GENOGENIX™
To Good Health

Compound Overview

UCM-X Exosomes

Executive summary

UCM-X Exosomes can help repair damaged tissues and calm down inflammation. They're derived from young, healthy cells, which are rich in regenerative properties.

UCM-X Exosomes are a type of exosome therapy derived from umbilical cord mesenchymal stem cells (UCM). Exosomes are small vesicles released by cells and play a crucial role in cell-to-cell communication. They are being researched for their potential in regenerative medicine due to their ability to carry and transfer molecules like proteins, lipids, and RNA to other cells.

- **Cellular Communication:** Exosomes function as nano-messengers, carrying and delivering important molecules between cells, which can instruct cells to behave in certain ways, like reducing inflammation or beginning repair processes [1,2, 15].
- **Regenerative Mechanisms:** They can carry regenerative molecules from stem cells to damaged tissues, potentially aiding in repair and recovery [1,2, 15].
- **Immunomodulation:** By transferring specific biomolecules (such as proteins or RNA), they can help in modulating the immune system response, balancing from helping in cell co-stimulation to secretion of pivotal factors (such as cytokines) [1,2, 15]

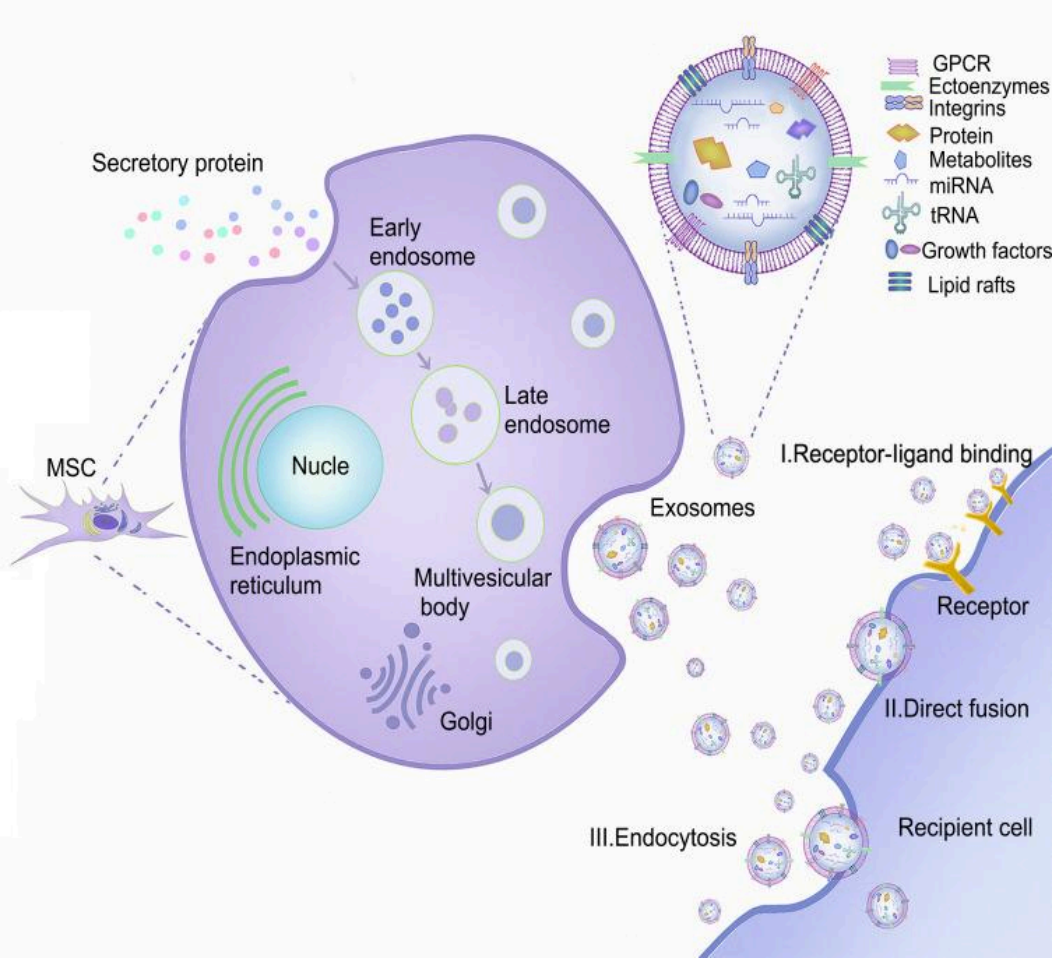
Benefits

Regenerative Properties: They may aid in tissue repair and regeneration, which is promising for recovering from injuries and diseases.	**Anti-Inflammatory Effects:** UCM-X Exosomes could help reduce inflammation, beneficial in treating various inflammatory conditions.	**Immunomodulatory Impact:** They might modulate the immune system, which can be crucial in autoimmune diseases and in enhancing the body's recovery processes.	**Skin Rejuvenation:** In cosmetic medicine, they're being explored for skin rejuvenation and anti-aging treatments.

Technical Overview

Exosomes are extracellular vesicles with a nanoscale size (from 40 to 160 nanometers in diameter) that are produced and secreted by cells [1]. Potentially all cells of different species can produce and release exosomes in their living milieu. Exosomes are single-membrane nanovesicles, with their external part composed of a lipid bilayer with proteins inserted in. The internal part of exosomes contains cell constituents and produced biomolecules, such as nucleic acids, amino acids, proteins and intracellular cell metabolites. All exosomes constituents are acquired from their producer cells. Each exosome size, shape, and density are primarily determined by its specific content of protein, lipid, enzymatic, and mineral, consequently we find a heterogeneous population of these nanovesicles in every extracellular environment.

