Placental Mesenchymal Stem Cells-derived Extracellular Vesicles (plEVs) Use for Wound Healing in diabetic conditions

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Abstract

Objective 1: To examine the effectiveness and mechanism of placental MSCs-derived plEVs in wound healing in a diabetic skin model using human keratinocytes.
Objective 2: To test the immunomodulatory effects of plEVs in human keratinocytes wound model and their potential impact on dysregulated inflammatory cytokines in diabetes.
Background: Diabetes is associated with complications such as delayed wound healing. Current therapeutics to restore wound healing in diabetic patients are inadequate and warrant novel approaches. Mesenchymal stem cells (MSCs) and their derived extracellular vesicles (EVs) play a major role in tissue regeneration. Placenta MSC-derived EVs (plEVs) may have a therapeutic effect in enhancing diabetic wound healing.
Methods/Design: MSCs from healthy full-term placentae and Wharton's jelly (WJ) were isolated and expanded in vitro. plEVs were isolated from WJ MSCs. Experiments used normal human keratinocytes in high glucose medium to mimic diabetes. Effectiveness of plEVs was measured by wound/scratch closure, cell proliferation, and cytokine levels (ELISA).

Introduction

- **1 in 3 Americans suffer from Diabetes and it is one of the leading chronic diseases in America**
- **Delayed wound healing is a common complication of diabetes**
- **Placental tissue and Wharton's jelly are considered rich sources for mesenchymal stem cells (MSCs).**
- **Stem cells were shown to mainly work locally by paracrine effects via their secreted extracellular vesicles (EVs)**
- **Our lab has previously shown that fetal Placental side is a rich source of EVs that promote cell viability, so our approach has shifted towards examining wound healing (in vitro)**
- **In this study, we examined the potential benefit of placental-derived plEVs in diabetic wound healing conditions**

Objective

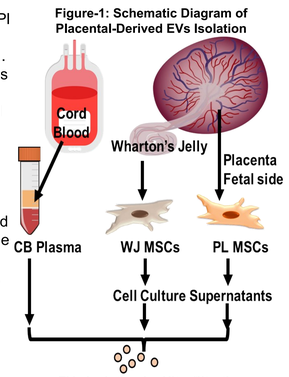
To examine the effectiveness of WJ and PI MSCs derived EVs isolated from healthy full-term pregnancies in wound healing using a diabetes mimic condition using high glucose media in Keratinocytes (HaCaT cells) in vitro model

Methods and Experimental Design

1. Isolation and Characterization of EVs:

- Placentas were collected from full term (FT) pregnancies for neonates born at Sparrow Hospital, Lansing, MI, USA, after obtaining informed consent and following protocols approved by the institutional review board.
- EVs were isolated from PI MSCs cultures conditioned media (CM) . Briefly, aliquoted samples were centrifuged to remove cells, debris and apoptotic bodies. Supernatants were filtered through 0.22-µm membrane filters to isolate large vesicles. Exosomes were collected using 0.05-µm membrane filters.
- Size range, morphology, and surface markers profile of the collected EVs were analyzed by **Nanoparticle tracking analysis (NTA), Transmission Electron Microscopy (TEM), and western blotting (WB).**

Figure-1: Schematic Diagram of Placental-Derived EVs Isolation



- The EVs total protein concentrations were measured by BCA protein assay to be used for subsequent cell culture treatments.
- For our in vitro experiments, we used **400 mg of EVs** per experimental group

2. Scratch Assay and use of EVs:

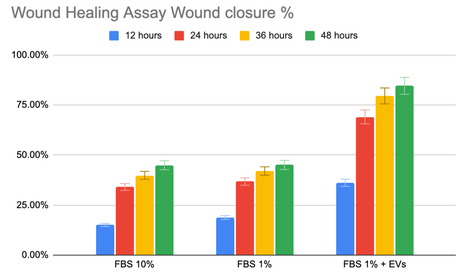
- We placed 100,000 Keratinocytes (HaCaT cells) into each well in a 48-well plate with complete high glucose DMEM until they reached confluency
- Once reached confluency, a scratch assay was performed using a 200 ul pipette and 500 ul of respective media was added into the wells
- 3 types of high glucose media were used in the wells, 10% EV depleted media, 1% FBS complete media, and 1% FBS EV depleted media
- High glucose was used to mimic the diabetic conditions (400 mg/mL)
- Control group was the 1% FBS complete media and the experimental was the 1% FBS complete media in addition to the placental-derived EVs (400 mg).

