exosmart: Autologous Platelets Exosomes

INTRODUCTION:

Plasma contains a high amount of extracellular vehicles (EVs) produced by different cells such as leucocytes, erythrocytes, dendritic cells (DCs), platelets, mast cells, epithelial cells, endothelial cells and neurons.

Extracellular vehicles (EVs) are small membrane-enclosed structures with various functions and different origins. Three major types of extracellular vesicles are: apoptotic bodies (>1000 nm), macrovesicles (100–1000 nm), and exosomes (30–100 nm).

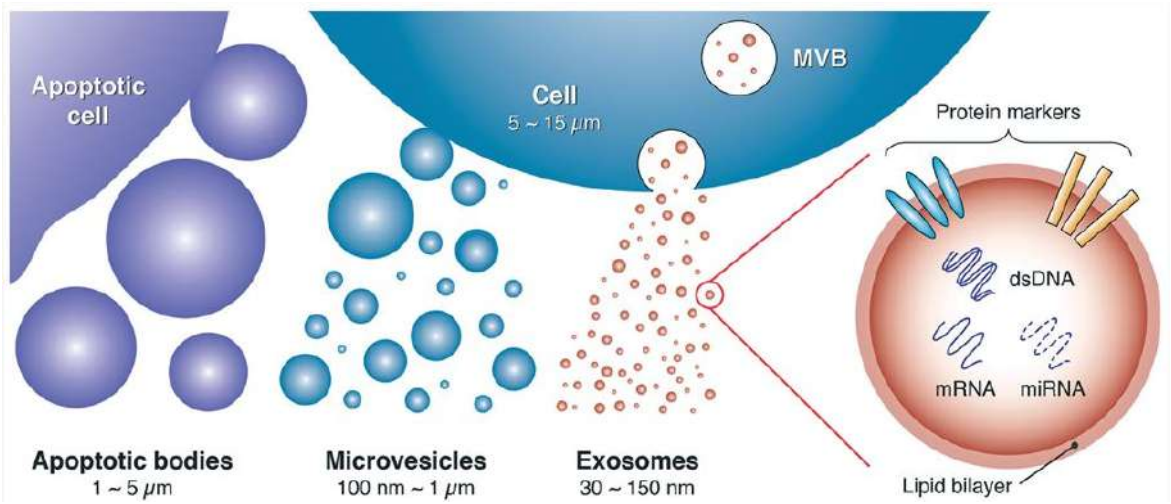

Fig.1 - Types of extracellular vesicles.

Of these vehicle types, exosomes have received a large amount of interests in research because of their roles in intercellular communication, immunomodulatory function, and their potential for use in identifying and treating diseases.

The exosome composition is variable and seems to reflect the physiological status of the secretory cell. The exosomes can hold approximately 100 proteins and 10,000 net nucleotides of nucleic acid. They may also have a unique protein, lipid, and carbohydrate composition.

Characteristic markers of the exosomes exposed on the membrane are Alix, Tsg101, Hsc70, CD63, CD81 and CD9.