Currently we know that exosomes are very important mediators in cell-to-cell communication (also referred as intercellular communication), once these nanovesicles are able to transport and deliver molecular cargo from donor to recipient cells located in either short or long distances inside our body. Exosomes are found in almost all bodily fluids, such as urine, saliva, breast milk, blood and interstitial fluids [2]. When traveling in blood, they can reach target cells located far away from the region where they were originally secreted by their producers. These extracellular vesicles work like nano-messengers, since they are uptaken by specific target cells (also known as recipient cells) at their final destination. Exosomes uptake is topologically similar to well-established models of virus–cell interactions, meaning that their internalization occurs after interaction between the proteins on the vesicle surface with their respective ligands on the target cell. It is not surprising that exosomes are taken up by a plethora of mechanisms, including macropinocytosis, phagocytosis, clathrin-dependent endocytosis, and clathrin-independent endocytosis [1, 2,15]



Schematic representation of exosome-producer cells, exosome nanospheres and recipient cells. The producer cell (left) synthesizes and releases exosomes (in the middle). Exosomes are composed of lipid bilayer with several molecules encapsulated inside (eg. Protein, miRNA, Metabolites, tRNA, Growth Factors) and some inserted into the membrane (eg. GPCR, Ectoenzymes, Integrins). Once exosomes find their target cells (recipient cells), they are uptaken by endocytosis (III in the illustration), direct fusion (II in the illustration) or triggering a signaling pathway through receptor binding (I in the illustration). This illustration was adapted from Wang S et al (2022) *Int J Nanomedicine* **17**:1757-1781. Reproduced under terms of creative commons

Relevance of exosomes in the body

Exosomes, also referred here as Extracellular Vesicles (EVs), have already been implicated in several biological processes, such as immune responses, pregnancy, cardiovascular diseases, viral pathogenesis, cancer development/metastasis and central nervous-related diseases. As mentioned above, proteins, nucleic acids, metabolites are carried inside EVs,

so when these vesicles reach their recipient cells, a transfer of the cargo frequently occurs to their targets, ultimately altering their signaling pathway, metabolism or gene expression.

In this context, these exosome-mediated components can promote or restrain a disease, thus making them an important tool in therapeutics. Importantly, exosomes can target very specific cells (or recipient cells) in the body, thus different approaches are being developed to engineer exosomes carrying specific deliveries with therapeutic purposes. Some examples encompass the use of exosomes to deliver interfering RNAs to silence the expression of genes (like genes involved in cancer process) or even the use of exosomes to deliver chemotherapeutic drugs to target cancer cells. In addition, studies have been conducted to increase the specificity of exosomes to their target cells. The universe of studies on exosomes is still expanding and far away from the end.

The different types of Exosomes

Exosomes contain a broad array of transmembrane proteins, lipid-anchored membrane proteins, peripherally associated membrane proteins, as well as their soluble proteins present in their lumen. Considering that different cells can produce different metabolites over a time period, we can easily conclude that the pattern of biomolecules inside EVs are directly correlated to the metabolism state of their producer cells at the moment of secretion. This rationale is also valid for the surface molecules anchored on exosome membranes, making the specificity of an exosome target be dictated by the exposed biomolecules supplied by the producer cells.

A good example for this statement was observed when exosomes were isolated from antigen-presenting cells, an immune cell responsible to start the immune response: Inserted on their surface, these purified nanovesicles were expressing MHC-II molecules coming from these important immune cells [3]. As a consequence, these vesicles could only be recognized by its natural ligands TCR, which is expressed on the surface of the target T cells [2]. Because MHC-TCR is a good example of a very specific interaction between molecules, scientists observed that cargo EVs from antigen-presenting cells could be delivered to specific sets of T cells, similarly to a cargo specifically addressed to an unique place.

To better define how exosomes are different from each other, scientists from the US and UK analyzed the protein profile in exosomes produced by different cell types [4]. They

quantified a total of 1,212 proteins with high variability between vesicles from different origins, suggesting that exosomes carry proteins which are related to their producer cells. Nowadays, we know that tetraspanin proteins (CD81, CD82, CD37, and CD63) are highly enriched in exosomes, with CD81 as the most highly enriched protein.

Also interesting is the fact that not all exosomes present the same abundance of a given cargo [5], suggesting a high heterogeneity in the amount of biomolecules per nanovesicle particle. In addition, proteomic and RNA library analysis revealed that a purified population composed of the same cell can secrete heterogeneous exosomes [24].

Overall, we can conclude that exosomes are very heterogeneous and their contents are directly related to their cellular origin. This means that exosomes are a partial portrait of its cell producer.

Exosomes can help people in various ways

Exosomes can be implicated in the pathogenesis of several diseases, such as viral infections, cancer, neurodegeneration, cardiovascular dysfunction.

Due to the EVs ability to interact with target cells and to exert a specific function, several researchers investigated the biological role played by exosomes in the genesis of several disorders. Exosome-mediated transfer of molecules is exploited by viruses and tumor cells, since this is a smart way to protect molecules from degradation by circulating/tissue extracellular enzymes. Because our main goal is to highlight the therapeutic effect of EVs, we will cite only a few examples to better illustrate how EVs can influence cell biology in the context of diseases.