3. Cytokine Assay:

- Using same procedure of scratch Assay, supernatant was collected and sent to Eve tech for analysis of cytokines (48-plex).

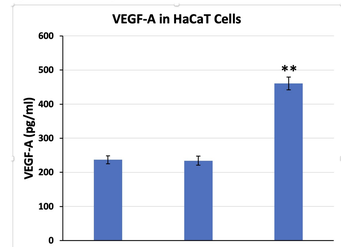
Results

Figure 2: Effect Of Placental Extracellular Vesicles (plEVs) on wound closure of the scratch Assay



- Control and experimental (plEVs) groups measured surface area (µm²) of the scratch assay
- Isolated EVs derived from placenta were added into the experimental groups (400mg/ml) and watched over 12-hour time periods up to 48 hours
- Enhanced surface area wound closure in the experimental EVs groups

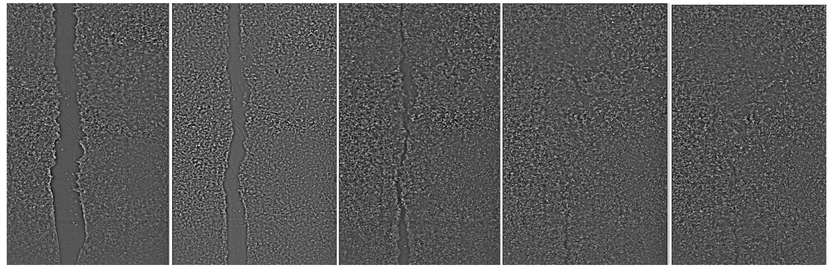
Figure 4: VEGF Cytokine Levels at 24 hours after scratch



** p<0.01 by ANOVA and Tukey post test

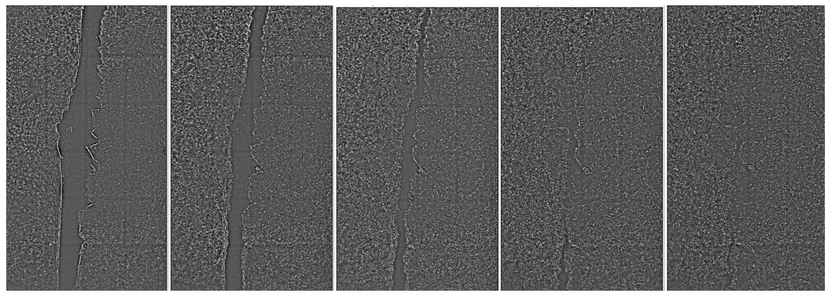
- Isolated plEVs derived from placenta were added into the experimental group (400mg/ml) and measured after 24 hours
- plEVs-treated group showed higher VEGF levels, indicating that extracellular vesicles enhanced angiogenic signaling during wound closure.
- TGF-β and RANTES showed similar results.

Figure 3a: Scratch Assay of Experimental (plEVs-treated) HaCaT Cells



- plEVs-treated HaCaT cells images at 5 time points: 0h, 12h, 24h, 36h, and 48h
- Scratch is fully healed at or before the 36-hour mark

Figure 3b: Scratch Assay of control (not treated) HaCaT Cells



- HaCaT cells (No EVs) images at 5 time points: 0h, 12h, 24h, 36h, and 48h
- Scratch is not fully healed even at the 48-hour mark
- **10% was not included in the results due to insignificance

Conclusions

- In diabetic conditions, Keratinocytes (HaCaT cells) treated with Placental derived EVs (plEVs) showed a promise in enhancing cell migration and wound healing. The use of the placenta as a source of EVs have the advantage of being rapidly isolated and can obtain large quantities to enhance wound healing.
- These findings suggest that EVs facilitate wound healing by upregulating cytokines such as VEGF, TGF-β and Rantes. These cytokines play an important role in promoting fibroblast activation, ECM production, recruitment of immune cells, wound contraction, promote angiogenesis and tissue regeneration.
- Placental EVs is a promising safe, cell-free therapy for clinical use to provide regenerative benefits without the risk of tumorigenicity that may be associated with stem cell therapy.
- Future work is needed to develop current Good Manufacturing Practice (cGMP) compliant Placental EVs to promote wound healing.