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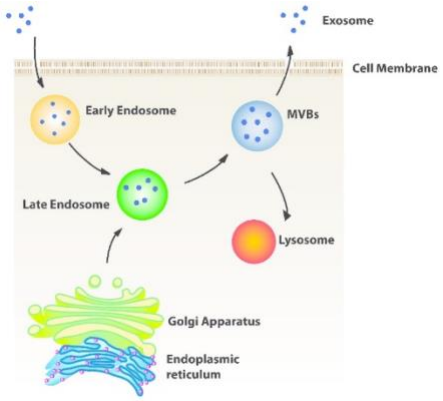
PRODUCTS

Exosomes based cure in the treatment of patients suffering from the novel Corona virus (Covid-19 or SARS-CoV-2)

Exosomes are small naturally occurring, bio membrane nano-sized extracellular vesicles (EVs) which contain various biomolecules such as proteins, ribonucleic acids (RNAs), deoxyribonucleic acids, lipids and microRNAs (miRs). Pioneering studies documented the generation of exosomes in differentiating reticulocytes as a result of the fusion of multivesicular endosomes/MVBs with the plasma membrane. Later studies have shown that exosome plays a critical role in the regulation of physiological processes (**C. Thery, 2018**). These nanoparticles have been characterized to be between 30 to 120 nm (**A. Marote, 2016**). Exosomes are naturally produced



within the body and is involved in cell-to-cell communication which can be a critical component for cancer Therapy (**N. L. Syn, 2017**), osteochondral regeneration (**S. Zhang, 2016**) and myocardial ischemia/reperfusion (I/R) injury (**R. C. Lai, 2010**). Exosomes are naturally produced in the body by two separate mechanisms, the endocytic pathway and the biosynthetic mechanisms. Exosomes biogenesis proceed in four steps: origination, endocytosis, multivesicular endosomes (MVE) generation, and secretion (**C. Théry, 2002**). Exosomes contain conserved proteins such as CD81, CD63 (membrane associated proteins like LAMP-3), and CD9, Alix and tumor susceptibility gene 101 protein (**A. Luarte, 2016**), as well as tissue/cell type specific proteins that reflect their cellular source. The exosome membranes are enriched with cholesterol, sphingomyelin, and ceramide (**C. Thery, 2006**). Most proteins within exosomes derive from parent cell membranes, the cytosol, and Golgi, but rarely from endoplasmic reticulum or mitochondria (**M.A.Lopez-Verrilli, 2013**). It is notable to note that exosome proteins participate in antigen presentation, cell adhesion, cell structure and motility, and are stress regulators, involved in transcription and in the participation of protein synthesis (**A. Lakkaraju, 2008**).

Generation of exosomes involves the creation of the endocytic pathway with extrinsic or intrinsic signals from the local milieu. The early endosome is formed by the invagination of the plasma membrane, thus becoming the late endosome under the regulation of multiple cell signaling pathways. The Golgi apparatus and the Endoplasmic Reticulum (ER) also participate in the secretion of exosomes.

Usage of Exosomes in Therapy has several distinct benefits which can be stated as follows: -

1) The exosomes can avoid problems associated with the transfer of cells that have a mutated or damaged DNA (**F. G. Teixeira, 2013**).
2) Most of the exosomes are small that can easily circulate through capillaries and these are highly advantageous since cells used in cell-based therapies are too large to enter into capillaries and are unable to pass the capillary beds that enable it to travel to lungs (**A. Marote, 2016**).
3) Cell based therapies are less effective as the efficacy may quickly diminish after transplantation, however, exosomes are much more effective due to its potential efficacious effect in areas far from the transplant site and thus achieving a "higher dose" than most cell based therapies (**D. G. Phinney, 2017**).
4) Exosomes have also been utilized in biomaterial treatments such as nanoparticles which can cause toxicity and other immunogenicity issues (**A. Fleury, 2014**).

Exosomes, unlike cells do not require cryogenic storage and can be stored at a suitable temperature of -20°C (**B. Yu, 2014**). Studies have shown that there is a deterioration of surface characteristics, morphological features and protein content of exosomes upon storage at -20°C and thus it was proposed to perform the representative functional analysis (including identification of morphological features) immediately upon fresh isolation (**R. Maroto, 2017**). Cryoprotectants such as Dimethyl Sulfoxide (DMSO) have also been used to preserve the morphological features of exosomes but the quality of the product cannot be established to be equal to a fresh isolate (**Y. Wu, 2015**).