For example, the oncogenic receptor EGFRvIII can be transferred between cells through exosomes-derived from glioma tumor cells [16]. In an interesting way, tumor cells expressing the receptor EGFRvIII can produce exosomes-bearing receptors and subsequently transmit them to different cancer cells lacking the molecule, leading to the transfer of oncogenic activity to the recipients [16]. This event generates tumor cells competent to activate transforming signaling pathways (eg. MAPK and Akt), changes in expression of EGFRvIII-regulated genes (eg. *VEGF*, *Bcl-x_L*, *p27*), morphological transformation and increase in anchorage-independent growth capacity [16]. Similarly, viruses are able to nano-deliver noncoding genetic material (miRNAs) through secreted

exosomes from infected cells [17]. For example, exosomes from cells infected with Epstein-Barr virus (EBV) were able to deliver the viral miRNA to uninfected cells, repressing well-known EBV target genes into the recipient cells [17]. This action might amplify the number of potential targets to the virus.

Exosomes function has already been described in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, as well as amyotrophic lateral sclerosis [18]. In this context, evidence indicates that exosomes derived from the neurons of patients with neurodegenerative disease could amplify the progression of neurodegenerative disorders by delivering their contents to recipient cells [18]. Also, changes in exosomal content may serve as early biomarkers for diagnosing neurodegenerative diseases.

In cardiovascular disorders, cardiac endothelial cells can shed vesicles that facilitate the disease progression [19]. For instance, atherosclerotic plaque-derived EVs have already been reported to transfer adhesion molecules, such as ICAM-1, to endothelial cells, supporting plaque progression *via* the recruitment of immune cells [19]. Overall, these adverse pathologies exemplify how exosomes mediate an intricate system of cell communication.

However, the therapeutic use should be emphasized as one of the most interesting parts in the biomedical application of exosomes. These extracellular vesicles have some advantages in therapy: they can carry both hydrophilic and hydrophobic molecules; the internal cargo is shielded by a lipid bilayer that protects against enzymatic and immune components (like antibodies); exosomes can travel long distances in our body; they are easily loaded with drugs and easily uptaken by cells; they present low immunogenicity and the specificity of exosomes to their target cells can be ameliorated with the use of gene engineering. By the end of 2023, 93 ongoing clinical trials with exosomes were identified on clinicaltrials.org (criteria: other terms: "exosome"; study phase: "not applicable"), with most of them in the beginning phase (phase I and II). These clinical trials are mostly performed with stem cells-derived exosomes and addressed to identify potential therapies or disease biomarkers. Below we will cite some advantages of exosomes in therapy.

For prodrug delivery:

Exosomes are being modified to be used as a nanoplatform for delivering drugs to target cells, as in cancer, angiogenesis and stimulation of immune response [6]. The specificity of the exosomes to their target cells is usually dictated by ligand-receptor interaction, thus improving this characteristic can be advantageous in therapy. Together with its lipophilic nature, exosomes can rapidly deliver drugs to cancer cells.

For instance, in experimental models with rodents, these vesicles were modified to deliver small nucleic acids (also referred as small interfering RNA) to cancer cells for silencing their cancer genes [6]. The small interfering RNA against BCR-ABL and galectin-9 is one example of this use [6]. In addition, exosomes can be loaded with chemotherapy, and Paclitaxel, Methotrexate, Oxaliplatin and Imatinib are also examples of anti-cancer prodrugs loaded to exosomes after some chemical modifications. The fact that exosomes can deeply penetrate in the body tissues is also another advantage for drug deliveries.

Clinical trials for induction of antitumor immunity:

In a smart way, cancer cells can release exosomes which are directly addressed to immune cells, aiming the modulation of the host immune response in the favor of tumor growth and its metastasis. To boost anti-cancer immunity in clinics, several scientists are engineering exosomes for the delivery of the FDA-approved Immune Checkpoint Inhibitors, such as PD1, PD-L1 and CTLA-4 to patients [6]. Due to the high complexity in the execution and ethical issues related to human studies, very few clinical trials (only three registered in 2023 at clinicaltrials.gov) are in progress for testing exosomes as intensifiers of traditional cancer therapy. Nowadays, exosome studies in humans are restricted to examining their function as predictive biomarkers in cancer diagnosis and prognosis.

For vaccines:

New generations of vaccines, such as those for COVID-19 from Pfizer and Moderna, are being developed using the principles of exosomes studies.

These vaccines are composed by a modified messenger RNA for producing the virus protein in our body. And this vaccine RNA is encapsulated in lipid nanoparticles for fast entering into antigen-presenting cells [7]. In this context, the efficiency of the vaccine can be increased if its RNA is enclosed into nanovesicles carrying ligands for direct contact with antigen-presenting cells.

Dermatological effects

Regenerative aesthetics is a large and expandable area in the dermatology field.

Regenerative medicine, in special skin regeneration, is an important topic of study and Mesenchymal stem cells-derived exosomes are gaining attention worldwide in the past 10 years since initial studies have reported beneficial actions of these nanovesicles in the aging process. Intrinsic factors, such as genome mutations, as well as extrinsic factors, such as sun radiation, air pollution and certain lifestyles can speed up the skin aging process. The use of mesenchymal stem cells in wound and skin therapies is based on their ability to secrete cytokines and growth factors that contribute to the wound repair and skin biogenesis. Both are very complex processes which involve multifactorial steps, thus we will briefly comment in the respective sections.

