EXOSOMES PRODUCED BY ADULT STEM CELLS AS A THERAPEUTIC TOOL

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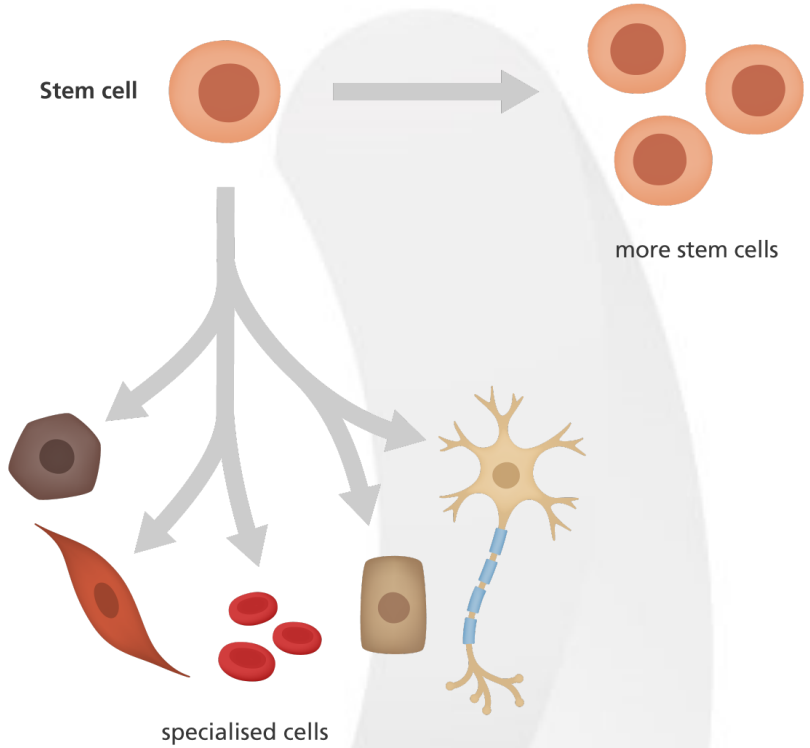
To talk about Exosomes, we must first start by talking about stem cells.

WHAT ARE STEM CELLS?

They are cells with the capacity to multiply, which can also develop functional and structural characteristics of specialized tissues, including cartilage, tendons, muscles and ligaments.



WHY ARE STEM CELLS USED AS A THERAPEUTIC TOOL?

Given their ability to multiply and differentiate, it was inferred that they could replace cells lost due to disease or degenerative conditions.

Clinical and preclinical experiments have shown that these cells also have an important capacity to modulate inflammatory activity, induce neovascularization, modify the extracellular matrix and modulate the immune response.

ARE STEM CELLS SAFE AS A THERAPEUTIC TOOL?

Yes they are, as long as they are produced under suitable conditions, by trained personnel and following strict procedures in terms of quality and safety.

Safety of Cell Therapy with Mesenchymal Stromal Cells (SafeCell): A Systematic Review and Meta-Analysis of Clinical Trials

Manoj M. Lalu[1,5], Lauralyn McIntyre[2,5]*, Christina Pugliese[5], Dean Fergusson[5], Brent W. Winston[6], John C. Marshall[7], John Granton[8], Duncan J. Stewart[3,4], for the Canadian Critical Care Trials Group

- *Thirty-six clinical trials were included, recruiting 1012 patients for the treatment of conditions such as: stroke, Crohn's disease, cardiomyopathy, AMI, GvHD, and healthy volunteers.*
- *There was no association found with toxicity, complications or organ failure, infection, death or cancer.*
- *A direct relationship was found with the development of early and transient febrile peaks.*

DO HUMAN ADULT STEM CELLS HAVE ANY TUMOR-FORMING CAPACITY?

No. Tumor formation has been reported only when cells obtained from rodent tissue samples are used.

