	[36]
Primary human dendritic cells	Bone regeneration model	Exosomes enriched Evs	*In vitro* (hMSC)	[37]

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