Skin undergoes several biophysical changes over the years, including the decrease in the production of collagen and elastic fibers, which all keep the skin elasticity and hydration. As the result of aging on skin, low hydration and elasticity can result in the formation of wrinkles. In this scenario, stem cells-derived exosomes have emerged as new therapy for treating these age-related conditions. These vesicles are being directly applied to skin in the form of topical creams, serums, masks or ointments and their use is considered a cell free-based therapy [8, 10]. Topical application of exosomes (eg. as ointment) is demonstrated to be safe and tolerable in human phase 1-trials (ID NCT05523011). Because exosomes can disappear from the body only 2 minutes after injection, researchers are improving exosomes-loaded hydrogels that permit slow and controlled release overtime.

A particular study *in vivo* observed that needle-free treatment with human fibroblasts-derived exosomes ameliorates skin sun damage by mechanisms that involve gene expression, in particular the upregulation of TGF-beta 3 and pro-collagen type 1, and downregulation of TNF-alpha in a model of photoaging mice [10, 21]. Also, this study has demonstrated collagen biosynthesis and deposition in the dermis, ameliorating skin aging [8,10]. Also, exosomes can decrease the production of Reactive Oxygen Species (ROS) by cells, diminishing the chance of damage in our DNA. All together, an emulsion with exosomes or exosomes-loaded hydrogels can improve skin hydration, texture, tone and wrinkles *in vivo*.

Wound recovery:

Wound recovery is a dynamic process consisting of four continuous phases that involve platelet/fibrin systems (thrombus), inflammation (with infiltration of several immune cells), proliferation of epithelial cells/collagen synthesis and remodeling . Some studies have already reported the positive effect of exosomes in wound recovery. For example, subcutaneous injection in mice of macrophages-derived exosomes enhanced fibroblast migration, collagen deposition and endothelial cells formation, ameliorating the wound recovery [10].

Similarly, exosomes from keratinocytes induce the expression of VEGF and FGF by human fibroblasts, contributing for angiogenesis and wound recovery. Finally, exosomes released by Mesenchymal Stem Cells (MSCs) down-regulated the expression of the genes TGF-beta 1, Smad2, Smad3 and Smad4, whereas upregulate TGF-beta 3 and Smad7 expression, which together culminates in a shift of cell signaling. This shift promotes human dermal fibroblasts proliferation, re-epithelization and dermal angiogenesis [10].

Skin inflammation / dermatoses:

Besides that, skin inflammation is also altered over years and could trigger several associated diseases. These diseases are commonly associated with immune disorders and recognition of our own proteins (called autoimmune diseases). This phenomenon results in elevated immune activation, high inflammation and attack of our own cells, leading to destruction of some cells, tissues or organs.

Due to the importance of exosomes in intercellular communications, studies have demonstrated that these nanovesicles can partially orchestrate the immune response, such as immune activation, antigen presentation and immune suppression.

To make a long story short, exosomes can play an important role in the development of Systemic Lupus Erythematosus, Psoriasis, Vitiligo, Atopic Dermatitis, Systemic sclerosis [10]. To cite very few examples, plasma of Lupus patients present high levels of extracellular vesicles when compared to healthy patients and their levels were correlated to disease outcome. In this case, these exosomes induce the secretion of cytokines (such as IFN-alpha, TNF-alpha, IL-1 beta and IL-6) by some target cells, amplifying an abnormal immune response. Corroborating this, when these patients' exosomes are purified and tested in isolated cells in the lab (called in vitro experiment), they were also able to induce cytokine secretion by antigen presenting cells [10]. When injected in a model of psoriasis in

mice, the keratinocyte-derived exosomes exacerbated skin lesions and the disease progression.

Similarly, exosomes obtained from systemic sclerosis patients promoted expression of type I collagen and fibronectin from human fibroblasts in vitro, which can potentially contribute to disease. More interestingly, patients with systemic sclerosis have decreased amounts of exosomes in plasma and these vesicle cargoes present a distinct pattern (in particular CD63, CD9 and CD81), which can serve as potential biomarkers for future diagnosis [10].

In skin pigmentation:

Our body has melanocytes in the outermost layer of the skin. These cells synthesize melanin under solar irradiation, giving the pigmentation aspect to the skin and acting as a cutaneous photoprotective defense.

Exosomes are able to interact with melanocytes and their cargo can signal to modulate gene expression and enzyme activity. For example, exosomes from UVB-irradiated keratinocytes (a process similar to sun irradiation) overexpressed micro-RNA 330-5p, which significantly activates melanocytes and their production of melatonin and pigmentation, via a signaling pathway dependent of tryrosinase. This result suggests a cross-talk between sun-exposed keratinocytes and epidermal melanocytes through the action of soluble exosomes [10].

In hair restoration:

Hair loss, also known as alopecia, can be induced by several factors, including aging, diseases, genetic factors and medications. Hair follicle stem cells can be stimulated by exosomes and can regenerate our hair growth.

For example, when exosomes-derived from human dermal papilla cells were cutaneously injected into mice, the scientists observed hair follicle growth and longer hair shafts in the rodent skin. However, this effect can only be observed with several subcutaneous injections of exosomes, due to the short-term retention in vivo [10]. Currently there are some ongoing clinical trials (all in the initial stage) to investigate the effect of exosomes in hair regeneration. In one of them (ID NCT05658094), the authors intend to investigate if Mesenchymal Stem Cells-derived exosomes could interfere with the Wnt-β-catenin pathway in the pathogenesis of alopecia.