The rodent´s stem cells apoptosis system, is activated by increasing oxygen concentration; what occurs during in vitro culture. Only cells that have inactivated the P53 protein, the guardian of the genome, survive the increase in oxygen concentration during in vitro culture and can be used in experiments. The P53 inactivation, make them so prone to the accumulation of mutations and therefore to malignization.

In conclusion, in almost all the stem cells experiments with rodent stem cells, uses malignan cells.

WHAT ARE MESENCHYMAL STEM CELLS (MSCs)?

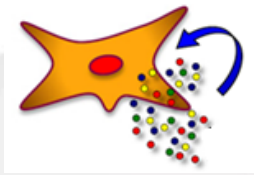
These are cells located in the vascularized stroma of tissues of more than nine weeks of gestation. Their name is due to the fact that when they were described, it was inferred they were remnant cells of the mesenchyme, embryonic supporting tissue.

They can be obtained from tissues as varied as bone marrow, adipose tissue, dental pulp and umbilical cord.

WHY ARE MSCs USED FOR THE TREATMENT OF DEGENERATIVE DISEASES SUCH AS OSTEOARTHRITIS, DIABETES AND ALZHEIMER'S DISEASE?

Because they could lead to a significant and long-lasting decrease in symptoms through different mechanisms, including inflammation control, new blood vessels formation, local cells activation, modulation of the immune response and replacement of lost cells in the tissue; these cells induce regeneration of a wide group of diseased tissues, as well as decreasing their inflammation and restore adjacent structures.

Figure 1.



Autocrine stimuli

Release of Soluble Factors

Endothelial/Smooth muscle Differentiation or Fusion

Differentiation into tissue lineage cells of the recipient tissue Fusion with recipient tissue cells

PARACRINE EFFECT

Proliferation of local SCs

Activation of local SCs

Extracellular Matrix modification

Vasculogenesis

Angiogenesis

Arteriogenesis

Cytoprotection and metabolic modulation

Immunomodulation

VASCULAR REGENERATION

Tissue Regeneration

DIRECT RECOVERY OF LOST CELLS

MSCs THERAPEUTIC ACTION MECHANISMS

The therapeutic capacity of MSCs is related to three groups of biological processes, which can be dependent on the differentiation capacity of MSCs and therefore, the direct replacement of lost cells; or on the paracrine trophic capacity of molecules produced by MSCs, which can be inside secretory vesicles (exosomes and microvesicles) or free.
Neovascularization is the result of vasculogenesis, angiogenesis and arteriogenesis. The differentiation capacity of MSCs is directly related to their capacity to favor vasculogenesis. While their capacity to produce trophic molecules with a paracrine effect (within secretory vesicles or not) is directly related to their capacity to induce angiogenesis and arteriogenesis.
The direct replacement of the cell population in the diseased tissue, can naturally be favored by MSCs due to their differentiation capacity, acquiring phenotypic characteristics particular to the cell species of the tissue, or it can also be encouraged by the induction of associated biological processes, leading to the activation of resident cells in the diseased tissue, thus causing their multiplication and migration.
Another large group of biological functions, including metabolic modulation, cytoprotection and immunomodulation, depend exclusively on the paracrine effect of the trophic capacity molecules produced by the cells, not on their differentiation capacity.