As it has already been established that cell-based therapies are too large to enter capillaries thus making exosomes a better choice (**A. Marote, 2016**), it is proposed that exosomes have an excellent potential as a natural therapeutic drug delivery vehicle (**H. Choi, 2020**). Recent laboratory reports have shown promise that mesenchymal stem cells generated secretome could offer a new therapeutic approach in treating COVID-19 pneumonia, due to the broad pharmacological effects, including anti-inflammatory, immunomodulatory, regenerative, pro-angiogenic and anti-fibrotic properties (**E. Bari, 2020**).

Recent outbreak of COVID-19 (SARS-CoV-2) that has impacted the world immensely had a significant impact on health care and the economy. The COVID-19 virus lead to more than 100,000 deaths in countries like the United States, Canada, Italy, Spain and Russia and plunged many leading economies into a financial crisis with many business shutting down and people forced to adhere to social distancing in order to stop the spread of this virus through person to person contact. At present there are no approved treatments as present measures are supportive and not curative in nature. Multiple approaches used so far include vaccines, medications Remdesivir and hydroxychloroquine and potentially combination therapy. The cure of COVID-19 is essentially dependent on the patients' own immune system. When the immune system is over activated in an attempt to kill the virus, this can lead to the production of a large

number of inflammatory factors, resulting in severe cytokine storm (**S. Atluri, 2020**). New approaches include the usage of expanded umbilical cord mesenchymal stem cells or (UC-MSCs) that may have a role in mitigating COVID-19 is being actively studied. However, this approach is questionable (**A. Marote, 2016**) since the size of these cells are too large to enter into the capillaries of the lungs and thus eliciting any kind or notifiable therapeutic action.

It is now available in the literature that SARS-CoV-2 is a positive-sense, single-stranded RNA virus, belonging to the genus betacoronavirus. It has an 88–96% sequence homologue sequence identity to three bat-derived SARS-like coronaviruses (bat-SL-CoVZC45, bat-SL-CoVZXC21, RaTG13) and to a coronavirus strains isolated in pangolins -a scaly, ant-eating mammal highly trafficked for its presumed medicinal virtues and clandestinely sold in live animal markets such as Wuhan- which possesses the same six key residues of the angiotensin converting enzyme 2 (ACE2) receptor binding domain as SARS-CoV-2 (**D.U. De Rose, 2020**)

Main hypothesis for the entry of SARS-CoV-2 into the lungs is the angiotensin-converting enzyme 2 (ACE2) which is a membrane-bound aminopeptidase that is highly expressed in lung alveolar epithelial cells. ACE2 represents a major gateway to the SARS-CoV-2 infection, although the specific mechanisms are still uncertain. As with other coronaviruses, the N-terminal portion (S1, receptor binding domain) of the viral spike (S) glycoprotein binds with high-affinity to receptor(s) on the surface of susceptible cells (**D.U. De Rose, 2020**). Although ACE2 has been identified as a viral receptor, there might be other receptors or co-receptors for this virus yet to be discovered (**A.J. Turner, 2004**).

The ability of exosomes to percolate within the capillaries and thus enter deeply within the lung alveolar epithelial cells, has made it an excellent candidate in a candidate of choice to act as a vector to transfer RNA and miRNA content that are considered a relevant cargo in exosomes in terms of the ability of a small number of molecules to influence several proteins/enzymes within one or more cellular pathways in target cells (**H. Ling, 2013**).

Exosomes have a tremendous potential to act as a vehicle to transport small molecules such as RNA, proteins to the target site since they can percolate very easily through small vesicles such as capillaries. In the case of SARS-CoV-2, exosomes can be an excellent vehicle and its efficacy, safety and pharmacological benefits can be utilized and truly exploited through research and Clinical Trials. Exosome research is truly changing the mode of action of therapeutic effect of drugs, as well as bringing about a significant change to the pharmacokinetic and pharmacodynamic effect of drugs.

Note- Exosomes are still in its experimental stage and use of this molecule on humans is strictly regulated by the FDA under proper safety and efficacy studies. Exosomes are categorized as minimally manipulated under PHSA 361 or drugs that require premarket approval. Thus, exosomes used to treat diseases and conditions in humans are regulated as biological products under the Public Health Service Act and the U. S. Food and Drug Administration and are subject to premarket review and approval requirements by December 1, 2020. RichSource represents the new exosomes product – Amniosomes™ - that has an FDA IND filed April 14, 2020 for 351 status.

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