TWIN HELIX

from experience toward innovation

LYOSECRETOME

the powerful driver of cells

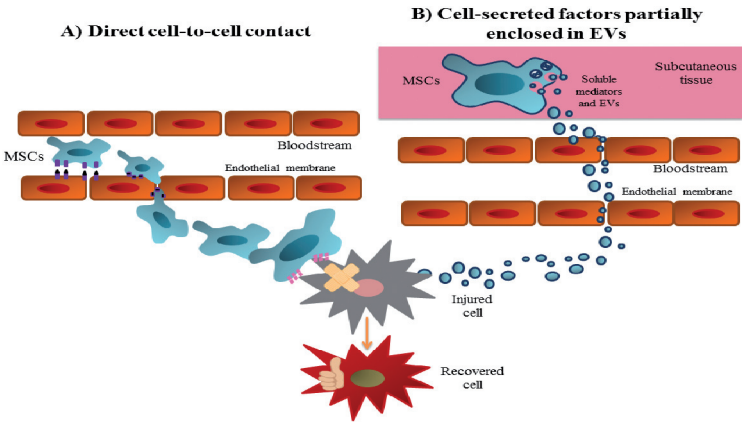
Advantages

- Off-the-shelf, fully standardized and lyophilized human MSC-derived secretome
- Dried sterile powder
- Good rheological properties
- Stable
- Standardized isolation, purification and lyophilization procedures
- Easy pharmaceutical management
- Small warehouse
- Active Pharmaceutical Ingredient-like
- GMP-grade
- Xeno-free

PharmaExceed
TRANSLATIONAL RESEARCH

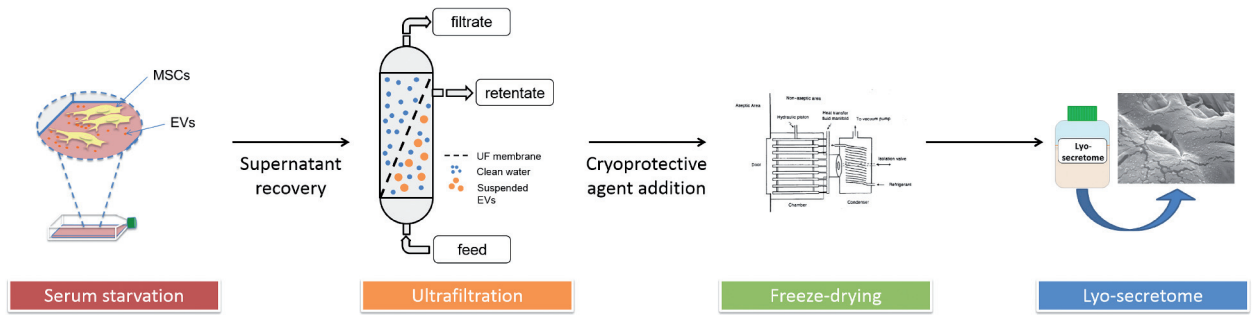
Applications - *In vitro cell culture*

Lyosecretome is a MSC-derived product obtained starting from the conditioned media by standardized protocols of concentration, purification and lyophilization. The reproducibility and full characterization made Lyosecretome suitable to be used as a standard for secretome-based research.
Lyosecretome can be used as reference standard to evaluate characteristics and potency profile of microvesicles and exosomes obtained with specific protocols (e.g. cytokines treatment or hypoxia) developed in your lab.
For example, Lyosecretome can be used as control to compare the immunomodulatory properties of cells and cell-derived products. Lyosecretome is a reservoir of cytokines and growth factors which can improve the regenerative process.