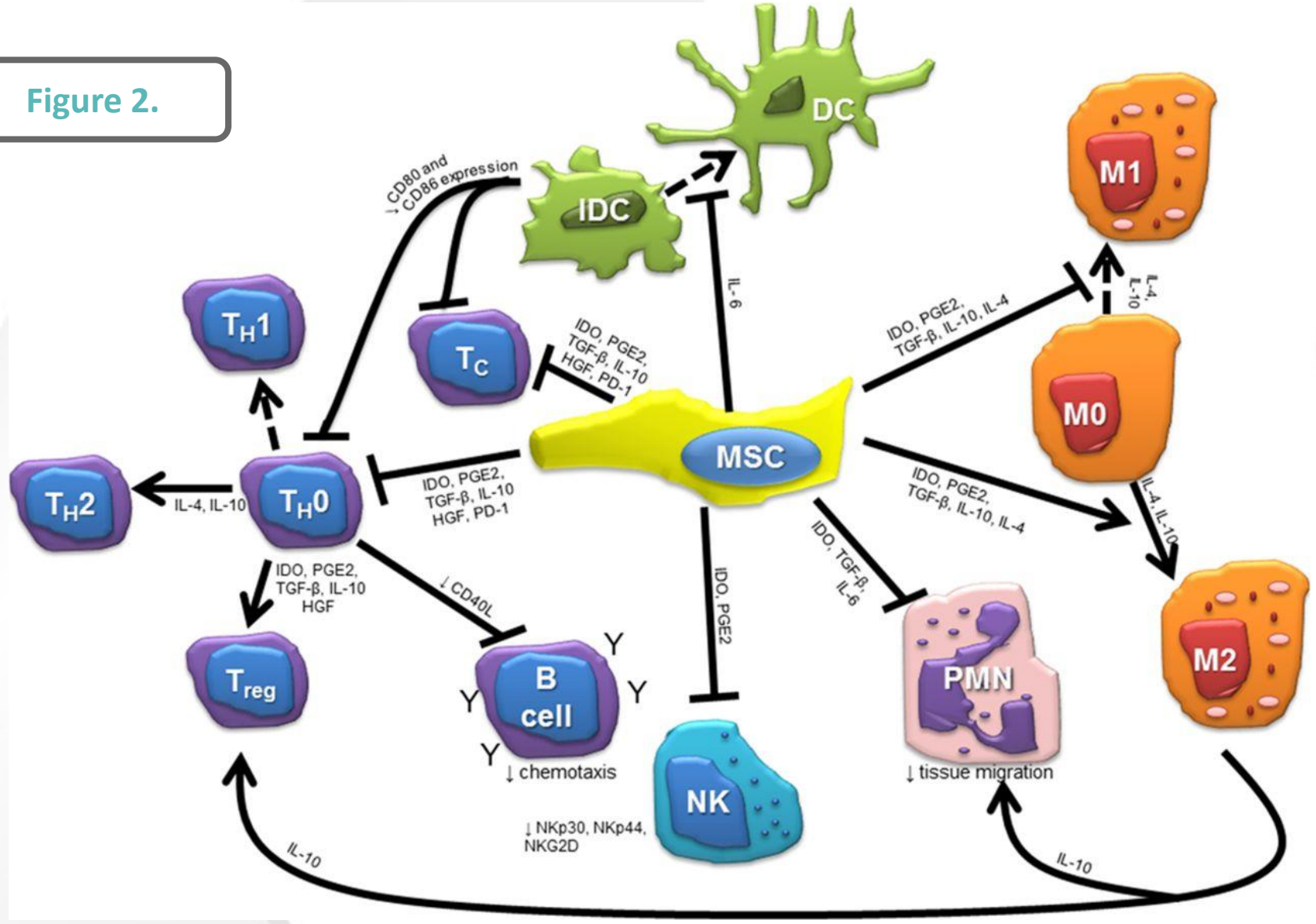
Modificado de: Gnecchi M, et al. Vascul Pharmacol. 2012

IT IS ACCEPTED THAT MSCs DO NOT REPLACE LOST CELLS, AT LEAST NOT TO A LARGE EXTENT. THEY MODULATE THE TISSUE NICHE, FOSTERING TISSUE REGENERATION.

MSCs do not replace the orchestra musician. They actually replace the orchestra conductor.

IMMUNOMODULATORY CAPACITY OF MSCs

Figure 2.