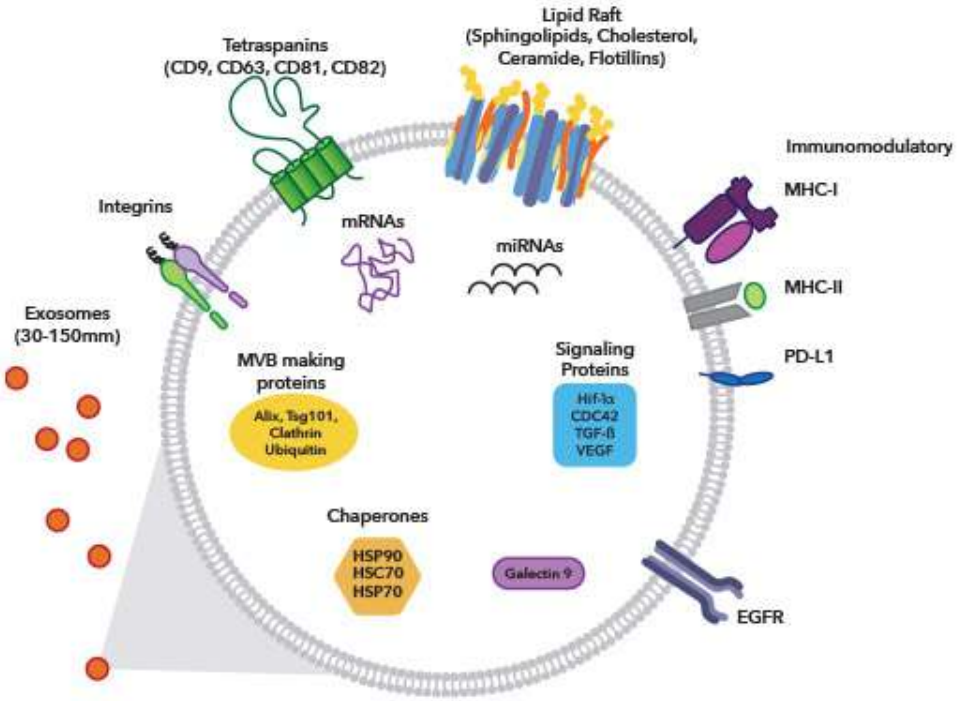

Fig.2 - Protein composition of an exosome

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. The methods of communication vary greatly because of the different origin cell and content of exosomes.

PLATELETS/PRP EXOSOMES

Platelets, originating from megakaryocytes in the bone marrow, are an important part of the blood, and are involved in various pathological processes, such as hemostasis, thrombosis, and the immune response.

Platelet-rich plasma (PRP) represents a well-known autologous derivative of whole blood, which aims to increase the number of platelets and the concentration of molecular mediators that exert therapeutic effects, while eliminating unwanted elements such as red blood cells.

Thus, the selective enrichment in growth factors and anti-inflammatory cytokines is considered to be responsible for the effects of PRP in improving clinical conditions in a variety of disease situations.

However, in addition to releasing free proteins into the affected environment, it is also known that platelets release proteins encapsulated in extracellular vehicles (EVs) known as exosomes. Similar to PRP, exosomes exert restorative effects, although their contribution to the healing and regenerative capacity of PRP is still unknown.

In recent years, exosomes isolated directly from PRP (PRP-Exo) have attracted increasing attention as potential mediators of the effects of PRP and platelet lysates in **tissue regeneration**.

In fact, there is evidence that PRP-Exo are rich in the molecular mediators responsible for the healing effect of PRP. Indeed, human PRP Exo have shown to increase cell proliferation and migration of

mesenchymal stromal cells (MSCs) from human bone marrow, to promote re-epithelization of chronic ulcers in a diabetic rat model and to support proliferation and inhibit apoptosis of rabbit chondrocytes.
Thanks to their unique features, such as high biocompatibility as well as low cytotoxicity, tumorigenicity, and immunogenicity, PRP-Exos could present advantageous therapeutic properties, including homologous administration in the clinical setting, thus overcoming the restrictive requirement of other biological products. Furthermore, in comparison with other cell sources and fluids used successfully to obtain exosomes, platelets allow obtaining higher amounts using minimally invasive procedures.

APPLICATION OF PLATELET-DERIVED EXOSOMES

Platelets and platelets-derived extracellular vesicles (pEVs), including exosomes, are natural mediators of different physiological processes and contribute to the immune system response functions and regenerative process. However, only a few articles have evaluated the potential of pEVs as therapeutic regenerative tools.