To overcome this problem, several biomaterials are being developed for better adsorption and bioavailability of exosomes in vivo [11]. Interestingly, implantation in wounded mice of one of those materials, a cryogel gel containing antioxidant polyurethane mixed with exosomes, reconstituted the epithelial structures with hair follicles and epidermal morphology similar to that of healthy skin [11]. Taken together, these results and others suggest that exosome application can repair the hair growth by stimulation of hair follicles.

Importance of Stem cells-derived exosomes in therapy and Cosmetic Dermatology

Recently too much focus is given on particular exosomes: the exosomes produced by Stem Cells, in particular those produced by Mesenchymal Stem Cells (MSCs). The MSCs can be obtained from bone marrow, fat, teeth or umbilical cord, They have the ability of self renewal and also retain the capacity of differentiating into multiple cell lineages, such as cartilage (chondrocytes), bone (osteocytes) and fat tissue [12]. MSCs can also be expanded in lab flasks (*in vitro*) and components (like cytokines and growth hormones) released by MSCs can favor tissue regeneration in determined conditions [12]. Differentially from the others, MSCs-derived content usually carry several biomolecules that are only produced by them. Grow MSCs *in vitro* requires attention to some important details: cell purity; special medium formulation; retention of their differentiation capacity and telomere size. Telomeres are the end region of each chromosome. Each time a cell divides, the telomeres become slightly shorter due to the cell inability of copying DNA in these regions. When telomere size is critical, cells stop dividing (called senescence) and implicates in genomic instability, oncogenesis and metabolism changes. Therefore telomere attrition should be accompanied during proliferation of MSCs in culture.

Several recent reports have shown that MSCs-derived exosomes exert a greater therapeutic effect when compared to MSCs alone, thus the number of clinical trials using exosome-based therapeutics is rapidly increasing over years. In the following, we will describe some potential use of stem-cells exosomes in therapy.

In cancer therapy and bone regeneration:

Due to the high complexity in producing considerable amounts of exosomes and to perform clinical studies, the progress of human trials is being slow, with very few studies

concluded so far. With the advent of methods for scalable productions of these nanovesicles, we'll probably see an increase in clinical studies in the upcoming years. Recent advances have confirmed that exosomes therapy is safe and feasible. One study described that MSC-derived exosomes loaded with the chemotherapeutic drug Paclitaxel could traffick to the tumor tissues and exert anti-tumor action. Additionally, MSC-derived exosomes can impair Cisplatin resistance in breast cancer cell lines by downregulating the plasma membrane transporter SLC9A1 [13]. In a different study, MSCs exosomes can reverse the resistance of ovarian cancer cells to Docetaxel [13]. Besides that, MSC-derived vesicles can help in the bone regeneration and differentiation, as observed in rats with osteoporosis treated with these exosomes [14].

In Central Nervous System (CNS) diseases

The current treatments for neurodegenerative disorders are very limited and only work for symptomatic relief. In animals, repeated injection of exosomes isolated from stem cells improved motor performance, diminished glial activation, and reached the lesion sites in a mouse model of amyotrophic lateral sclerosis [18]. Another study also described that MSC-derived exosomes were shown to alleviate motor, learning and memory impairments in a progressive Parkinson's disease model in mice. The authors also observed that these exosomes were able to alter phospholipid composition and cholesterol metabolism in hippocampal neurons [18]. To finalize, exosome injections are undergoing clinical studies, thus safety and efficacy of injections have not been evaluated in humans so far. Therefore, it is important to emphasize that the Code of Federal Regulations of the United States Food and Drug Administration (FDA) does not approve exosomes for intravenous or intramuscular injection in humans.

Intranasal administration is a promising field to directly deliver stem cell-derived exosomes into the central nervous system (CNS) [24].

Intranasal has several advantages over injection routes: it's a non-invasive route, can be performed by non-trained individuals, can fastly deliver xenobiotics to the brain and systemic circulation, it avoids first-pass metabolism and it has very few adverse effects [24, 26].

Notably, any molecule that reaches the blood must cross the blood brain barrier (BBB) to achieve the central nervous system (CNS). However, the semi-permeability of BBB poses a

significant challenge to orally- and intravenously- administered xenobiotics, since it has been estimated that greater than 90% of all small-molecule drugs cannot overcome this physical barrier between blood and brain [25]. In contrast, this hindrance to the CNS does not occur when a xenobiotic is administered in the intranasal mucosa. Our intranasal mucosa is composed by the olfactory epithelium enerved with olfactory sensory neurons that are anatomically connected to the brain. Our olfactory neurons have their receptor terminals exposed in the lumen of the nasal cavity, and these cells traverse the whole nasal mucosa, reaching the olfactory bulb already located in the forebrain [26].

Compounds deposited into our nasal cavity may access the brain after they interact with our olfactory neurons which have their axons elongating to the CNS [26]. Several therapeutic molecules, nanoparticles and even mesenchymal stem cells can exploit an intra-neural transport or even our perivascular and perineural spaces to arrive at the brain [26]. In this point, surprising observations indicate that different stem cells can be delivered to the brain by intranasal route [26]. To enter into the brain following intranasal administration, these cells exploits a perineural space, crossing an anatomical barrier called cribriform plate, which separates the nasal cavity from the olfactory bulb [27].