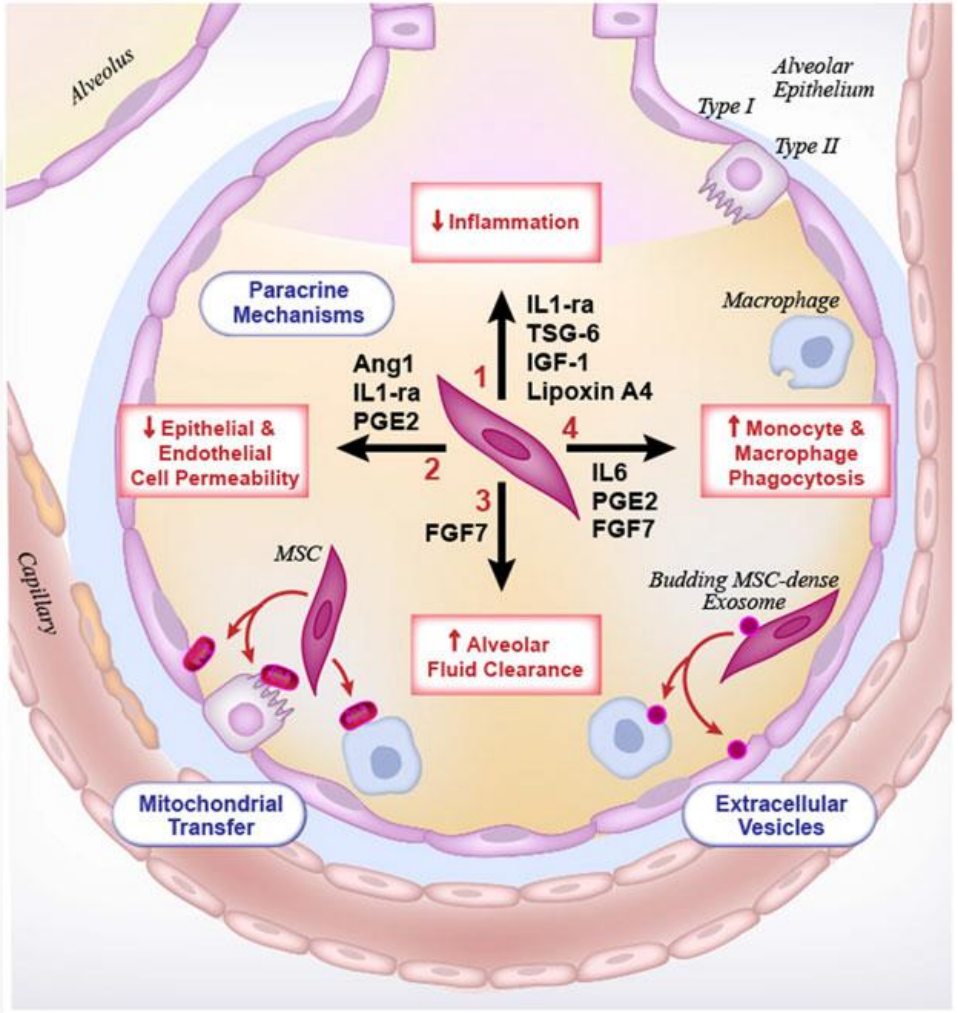


MSCs modulate the functioning of the innate and adaptive immune system, due to the trophic capacity of molecules with paracrine activity, released by them.
IDO: Indolamine-pyrrol2,3-dioxygenase.
PGE2: Prostaglandin E2.
TGF-β: Transforming growth factor β.
IL-10, IL-6 and IL-4: Interleukins 10, 6 and 4.
HGF: Hepatocyte growth factor.

Vasileios Karantalis, and Joshua M. Hare Circ Res. 2015;116:1413-1430

ACUTE RESPIRATORY DISTRESS SYNDROME

Figura 3.



MSCs favor the correction of pulmonary tissue disorders, characteristic of acute respiratory distress syndrome, such as that presented by people who develop severe forms of COVID-19.

PGE2: Prostaglandin E2.

IL-6: Interleukin 6.

FGF7: Keratinocyte growth factor.

