Human Mesenchymal Stem Cell-Derived Giant Plasma Membrane Vesicles(GPMV) As A Novel Candidate for Liver Regeneration

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ABSTRACT

Giant plasma membrane vesicles (GPMV) are micron-sized vesicles derived from the plasma membrane of mammalian cells under the influence of chemical vesiculants. In this work we have made an attempt to explore the regenerative potential of mesenchymal stem cell derived GPMV. For this purpose, in vitro cell culture based drug-induced liver disease model was adopted. We synthesized GPMV from adipose tissue derived mesenchymal stem cell using chemical vesiculants namely dithiothreitol (DTT) and paraformaldehyde (PFA). The synthesized GPMVs were found in the size range of 2-15µm. We confirm the presence of cytosol in the GPMV by calcein-AM staining. Fluorescence recovery after photo bleaching assay further showed that solute mobility inside the GPMV was almost 2 fold higher in comparison to that of inside the stem cell. Using a set of dyes, we prove that GPMVs contain proteins and RNA thus can work as packaged cellular cargo for the delivery of bioactive molecules but are devoid of DNA. The proteins and RNA are homogeneously distributed. To evaluate the regenerative potential of GPMVs, we created an acetaminophen (APAP) induced diseased liver model in vitro. The restoration of the functionality of the APAP-treated cells under the influence of GPMV was evaluated by checking the expression of albumin by immunocytochemistry. This study suggests that the mesenchymal stem cell derived GPMV can be used as a new stem cell derived therapeutics.

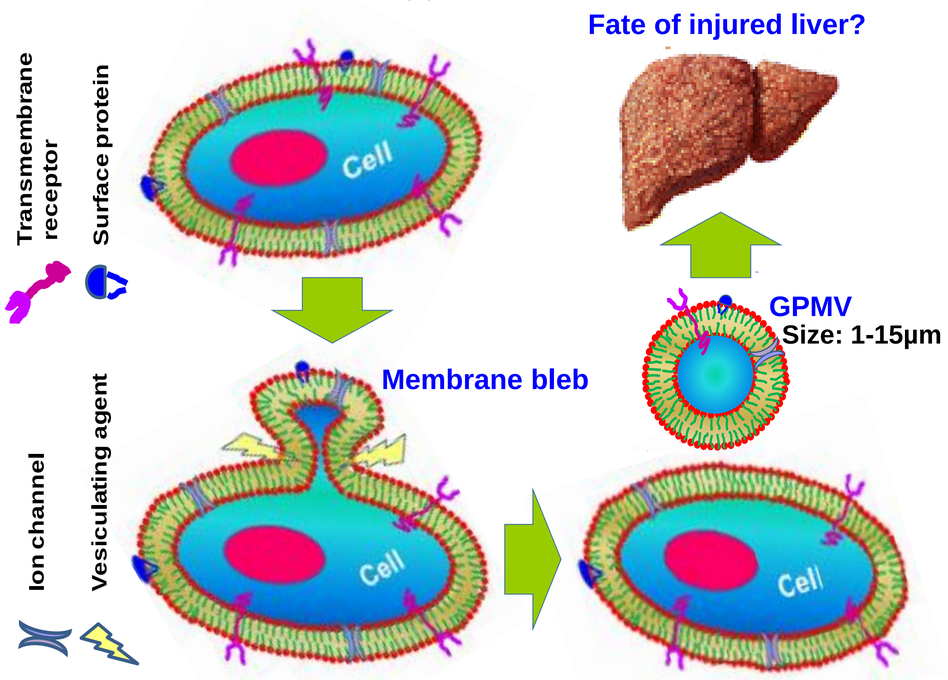
INTRODUCTION

- Liver has remarkable regenerative capacity after injury
- Hepatic diseases, like viral hepatitis and non-alcoholic fatty liver disease (NAFLD) , liver fails to perform regeneration up to its potential
- Conventional methods available for the treatment includes the usage of drugs ofosbuvir and simeprevir that have been approved by the FDA
- The upcoming treatment options are cell based therapeutics including exosomes
- The major challenge in the cell based therapeutics is a lack of control over the composition

