Therapeutic Potential of Extracellular Vesicles Derived from Human Placenta for the Treatment of Corneal Transplant Graft Rejection and Corneal Injuries

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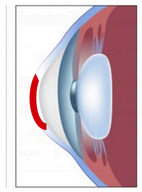
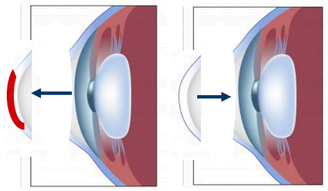
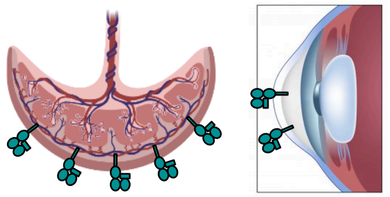
BACKGROUND



In response to trauma, infection or chemical exposure, the corneal immune tolerance breaks down and the cornea undergoes neovascularization and scarring.



There is a lack of therapies to treat corneal neovascularization and scarring. Corneal transplant can restore vision in these eyes, but comes with the risk of **transplant rejection**.



Human leukocyte antigen G (HLA-G) is a molecule expressed in the cornea and involved in maintaining the cornea's immune tolerance. HLA-G is also highly expressed in the placenta, and secreted in placental extracellular vesicles (EVs).

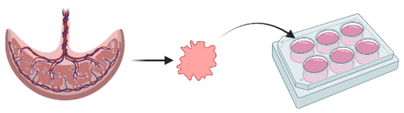
HLA-G gene therapy to the cornea has been shown to reduce fibrosis and neovascularization following chemical injury to rabbit corneas.[1]

OBJECTIVES

- Isolate **HLA-G+ EVs** from placental tissues and cells
- Study the therapeutic potential of placenta tissue and cell-derived HLA-G+ EVs to
  - –1) reduce corneal transplant rejection
  - –2) reduce corneal scarring and neovascularization after chemical injury

METHODS

Sources of HLA-G:



Conditioned media from placental explants

Placental tissue is dissected and placed in DMEM/F-12 media for 48 hours at 8% O_2.

Conditioned media from HTR-8 (immortalized trophoblast) cells

HTR-8 cells are grown to 100% confluency, then serum starved for 24, 48 or 72 hours at 3% or 20% O_2.

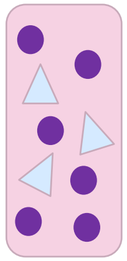
Add dextran and PEG

Enriching for HLA-G+ EVs:

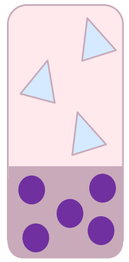
Aqueous two-phase separation (ATPS):

1. A mixture of dextran (DEX) and polyethylene glycol (PEG) is added to the conditioned media

2. The mixture is centrifuged at 200 g for 15 minutes, which allows for separation into two phases (DEX phase and PEG phase) by density



Centrifuge @ 200g



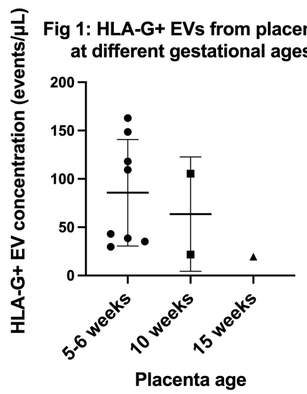
PEG phase

DEX phase: enriched for EVs

Nanoscale flow cytometry (nFC) to quantify HLA-G+ EVs

RESULTS

HLA-G+ EVs are detected in the conditioned media from placental explants and HTR-8 cells using nanoscale flow cytometry (nFC)



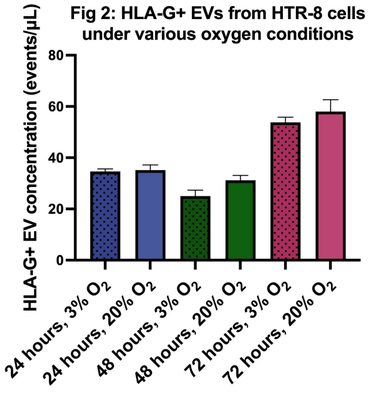


Figure 1: The concentration of HLA-G+ EVs was not significantly different at different placenta ages.

Figure 2: The concentration of HLA-G+ EVs from HTR-8 cells appeared higher in cells serum starved for 72 hours before media collection. No difference in hypoxic (3% O_2) vs normoxic (20% O_2) conditions. Error bars represent SEM around the mean of technical triplicate.

HLA-G+ EVs from placental explants are enriched 5.9X following ATPS

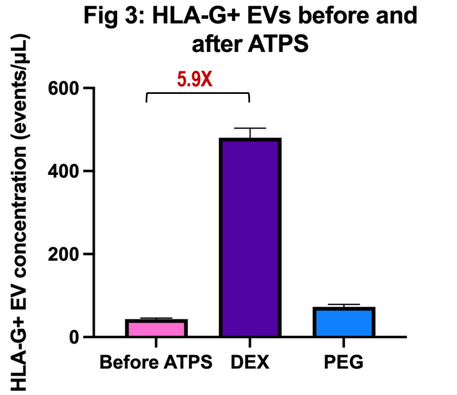
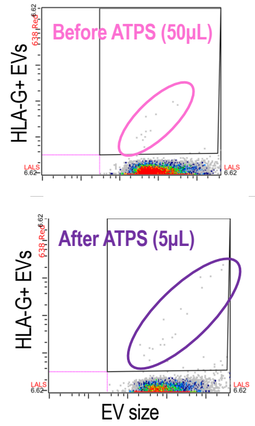


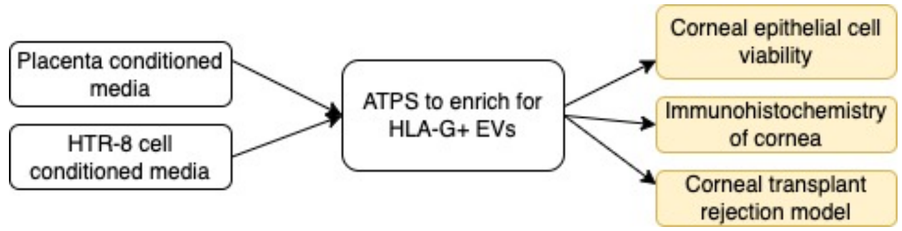
Figure 3: Following ATPS on placental conditioned media, HLA-G+ EVs are concentrated 5.9X compared to before ATPS. Error bars represent SEM around the mean of technical triplicate.

Figure 4: nFC diagrams show HLA-G+ EV populations before (50 µL loaded) and after (5 µL loaded) ATPS.



CONCLUSION

- HLA-G is involved in the immune tolerance of the cornea and placenta
- We have detected HLA-G+ EVs in the conditioned media from placental villous explants and a trophoblast cell line, and have enriched for HLA-G+ EVs
- Our next steps involve evaluating corneal epithelial cell viability, fibrosis and neovascularization with topically applied HLA-G+ EVs



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1. Gilger BC, Hirsch ML. Therapeutic Applications of Adeno-Associated Virus (AAV) Gene Transfer of HLA-G in the Eye. Int J Mol Sci. 2022 Mar 23;23(7):3465. doi: 10.3390/ijms23073465. PMID: 35408825; PMCID: PMC8998501.