IL1-ra: IL-1 receptor antagonist.

TSG-6: Tumor necrosis factor-induced gene 6 protein.

Ang1: Angiopoietin 1.

Lipoxin A4: Lipoxin A4.

IGF-1: Insulin-like growth factor 1.

Huperd L, et al. (2019) TRASNFUSION.

Now it is the turn of the Exosomes. To do so, let's start with the extracellular vesicles.

WHAT ARE SECRETORY VESICLES OR EXTRACELLULAR VESICLES?

Extracellular vesicles are lipid bilayer structures secreted into the extracellular medium by most of our cells.

These vesicles can be divided into three categories according to their size: exosomes, microvesicles and apoptotic bodies (Table 1).

Table 1. SECRETORY VESICLES PRODUCED BY HUMAN CELLS.

EVs	Size	Markers	Mechanism of biogenesis
Exosomes	30–150 nm	CD63, CD9, CD81, Tsg101	Generated by inward budding of the membrane of MVBs through ESCRT-dependent Or/and ESCRT-independent and released into the ECM upon fusion of MVBs with the plasma membrane
Microvesicles or ectosomes	100–1000 nm	ARF6, Annexin A1	pinching off from membrane protrusions/the plasma membrane shedding
Apoptotic bodies	50–5000 nm	Phosphatidylserine	Generated from apoptotic cells following stimulation of apoptosis-related pathways

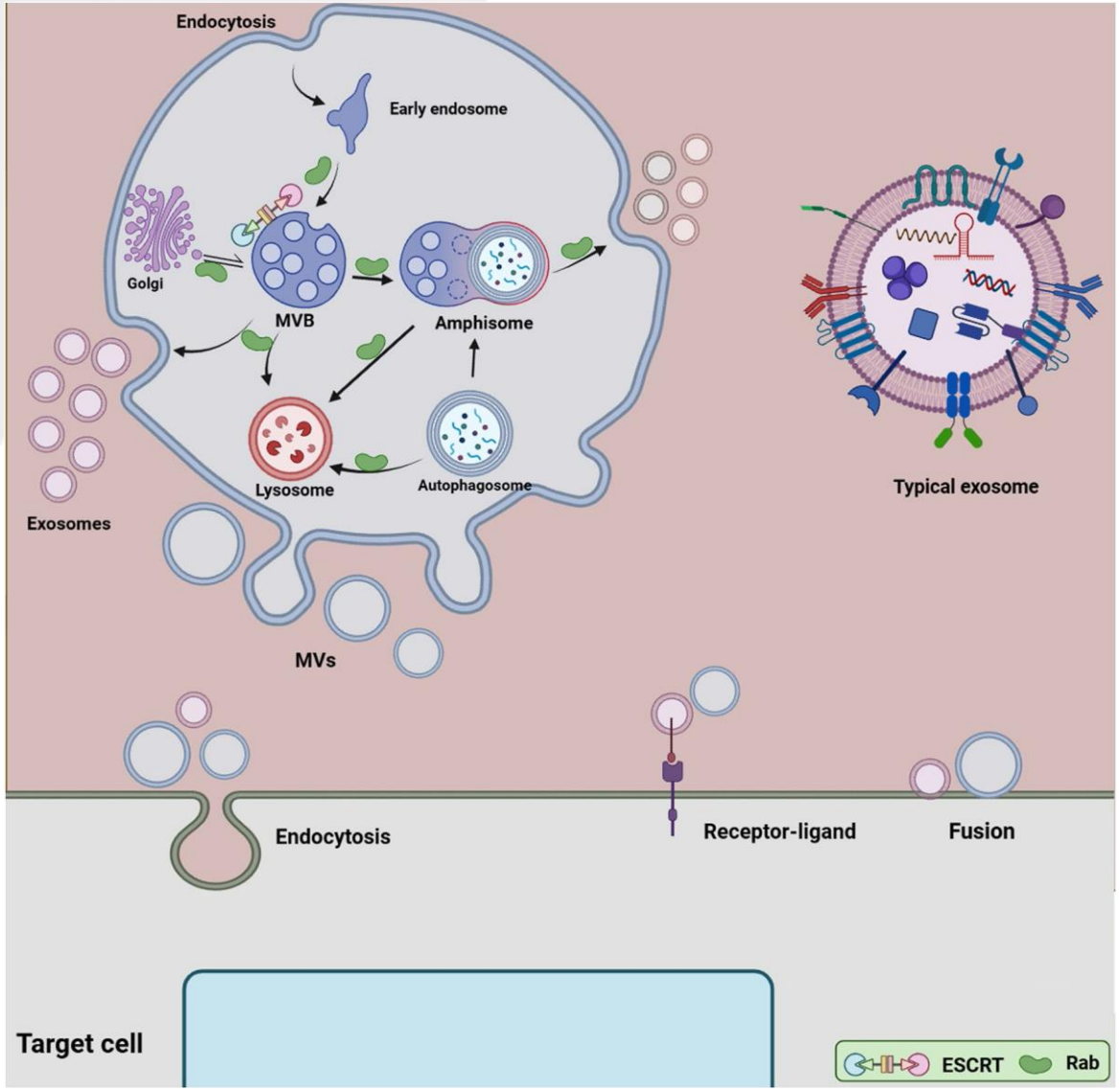
Lee B-C, et al. 2021. J Clin Med.