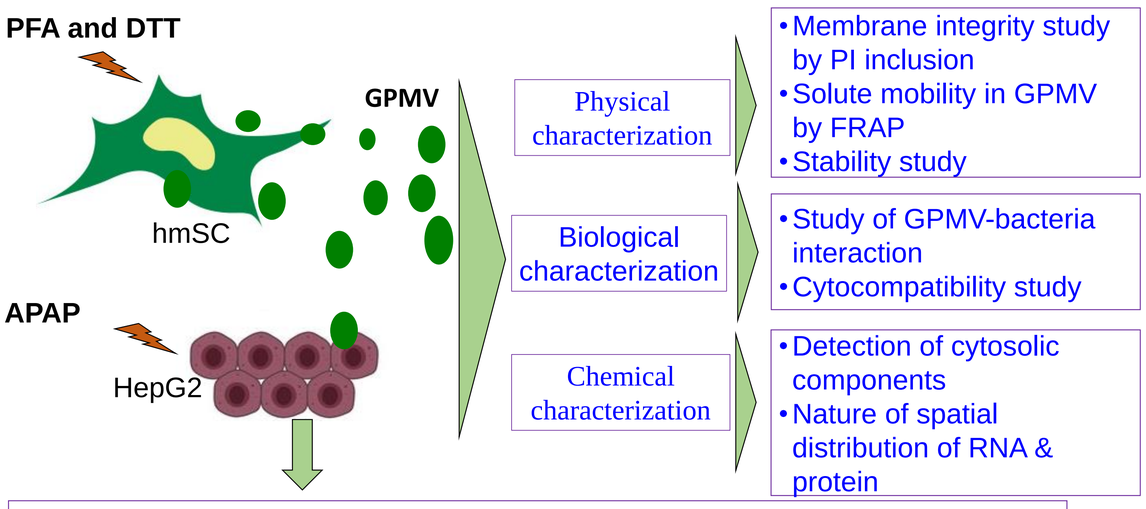
Here GPMV is an advantage

GPMVs are membrane vesicles derived from the membrane of live cell. They can be generated in vitro using a set of physical or chemical stimulus.

Our approach :Use of GPMV



METHODOLOGY



RESULTS

Physical characterization of GPMV

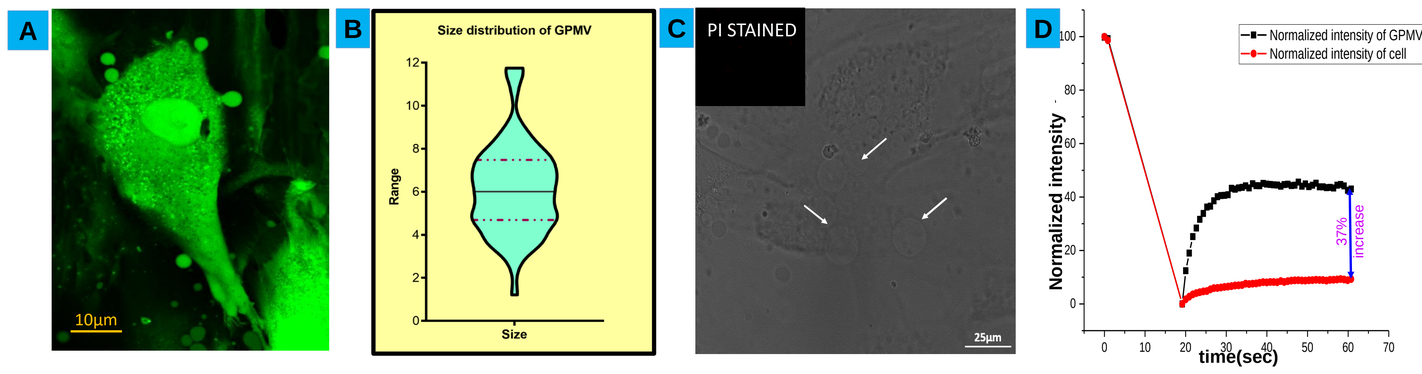
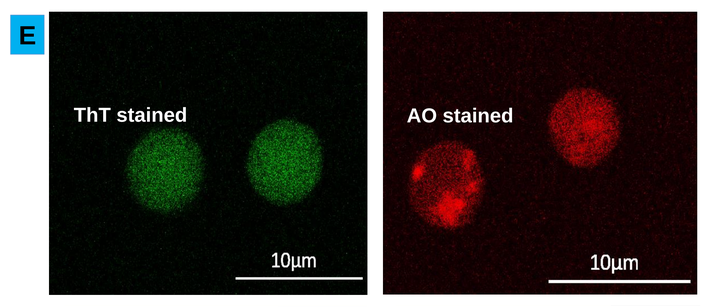


Fig1:A) Calcein AM stained hMSC generating GPMV under the influence of DTT & PFA
B) Violin plot depicting the size range of 1.5-12µm of generated GPMV
C) cell stained with propidium iodide(PI) generating GPMV depicts PI exclusion from cell & GPMV
D) Fluorescence recovery after photo bleaching in hMSC and GPMV