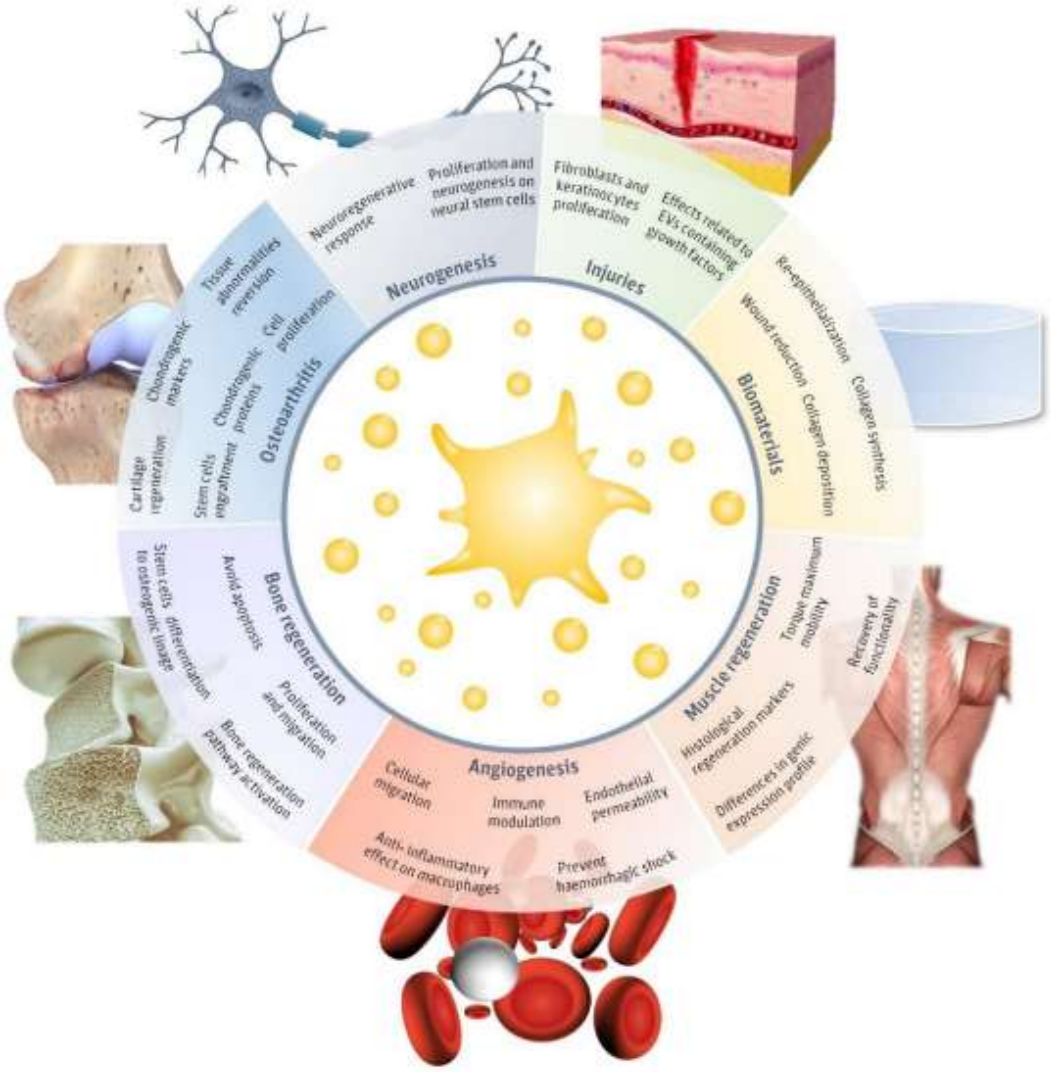

Fig. 3 - Main regenerative effects reported for pEVs in regenerative fields include: injuries, biomaterials, neurogenesis, muscle regeneration, angiogenesis, bone regeneration and osteoarthritis.

Based on their important regulatory role, PLT-Exos are expected to be a new method or target for the prevention and treatment of **atherothrombosis**. During atherothrombosis, platelet activation is accompanied by massive release of PLT-Exos, which in intercellular communication acting by transporting cargoes such as microRNAs and proteins. Studies have shown that the exosomes secreted by healthy volunteer can inhibit platelet aggregation and endothelial cell inflammation, while PLT-Exos derived from some patients promote endothelial cell apoptosis and the neutrophil-mediated inflammatory response. PLT-EXOS seem to have a fundamental role in the atherothrombosis, and their application could be effective as the treatment of coronary heart disease.

One of the main fields in which the applications of platelet derived EVs (pEVs) have been studied are **injuries and wounds**. Concretely, an increase of fibroblast and keratinocyte migration and proliferation in vitro has been reported, associated with the wound healing process. These effects may be related to the pEVs cargo, which was positive in different growth factors, including platelet-derived growth factor (PDGF), basic fibroblasts growth factors (FGF2), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF). Even more, the evaluation on a diabetic rat model confirms in vivo the wound regenerative effects observed for pEVs. It is suggested that pEVs induce angiogenesis (may be mediated through Erk and Akt pathways) and the reepithelization process (activating yes-associated protein (YAP)).

More creative experiments suggest that pEVs can be combined with biomaterials or active biomolecules to obtain improved regenerative results. PRP-Exo incorporated in thermosensitive hydrogel increased their retention in the joint and thereby playing a therapeutic role on subtalar osteoarthritis due to chronic mechanical instability established by transecting lateral ligaments.

