Clinical grade Exosomes of mesenchymal stem cells enhanced by ExoRAP and ExoPAN technology

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Introduction

Mesenchymal stem cells, or MSCs, are multipotent stromal cells that can differentiate into a variety of cell types, including: osteoblasts, chondrocytes, neurons, muscle cells and adipocytes. This phenomenon has been documented in specific cells and tissues in vivo and in vitro. MSCs are distributed all over the body and are responsible for regeneration. Commonly used tissues for the isolation of MSC are bone marrow, umbilical cord, cord lining and, increasingly, adipose tissue, which has a superior amount of MSCs.

All cells communicate and exchange information by different ways, including the secretion of soluble factors, the cell-to-cell adhesion contact, and the intercellular exchange of organelles. The secretome of mesenchymal stem cells includes paracrine substances, exosomes and microvesicles. Over 150 paracrine substances, also called cytokines and chemokines, can be released by mesenchymal stem cells. Two distinct populations of vesicles with peculiar membrane structure, mechanism of production, pathophysiological relevance, and different size have been described: exosomes and microvesicles. Microvesicles and exosomes contain biomolecules, including messenger RNA and micro RNA.

Exosomes and the whole secretome have been used for therapies in regenerative medicine to treat various illnesses such as osteoarthritis, cardiovascular diseases, neurological diseases, pulmonary diseases, diabetes and many more.

Objectives

Previous studies suggest that a hypoxic condition promotes self-renewal of undifferentiated mesenchymal stem cells and enhances their therapeutic potential.

Hypoxic exposure activates several signal transduction pathways including hypoxia inducible factor (HIF), a master transcription factor that regulates the expression of hundreds of genes to promote cellular adaptation to the hypoxic condition.

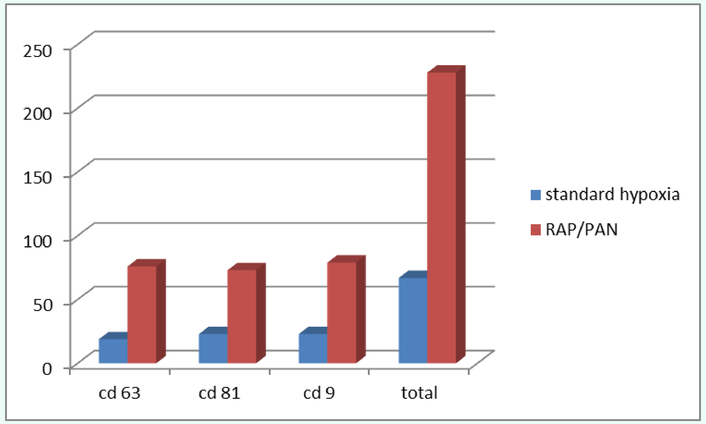
Our ExoRAP and ExoPAN technology uses a protocol of pre-treatment of the cultured MSC in special media and various anoxia conditions instead of hypoxia.

Materials & Methods

Adipose-derived mesenchymal stem cells were isolated, expanded and cryopreserved, according to standard procedure. One million stem cells were thawed in a T75 culture dish, washed with saline and covered with 10 ml of saline and then subjected to anoxia of variable duration. Micro and messenger RNA and Exosomes were measured using the Maestro Nano spectrophotometer and two ELISA assays (Exocet exosomes quantification kit, Signosis and Signosis EXOAB Antibody 9, 63, 81) . Results were compared to standard procedure to harvest Exosomes via hypoxia.

Results

Exosome quantification via CD 9, CD 63 and CD81

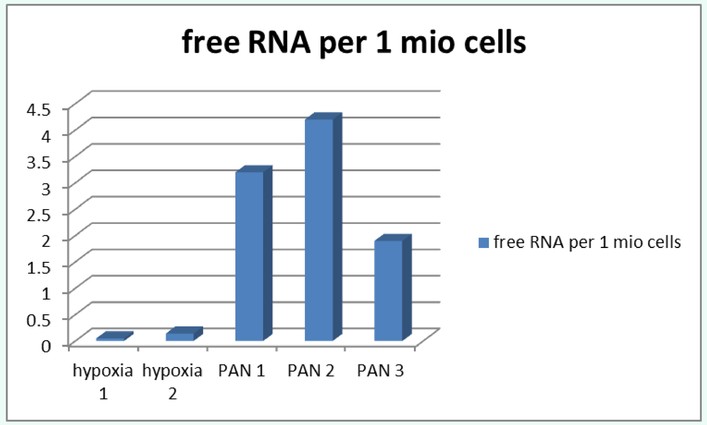


Increase of 4.5 times with EXO RAP technology compared with standard hypoxia procedure.

Our ExoRAP and ExoPAN technology uses a protocol of pre-treatment of the cultured MSC in special media and various anoxia conditions instead of hypoxia. With this protocol a 30 fold increase in RNA content per cell can be achieved.

The technology is successfully being used in regenerative treatments in human ExoRAP and ExoPAN technology and animal patients.

Total RNA measurements



30 fold increase of total RNA with the EXO PAN system compared with standard hypoxia procedure

Conclusions

- The ExoRAP and ExoPAN technology is easy to use and achieves a much greater amount of RNA. This will be beneficial for all regenerative treatments but especially for diseases where the success rate has not been huge so far, such as diabetes, multiple sclerosis and other neurodegenerative diseases, cardiovascular diseases and so forth.
- The secretome is devoid of all cells which will decrease to risk of unwanted reactions in allogeneic use.
- The secretome has been tested in an autologous setting in hundreds of patients with no side effects so far.
- The secretome can be stored at -800C with minimal loss of activity.
- Therapeutic doses can be achieved with only 1 mio MSCs saving owner's banked cells and enabling industrial up-scaling processes

References

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