Chemical characterization of GPMV



Chemical characterization of GPMV

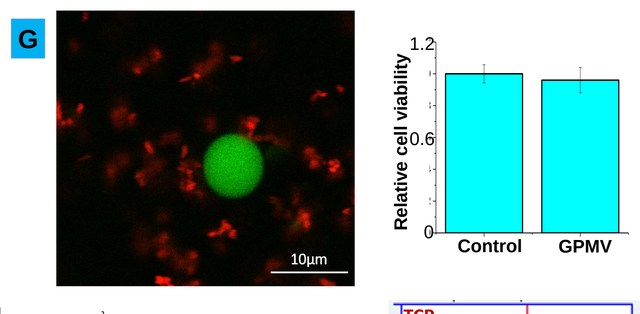


Fig 2: E)GPMV stained with ThT& acridine orange(AO) showing presence of proteins and RNA.
F)Normalized intensity of ThT & AO across the GPMV shows a homogeneous distribution of proteins inside GPMV

Fig 3:G)Relative number of GPMV post 48 hours E.coli interaction shows bacterial resistant property of GPMV
H)MTT and FACS based cytocompatibility of GPMV

Liver Functionality assay

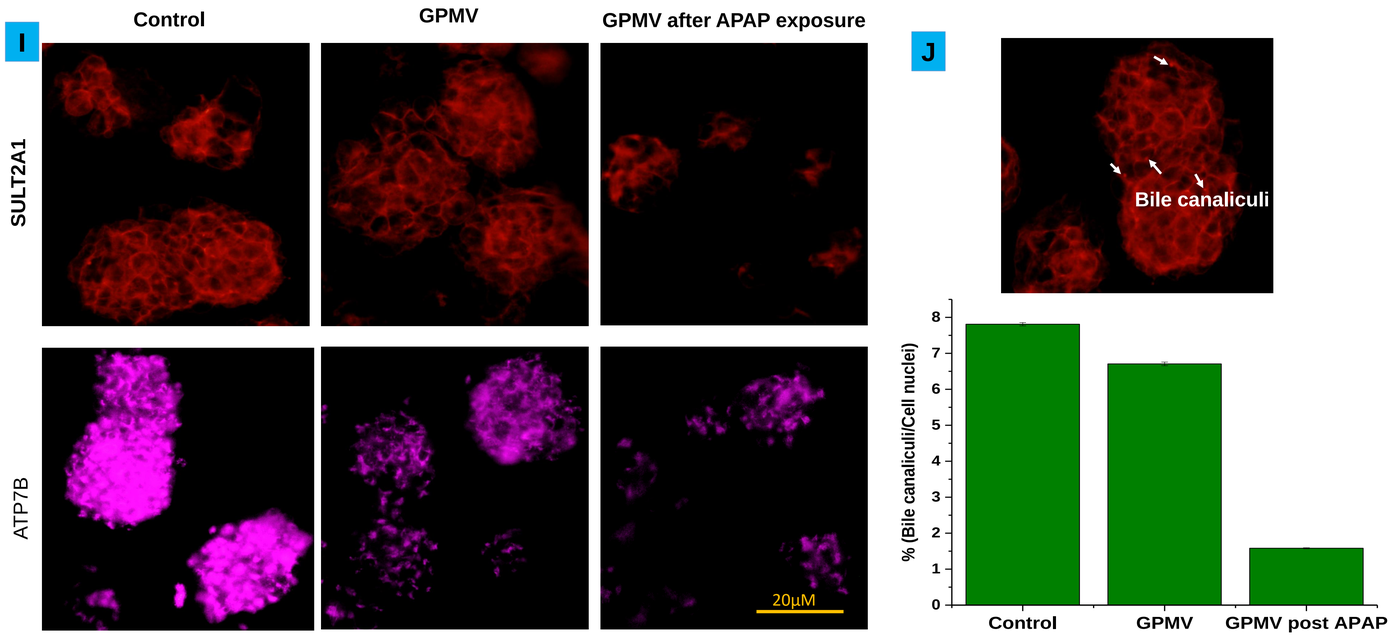


Fig 7:I)Fluoresecence micrographs of HepG2 cells treated with GPMV after 24 hours expression of ATP7B and SULT2A1 as liver functionality markers
J)Arrow indicating the presence of bile canaliculi in HepG2 cells

CONCLUSION

- GPMVs(1-15µm)were synthesized from hMSC using low concentration of DTT and PFA in a Ca^{2+} and Na^{+} containing buffer
- The generation of these vesicles is a time, dose and temperature dependent phenomenon
- GPMV contains cytosolic proteins and RNA but lacks DNA
- The expression of ATP7B and SULT2A1 and bile canaliculi was prominently observed in HepG2 cells treated with GPMV

REFRENCES

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- Gaur, Deepanjali, et al. (2018).Advanced Biosystems ,2.9: 1800093.
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