Furthermore, in addition to the wound healing properties, two rat model studies suggest that pEVs **prevent uncontrolled blood loss and hemorrhagic shock**. In fact, the pEVs dose-response performed in vitro suggests that pEV blood coagulation is dependent on EVs concentration. Even more, pEVs have an effect on endothelial permeability, which mitigates blood loss too. Further studies report that aggregates of thrombin activated pEVs decrease the bleeding time after in vivo injuries while decreasing the interleukin concentration too. Interestingly, pEVs have been used after being stored at −20 ◦C, proving to maintain the positive effects for hemorrhagic shock treatment and easing their use, thus being an attractive alternative to liquid platelet-rich plasma preparations that need to be kept at temperatures of 20–24 ◦C and with a short half-life (approximately 5 days).

Moreover, it is important to realize that pEVs are also involved in the **inflammatory response**. Some studies report that pEVs present an anti-inflammatory effect on stimulated macrophages, which decreased the release of cytokines, such as the tumor necrosis factor alpha (TNF-α) or interleukin 10 (IL-10). Even more, non-therapeutical studies have reported that pEVs may act as inflammation modulators, inducing pro-inflammatory or anti-inflammatory responses depending on the stimuli conditions. However, few studies have been performed to date on evaluating pEVs treatment effects on immune modulation, although the pEV role is known to be involved in the inflammation processes.

Another interesting property of pEVs treatments is their **angiogenic capability**, associated with cellular mobilization and migration. In fact, vaso-regeneration, and maintenance of arterial integrity after injury have been reported by different studies. These effects were attributed to pEVs protein cargo, such as PDGF, FGF2, and VEGF, and also to lipid growth factors, despite not being directly identified. Incorporation of pEVs into cells and later phenotypical changes were assessed through

in vitro studies [40]. Later in vitro and in vivo experiments confirmed an increase in cell recruitment and adhesion, followed by a regenerative effect. Even more, rat ischemic hearts were analyzed in vivo confirming the angiogenic effects of pEVs. A dose-dependent angiogenic effect has been reported for pEVs.

In more specific studies, pEVs have also been reported to be involved in the **neurodegenerative response**. First, in vitro studies suggest that pEVs induce proliferation and neurogenesis on neural stem cells, which have been associated with different proteins contained in pEVs, such as PDGF, FGF2, and VEGF. Even more, the use of pEVs induces higher increase on Erk and Akt pathways than the direct treatment with these growth factors alone. Secondly, in vivo studies show an increase in neural stem cells proliferation and differentiation, in addition to the angiogenic effect. Furthermore, the rat model evaluated improved the neurological functionality after ischemic stroke according to a motor disability test. Overall, it is interesting to notice that the neurodegenerative effects attributed to pEVs follow a dose-dependent response, as it has been reported.

Another field in which pEVs have been evaluated as therapeutical agents is **musculoskeletal regeneration**. To start, it has been suggested that pEVs may contain a functional miRNA profile that would benefit osteoarthritis regenerative therapies. Chondrocyte cell culture studies have shown that pEVs induce an increase on proliferation and cell migration (through the activation of the Wnt/β-catenin signaling pathway). Moreover, pEV treated chondrocytes have shown a decrease in the proinflammatory response and the apoptosis rate induced by inflammation conditions.

In bone regeneration, pEV miRNA suggested their potential use for **bone repair**. These predictions have been supported in some vitro studies, which report that pEVs promote the differentiation of mesenchymal stromal cells into the osteogenic linage expressing cellular osteogenic markers. The osteogenic effects in vitro have been attributed not only to the growth factors pEVs contained, like VEGF, PDGF, FGF2, or TGFβ, but also to their genetic material, such as RNA. It was shown that pEVs can be internalized by stem cells and induced proliferation and migration of stem cells in a dose-dependent manner.

Some models suggest that pEVs can promote proliferation and avoid apoptosis, inducing a bone regeneration effect through the activation of Akt/Bad/Bcl-2 pathway, other studies suggest instead an increase of angiogenesis, but no significant effects in bone formation. Therefore, it is necessary to perform further experiments, and proper pEVs characterization, to determine their real osteogenic effect.

Our vision.

Exosomes are transport systems with an information part and a nutritional part inside.

They can be seen as a “biovitalizer” produced by the patient and redistributed where the patient needs it most, for example in areas with little vascularity. Our theory is based on the fact that by using exosomes the use of live cells is bypassed and the biologically active nutrient / product is immediately given. Instead of collecting monocytes that become macrophages and begin releasing VEGF for vascularization, exosomes are used that have information inside them for vascularization.

Exosomes are currently being explored as “cell-free” therapeutic tools and have been extensively studied for their role in stimulating tissue regeneration by conferring proangiogenic, proliferative, anti-apoptotic and anti-inflammatory actions thorough transport of their protein cargo and RNAs.

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