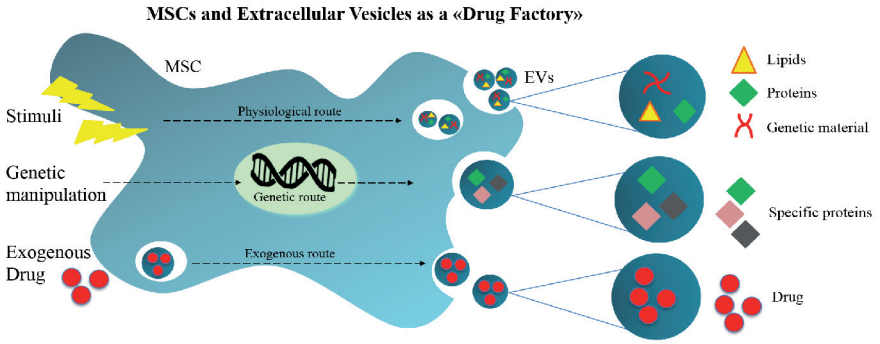
Lyosecretome production

Lyosecretome is obtained from Mesenchymal Stromal Cells (MSCs) isolated from Adipose tissue (AD-MSCs) or from Bone Marrow (BM-MSCs). Cell culture media are collected, after serum-free cell culture, and concentrated/purified using the ultrafiltration technique. With respect to ultracentrifugation, ultrafiltration allows to preserve microvesicles and exosomes integrity, preventing particle aggregation. Lyophilization is performed, after the cryoprotective agent addition, to obtain a stable, dry and ready-to-use powder (***Lyosecretome***). Reconstitution: addition of deionized water, phosphate buffer saline (PBS) or culture medium to reach the desired concentration.



Customized Lyosecretome

The composition of the MSCs' secretome can be modulated, according to the therapeutic/research purpose, by preconditioning the MSCs during *in vitro* culture. MSCs will be "educated" to produce the best secretome according to the desired goals.Lyosecretome can be produced "on demand" thanks to the developed production method which can be applied to all cell lines (e.g. immortalized cells, primary cells, tumoral cells) and to different culture conditions (e.g. stimulation with specific growth factors, drugs or cytokines; different culture supplements – FBS, Platelet Lysate or others; hypoxia).
Furthermore, ultrafiltration technique allows to select different fractions. Modifying the filtration unit we can obtain four different products:



- Lyosecretome (soluble factors, exosomes, microvesicles; particle size 10-1000 nm)
- Soluble factors (proteins; 5-300 kDa)
- Exosomes (particle size 10-200 nm)
- Esosomes + Microvesicles (particle size 10-1000 nm)

Case study

Cytotoxicity was tested by MTT test on human fibroblasts and human chondrocytes treated for 24 and 48 hours with Lyosecretome. The product resulted cytocompatible until a concentration of 25 mg/ml (2.5 x 10^6 cell equivalents per ml), as the cell metabolic activity always remained higher than 60%. The cell metabolic activity of human chondrocytes was higher after 48 hours of incubation rather than after 24 hours, suggesting that, for this cell line, the exposition to Lyosecretome stimulated cell proliferation (Figure 1A and 1B). The ability of Lyosecretome to protect cells from the damages induced by oxidative stress was evaluated on nucleus pulposus cells, a highly responsive model. For doses between 5 and 50 mg/ml (0.5 x 10^6 and 5 x 10^6 cell equivalents per ml), Lyosecretome showed to in vitro counteract the oxidative stress damages: cells not previously treated with Lyosecretome in presence of H_2O_2 die; instead cells previously treated with Lyosecretome are able to survive, even in the presence of H_2O_2 (Figure 1C). This efficacy is also supported by the proteomic characterization performed: proteins involved in immune and oxidative stress response, such as SOD1, SOD2 and SOD3, were among the most represented into Lyosecretome. At the functional level other proteins were involved in cytoskeleton, metabolism, protease-antiprotease balance and response to bacteria (Figure 3).