WHERE ARE EXOSOMES PRODUCED AND WHY ARE THEY OF THERAPEUTIC INTEREST?

Exosomes are produced in the Golgi apparatus and in the rough endoplasmic reticulum, by a process of evagination and targeted loading of specific molecules (figure 4), which in the case of MSCs, are the molecules with trophic capacity directly related to the therapeutic capacity of MSCs (figure 1)

EXOSOMES AND MICROVESICLES SYNTHESIS

Figure 4.



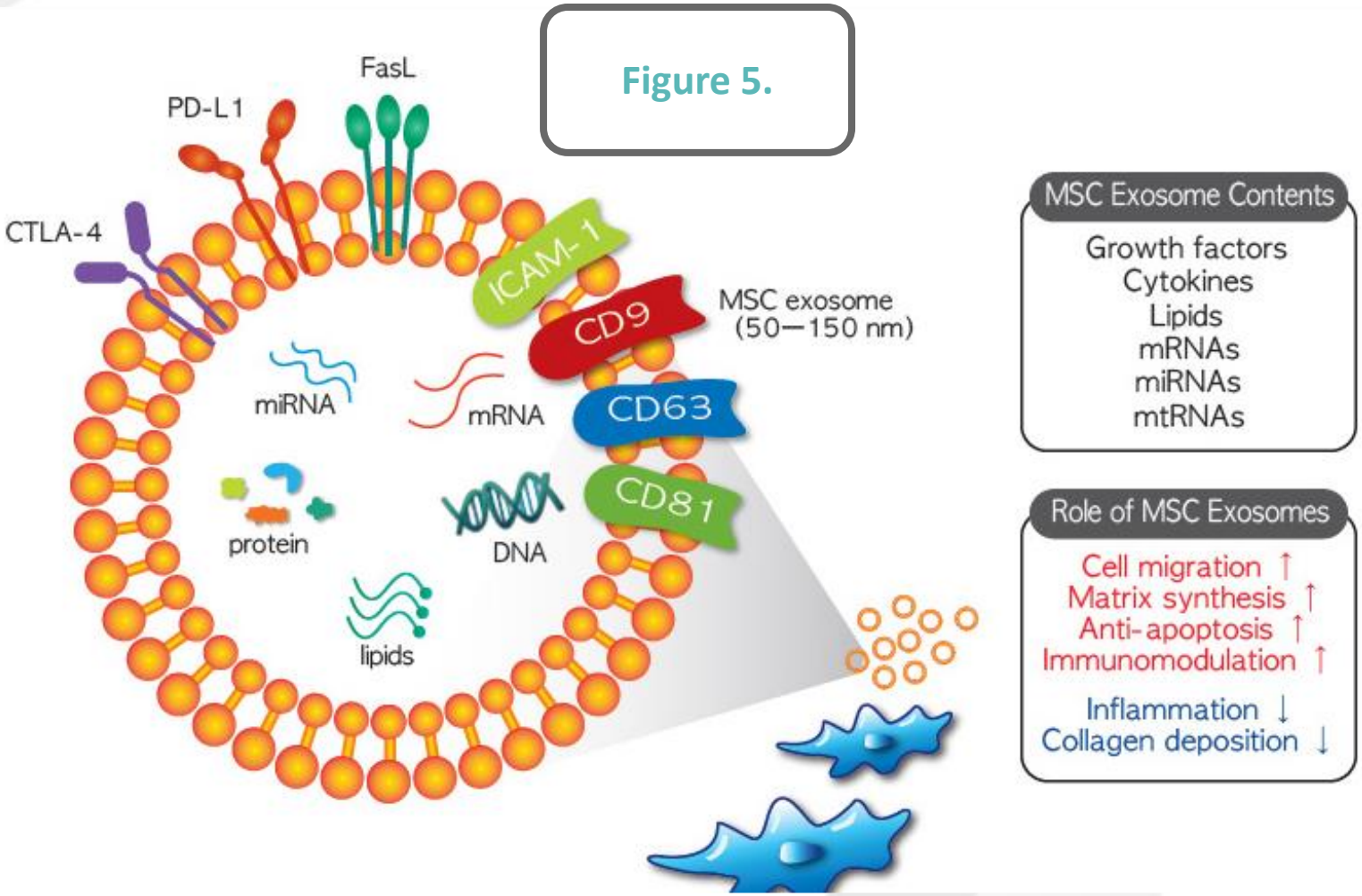
Exosomes are actively produced in the Golgi apparatus and the endoplasmic reticulum, where they are "loaded" with secretion molecules, which in the case of MSCs are the trophic capacity molecules related to the therapeutic capacity of MSCs. In the target or recipient cell, the microvesicles (MVs) release their contents by nondirected membrane fusion with the recipient cell, whereas exosomes release their contents by receptor-ligand interaction-mediated membrane fusion.
MVB: Multivesicular body.

Lee B-C, et al. 2021. J Clin Med.

SO THE EXOSOMES ONLY CONTAIN MOLECULES OF PROTEIN NATURE?

No. The list of molecules that have been found in the exosomes of our cells, is very broad and heterogeneous, as shown in Figure 5.

Figure 5.



Lee B-C, et al. 2021. J Clin Med.

ARE EXOSOMES A SUBSTITUTE ALTERNATIVE TO STEM CELLS?

No. They are complementary alternatives, and both are frequently used as part of therapeutic protocols. Both alternatives are for parenteral use, however, exosomes can also be nebulized. Their handling requires much simpler technical and logistical conditions than those of the cells.

ARE THERE ANY UNIQUE APPLICATIONS FOR EXOSOMES?

Yes. Exosomes are exclusive for:

- ✓ ***Aesthetic medicine***
- ✓ ***Treatment of striated muscle injuries***
- ✓ ***Treatment of pulmonary problems by nebulization***

HOW ARE THE EXOSOMES APPLIED TO THE PATIENT?

The process depends on the characteristics of each patient and is influenced by different factors including, of course, the disease and its severity.

They are usually applied directly to the affected area, although they are also frequently used intravenously considering the benefit they can generate in the whole body.

CAN ALL PATIENTS BE TREATED WITH THE EXOSOMES PRODUCED BY MSCs?

No. It must be understood that stem cells (and their exosomes) have an enormous capacity to stimulate the growth of other cells in different tissues.