Currently (in february 2024), there are more than 40 experimental studies already published showing that intranasally-administered exosomes can reach the brain, impacting several Central Nervous System diseases [24]. To cite some of these experimental models in rodents, intranasal administration of stem cells-derived exosomes has already been tested against ischemic stroke models, against traumatic brain injury and spinal cord injury models, against perinatal ischemic injury model, against cognitive impairment model, against Parkinson's disease model and against psychiatric and other neurological disease model. In addition, 3 clinical trials are ongoing without conclusive results. In experimental models overall, intranasally-administered labeled exosomes are able to reach the brain and exert positive effects in the improvement of the disease.

NOTE: Currently Exosomes is approved by the FDA for dermatologic use only.

In Cosmetic Dermatology:

Few years ago, dermatological use of exosomes for aesthetics purposes started after publication of the first preclinical results. Exosomes from stem cells can reduce the aging process of human dermal fibroblasts [14]. Also, they can regulate the expression of MMP-1 which contributes to the induction of type 1 collagen [14]. As already mentioned, exosomes from stem cells can participate in the recovery process via activation of several cell signaling pathways, such as Erk1/2, Notch, Wnt4/beta-catenin and NF-kB [14]. Also, *in vivo* results have shown that the injection of mesenchymal stem cells (MSCs)-derived exosomes significantly increased skin flap survival and capillary density, probably as a consequence of a decreased inflammatory reaction and cell death [20]. We would like to highlight the studies showing that the injection of exosomes into the skin can stimulate the production of collagen and elastin, two substances that are essential for skin regeneration. For example, Kim and co-workers labeled mesenchymal stem cells-derived exosomes and applied them on the outermost layer of human skin [19]. The results revealed that EVs approach the epidermis after 18h and increases the expressions of Collagen I and Elastin by Human Dermal Fibroblasts (HDFs) after 3 days of treatment [19]. Moreover, exosomes decreased the expression of MMP-1, which degrades Collagen, by more than 50%. Considering that Collagen I is one of the main proteins in the human extracellular matrix and that Elastin is pivotal to skin elasticity, these results reinforce the topical use of exosomes for skin rejuvenation.

Delivery methods

Numerous biocompatible and nontoxic nanoparticles have been employed as drug delivery systems. In this context, exosomes can deeply penetrate biological tissues and they are not rejected by our immune system.The biodistribution and pharmacokinetics of exosomes can be modulated by engineering various factors such as cellular origin and membrane protein composition. Because they are easily removed by cells, the bioavailability of exosomes is not long in the body, requiring several local applications. For example, systemic injection of radiolabeled exosomes in healthy animals show rapid clearance (~2 to 3 mins) from the blood [22]. To circumvent this problem, biomaterials are being developed to be mixed with exosomes to allow a slow release of these nanovesicles, thus increasing their bioavailability in the body after administration. These materials can extend the exosome storage time, stability and even change the release properties in the body.

Several biomaterials have already been tested with exosomes: metals, bioactive ceramics, hydrogels and synthetic polymers [14].

Exosomes can be injected in aqueous solutions or coupled to homogeneous gels and creams. Our company is studying different ways to improve exosome stability in our preparation.

Side effects of application

There are no described side effects for dermatological applications of exosomes. No redness, swelling or bruising in the skin site when topically applied.

Exosomes, either in an aqueous solution or in oil-in-water emulsion cream, topically applied to human skins remain confined to the site (in *stratum corneum*) and able to exert their effects, as demonstrated by studies with human skin explants [23]. This local retention minimizes potential systemic side effects that might occur if the particles travel to the blood.

JuveXO

Medical-grade, MSC-derived Premium Secretome

Our exosome-based product (JuveXO®) is a cell-free aqueous solution containing biomolecules naturally produced by purified human Mesenchymal Stem Cells (MSCs). Our cell isolation is in compliance with FDA and cGMP rules and the purity of our MSCs population is characterized by flow cytometry using different cell-specific markers.

Our MSCs-derived product was specially designed for dermatological procedures, such as skin rejuvenation; improving skin tone and textures; reduction of skin inflammation; and to give skin a smoother and luminous appearance. The cells and technology in JuveXO® are currently part of FDA-approved clinical trials. *Note: JuveXO® is approved and tested for topical use only and not for injection into blood or muscles.*

JuveXO®'s MSCs-derived secretome is a result of over 17 years of research and is produced by a patent pending technology that enables control of the biomolecules.

Powerful, natural, lasting effects

The number of exosomes in our product was evaluated several times and it is over 50 billion per milliliter. By combining the incredible power of exosomes, collagen, elastin, and hyaluronic acid, the secretome in JuveXO® offers you more natural-looking and rejuvenated skin and hair. Specifically designed to yield long-lasting effects, most consumers typically receive JuveXO® every 18-24 months.

JuveXO® can rejuvenate the skin

Developed to be the ideal treatment for human skin rejuvenation due to its high contents of Collagen Type I and III, Hyaluronic Acid, Chondroitin Sulfate, several growth factors.

Sagging and wrinkled skin are features of chronic sun-damaged and aged uncared skins, as a consequence of collagen and elastic fibers deterioration over years. Stimulation of these proteins synthesis by JuveXO® application can rejuvenate the face skin, promoting better tone and hydration levels.

Read more about the science behind JuveXO®

Advantages of JuveXO®
over competitors

Exosomes regenerative power to restore and sustain youth is dependent on three key factors: the source, processing, & purity.

Source: JuveXO® harnesses the transformative power of umbilical cord lining stem cells (ULSCs), nature's fountain of youth. Ethically sourced and bursting with regenerative potential, ULSCs unlock a unique blend of benefits for skin. All based on over 17 years of research and FDA clinical studies.