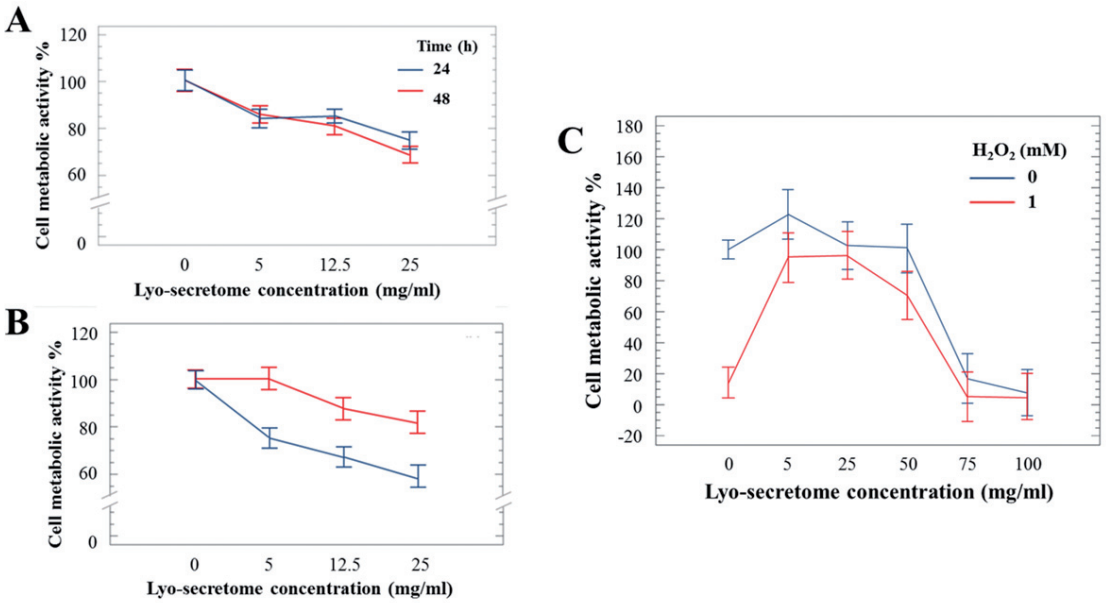


Figure 1. *Cell metabolic activity of human fibroblasts (A) and human chondrocytes (B) treated with different doses of Lyosecretome. (C) Cell metabolic activity of NP cells with and without H_2O_2. Mean values ± LSD, ANOVA.*

Immunomodulation

Lyosecretome immunomodulatory properties were compared with the ones of its parental stem cells. Blood mononuclear cells were activated to produce IFN-γ and treated with increasing doses of stem cells, expressed as cell number, or Lyosecretome (doses expressed as cell equivalents, that are the mg of powder corresponding to that precise number of cells). At the highest dose, Lyosecretome was able to reduce the IFN-γ production the same as stem cells

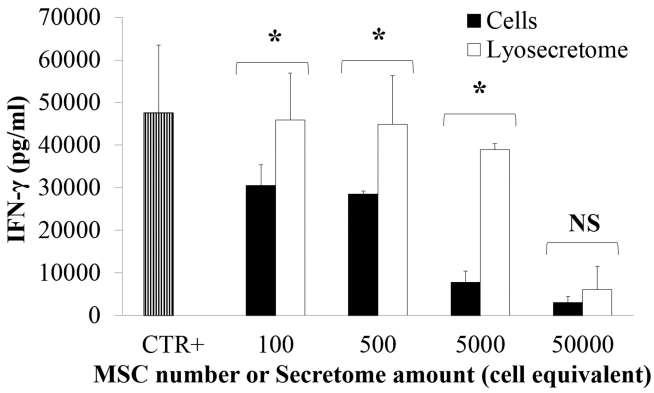


Figure 2. *IFN-γ production after PHA stimulation of PBMCs: MSCs versus their Lyosecretome. Untreated PBMCs were considered as control (0 cells or Lyosecretome cell equivalents); asterisks indicate significant difference ($p<0.05$, ANOVA).*

Products

Cat. N	Content (cell equivalent/mg)	Cell source	Size distribution (nm)	Active content
A005	10^5	AD-MSCs	0 – 1000	Secretome (soluble factors, microvesicles, exosomes)
A100	10^5	AD-MSCs		Soluble factors (e.g. proteins)
A300F	10^5	AD-MSCs	40 – 200	Exosomes
A300	10^5	AD-MSCs	40 – 1000	Microvesicles + Exosomes
B005	10^5	BM-MSCs	0 – 1000	Secretome (soluble factors, microvesicles, exosomes)
B100	10^5	BM-MSCs		Soluble factors (e.g. proteins)
B300F	10^5	BM-MSCs	40 – 200	Exosomes
B300	10^5	BM-MSCs	40 – 1000	Microvesicles + Exosomes

Technical Characteristics

Dry/stable powder
High purity
Certified cell donors
Storage at -20°C
Easily resuspension in water/PBS/culture medium
Size distribution: 40 – 1000 nm

For research use only

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