Therefore, a patient with an active cancerous process could become worse, since MSCs exosomes could stimulate the tumor cells and make the cancerous mass grow.

WHAT CHARACTERISTICS SHOULD AN EXOSOME PRODUCTION PROTOCOL HAVE TO COMPLY WITH GOOD MANUFACTURING PRACTICES (GMP)?

For a protocol to be executed within GMP parameters, verification and control mechanisms must be established for all raw materials, materials and reagents used during the production process, to ensure the absence of external contaminants that cause cross-contamination and therefore constitute a risk of rejection of the product by the patient (user).

The production process must be carried out in suitable facilities, equipped with appropriate equipment for manufacturing advanced medical therapy products.

The final product must be analyzed, prior to its release for clinical use, in order to verify its quality, suitability and safety for the patient.

HOW CAN THE SAFETY AND SUITABILITY OF EXOSOMES PRODUCED BY MSC BE DEMONSTRATED?

The expansion of MSCs is an in-vitro process in which conditions that favor cell growth are established, consequently, also the growth of other undesirable biological agents, such as microorganisms.

Hence, to demonstrate the safety and suitability of exosomes and their MSC-producing cells, microbiological, cytogenetic and phenotypic requirements must be defined, which must be evaluated by means of laboratory techniques to demonstrate their compliance.

WHAT POLLUTANTS ARE ALLOWED IN OUR EXOSOMES?

No microbiological agent is allowed either in the raw material or in the final product. For this purpose, in the human tissues used as raw material, the presence of viral, bacterial and fungal agents is ruled out.

The same applies to the ready-to-use exosomes (final product), which are evaluated by different laboratory techniques to demonstrate the absence of bacteria, fungi, yeasts, Mycoplasma sp. and the absence of toxic substances produced by bacteria, such as endotoxins.

HOW ARE THE SAFETY AND SUITABILITY OF EXOSOMES DEMONSTRATED?

The safety of exosomes is demonstrated through the analysis of the karyotype of the exosome-producing cells. This way their genomic normality is demonstrated.

Suitability is demonstrated through:

1. *a characterization by Nano Tracking, where their appropriate shape, size and concentration are assessed.*
2. *The expression of markers normally recognized for MSCs by the International Society for Cellular Therapy. At least 95% of theses cells must express positive markers (CD73, CD90 and CD105) and no more than 5% should express the negative ones (CD34 and CD45).*

IS THERE ANY ALTERNATIVE TO IMPROVE THE RESULT OF THE TREATMENT WITH THE EXOSOMES PRODUCED BY THE MSCs?

Yes, sometimes we combine them with growth factors from the same patient, which are purified under the strictest sterile conditions, in order to make the best use of their therapeutic capabilities and enhance the effect of the exosomes.

WHAT ARE THE POSSIBLE ADVERSE EFFECTS OF THE USE OF THESE EXOSOMES?

Actually they are scarce and insignificant.

Fever, redness and spasms on the application site may be experienced, which disappear in a couple of days with simple treatment.

WHAT IS TRUSTEM?

Trustem is a biotechnological laboratory focused on the development of advanced medical therapy alternatives for regenerative medicine, implementing the technology and knowledge developed over 10 years of work, with the scientific and academic support of the Fundacion Universitaria de Ciencias de la Salud-FUCS.

WHAT EXPERIENCE DO WE HAVE IN THE USE OF MSC AND ITS EXOSOMES?

Trustem was created with the objective of enabling access to advanced medical therapy alternatives.

Our team is made up of professionals in the health sciences, with master's and doctoral degrees. We count on a combined experience of more than 30 years in the development and implementation of advanced medical therapy alternatives for regenerative medicine. Our knowledge and experience allows us to provide you with technical and clinical support in the standardization and implementation of therapeutic protocols with these therapeutic tools.

We have experience in the management of different diseases, conditions and lesions, affecting the nervous, cardiovascular, pulmonary and locomotive systems, in people of different characteristics, including high performance athletes.

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