Processing: JuveXO® utilizes a patented technology that mimics the natural microenvironment of ULSCs, meaningour high quality MSC-derived secretome is specially engineered to contain high amounts of naturally occurring Types I and III Collagen, Elastin, Chondroitin Sulfate, Growth Factors.

Purity: Experience the ultimate peace of mind. JuveXO®'s exosomes are cell-free, pre-COVID, and organic, ensuring the purest, most potent formula for your skin. In addition to our superior production quality system, MSCs purity is frequently tested and cells with an elevated number of divisions are not used for product manufacturing.

The JuveXO® Difference:

Visible results: Experience tighter, brighter, and smoother skin as JuveXO® targets wrinkles, fine lines, and age spots.

Natural radiance: Restore your skin's youthful glow with the power of naturally occurring growth factors and hyaluronic acid.

Ethical choice: Embrace peace of mind knowing you're choosing a sustainable and ethically sourced product.

Don't settle for generic exosomes. JuveXO® is a meticulously crafted elixir, a testament to our relentless pursuit of scientific excellence and unwavering commitment to your beauty.

UCM-X SAFETY TEST DATA SHEET: Addendum UCM-X Certificate of Analysis (Lots UCMX-211116, UCMX-221121-JT, UCMX-230112-JT)

Measures are taken throughout UCM-X production to protect against the presence of pathogenic viruses or microbial contamination.

*** Note: Test results in this data sheet pertain to the cell line used for UCM-X production and the single donor of tissue source of that cell line.**

UCM-X lot release specifications pertain to all GMP lots of UCM-X; CCIT pertains to 2mL or 5mL vials.

Umbilical Cord Tissue Donor Screening and Testing (GTP) -- Specification: No infectious disease or risk factor for infectious disease.

Maternal blood samples were collected within 7 days of umbilical cord collection and tested at CLIA-certified lab on FDA approved tests.

Test Name	Result *	Method	Detection Target
Hepatitis B Virus (HBV)			
HEPATITIS Bs Ag	NONREACTIVE	EIA	HBV Surface Antigen
HEPATITIS Bc (Total) Ab	NONREACTIVE	ELISA	Antibodies to HBV Core Antigen
HBV ULTRIO	NONREACTIVE	Nucleic Acid Test (TMA)	HBV DNA
Hepatitis C Virus (HCV)			
HEPATITIS C Ab	NONREACTIVE	ELISA	Antibodies to HCV
HCV ULTRIO	NONREACTIVE	Nucleic Acid Test (TMA)	HCV RNA
Human Immunodeficiency Virus (HIV)			
HIV 1&2 Ab	NONREACTIVE	EIA	Antibodies to HIV-1 and/or HIV-2
HIV ULTRIO	NONREACTIVE	Nucleic Acid Test (TMA)	HIV-1 RNA
Human T-Lymphotropic Virus (HTLV)			
HTLV I/II Ab - AVIOQ	NONREACTIVE	Microelisa	Antibodies to HTLV I/II
Cytomegalovirus (CMV)			
CMV TOTAL Ab	NONREACTIVE	Solid phase red-cell adherence	IgG and IgM Antibodies to CMV
Treponema pallidum (Syphilis)			
SYPHILIS - NON-TREPONEMAL	NONREACTIVE	Rapid Plasma Reagin (RPR) Test	Reagin Antibodies (Syphilis screen)
West Nile Virus (WNV)			
WNV TMA:SINGLET RESULT	NONREACTIVE	Nucleic Acid Test (TMA)	WNV RNA
Trypanosoma cruzi (Chagas)			
CHAGAS ORTHO	NONREACTIVE	ELISA	Antibodies to T. cruzi (Chagas)

Cell Line Safety Testing for Biologics (GMP) -- Specification: No microbial (bacterial/fungal), mycoplasma, aventitious agent or viral contaminant.

Cells were expanded in culture, submitted to a third-party FDA-registered laboratory, and tested in accordance with GMP.

Test Name	Result *	Method	Detection Target
Sterility			
Sterility Testing for Final and Biological Products	PASS; No Growth	Direct Method: Inoculation into Test Microbial Media (FTM and SCDM/TSB) Incubated for 14 days per USP <71>	Bacteria and Fungi
and Baceriostasis/Fungistasis Test	No Inhibition		
Mycoplasma			
Cultivable and Non-cultivable Mycoplasmas	PASS; Not Detected	Cultivation for 28 days (Agar Cultivable) and Fluorescent Detection (Non-Cultivable) per USP <63> , EP <2.6.7>	Mycoplasmas
and Mycoplasmastasis Test	No Inhbition		
In Vitro Adventitious Virus/Agent Assay			
Tissue Culture Safety Testing	PASS; Not Detected	Cultures for 28 days on Indicator Cell Lines (MRC-5, Vero 76, HeLa) for hemadsorption, hemagglutination, and cytopathogenicity	Adventitious Viruses or Agents
Pathogenic Human Viruses			
Detection and Quantitation of Virus DNA or RNA:			
Hepatitis A Virus (HAV)	PASS; Not Detected	PCR Amplification of Nucleic Acid Sequences, Quantitation with Copy Number Standard, and Detection by Fluorescent Probe	HAV RNA
Hepatitis B Virus (HBV)	PASS; Not Detected		HBV DNA
Hepatitis C Virus (HCV)	PASS; Not Detected		HCV RNA
Human Cytomegalovirus (HCMV)	PASS; Not Detected		HCMV DNA
Epstein-Barr Virus (EBV)	PASS; Not Detected		EBV DNA
Human Parvovirus B19 (B19)	PASS; Not Detected		B19 DNA
Human Polyomavirus JCV (JC)	PASS; Not Detected		JC DNA
Human Polyomavirus BKV (BK)	PASS; Not Detected		BK DNA
Herpesvirus 6 (HHV-6)	PASS; Not Detected		HH-6 DNA
Herpesvirus 7 (HHV-7)	PASS; Not Detected		HH-7 DNA
Herpesvirus 8 (HHV-8)	PASS; Not Detected		HH-8 DNA
Human Papillomavirus types 16, 18 (HPV-16, HPV-18)	PASS; Not Detected		HPV-16, HPV-18 DNA
Endotoxin			
Determination of Endotoxin and Spike Recovery Test	PASS; 0.2 EU/mL (<0.3 EU/ml) PASS; 99% (>60%)	Kinetic-Chromogenic LAL Assay per USP <85>	Endotoxin

UCM-X Lot Release Safety Testing (GMP) -- Specifications: No microbial or mycoplasma contaminant; Low endotoxin; Evidence of container closure integrity.

UCM product material and filled vials are submitted to third-party FDA-registered laboratory and tested in accordance with GMP.

Test Name	Specification	Method	Detection Target
Sterility			
Sterility Testing for Final and Biological Products	PASS; No Growth	Membrane Filtration Method with Test Microbial Media (FTM and SCDM/TSB) Incubated for 14 days per USP <71>	Bacteria and Fungi
and Baceriostasis/Fungistasis Test	No Inhibition		
Mycoplasma			
Cultivable and Non-cultivable Mycoplasmas	PASS; Not Detected	Cultivation for 28 days (Agar Cultivable) and Fluorescent Detection (Non-Cultivable) per USP <63> , EP <2.6.7>	Mycoplasmas
and Mycoplasmastasis Test	No Inhbition		
Endotoxin			
Determination of Endotoxin	PASS; < 4 EU/mL	Kinetic-Chromogenic LAL per USP <85> Assay	Endotoxin
and Spike Recovery Test	PASS; No Matrix Interference		
Container Closure Integrity (CCI) - pertains to 2mL or 5mL vials			
CCI Test (CCIT) of UCM Container Closure System (CCS)	PASS; No Dye Ingress	Dye ingress under Vacuum with Detection/ Quantitation by UV-Vis spectrophotometry	Container closure system integrity breech or leak
and Qualification of CCIT for UCM CCS with Controls	PASS		

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CERTIFICATE OF ANALYSIS

PRODUCT CODE:	UCM-X
PRODUCT NAME:	ULSC Conditioned Medium-X
LOT NUMBERS:	UCMX-211116-2mL and UCMX-211116-5mL
ALIQUOT DATE:	17-NOV-2021
FILL VOLUME:	2 mL and 5 mL (fill volume indicated on vial label)

STORAGE CONDITIONS:	-20° C or below
EXPIRY OR RETEST DATE:	NOV 2023

RELEASE CRITERIA:	SPECIFICATION:	RESULT:		REPORT
pH Test	≥ 7.35 to ≤ 7.44	7.41		TBB Lot 211116 Batch Record UCMX-PBR-02
Sterility Testing of Final Containers and Biological Products (Membrane Filtration Method) per USP <71>	No Growth	No Growth		GP-V730: CRL 266748 RPT-13APR2022; CRL 297599 RPT-22APR2022; CRL 322925 RPT-05AUG2022.
Determination of Endotoxin Using Kinetic Chromogenic LAL-Testing per USP <85>	< 4 EU/mL	Dilution Factor: 1 (Neat)		GP-C555; CRL 266750 RPT-16DEC2021; CRL 266751 RPT-16DEC2021.
		Endotoxin Content:	Spike Recovery:	
		0.008 EU/mL	132%; 140%	
Testing for the Presence of Agar Cultivable and Non-cultivable Mycoplasmas in Accordance with US Pharmacopeia and EU Pharmacopeia Guidelines including Test for Mycoplasmastasis	Not Detected	Not Detected		GP-V611.20: CRL 266749 RPT-12JAN2022; CRL 277972 RPT-15MAR2022.
Container Closure Integrity Testing (CCIT) by Dye Ingress (UV-Vis) to ensure product conforms to sterility requirements specified in 21 CFR 211.167(a) and 21 CFR 610.12.	Pass (No Ingress)	Pass (No Ingress)		CP-9349/1.0: CRL 266704, CRL266705 RPT-220110-21.

Sterile filtered (0.2 µm). Quality Control (QC) Testing for Sterility, Endotoxin, Mycoplasmas, and CCI were conducted at a third-party lab in compliance with current Good Manufacturing Practices (cGMP) and in accordance with specifications set by TheBioBox, LLC Quality Assurance (QA).

INGREDIENTS FOR TOPICAL COSMETIC USE ONLY. INTENDED FOR DOWNSTREAM MANUFACTURING OR RESEARCH USE.
NOT FOR USE IN DIAGNOSTIC PROCEDURES, NOT APPROVED FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

This lot has been QC tested and QA reviewed. Test results meet release specifications per TheBioBox, LLC Quality System.

Marisol Castro-Paiz
Digitally signed by Marisol Castro-Paiz
Date: 2022.11.02 13:45:48 -07'00'

Reviewed by Marisol Castro-Paiz
Quality Systems Director
Review Date: 2-Nov-22

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