Engineering an immunomodulatory hydrogel using tolerogenic trophoblast exosomes

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Statement of Purpose: Immunosuppression is necessary for the survival of donor allografts[1], but systemic immunosuppression carries several life-threatening risks and limits the lifetime of the graft[2]. The induction of tolerance toward allografts would eliminate the need for chronic systemic immunosuppression and improve graft longevity.

Pregnancy is the only natural biological example of antigen specific tolerance toward allogeneic tissue[3]. Placental trophoblasts mediate this tolerance through the presentation of tolerogenic immunomodulatory molecules to immune cells[4]. One method of trophoblast signaling is via exosomes, which possess numerous immunomodulatory molecules[5,6].

We hypothesize that trophoblast exosomes delivered temporally and locally to an allogeneic graft site will induce a tolerogenic response in responding immune cells, prolonging graft survival in the absence of immunosuppression. To achieve localized, sustained delivery of exosomes, we engineered a hydrogel-based delivery platform designed to both tether and entrap exosomes, thereby potentially prolonging localized exosome delivery.

Methods: JAR and JEG-3 trophoblast representative cell line were used to isolate exosomes via Total Exosome Isolation kit. Exosomes were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), and liquid chromatography and mass spectrometry (LC-MS). Exosome proteome was analyzed via gene ontology enrichment analysis using PANTHER. Exosome loading within PEG-maleimide (dithiol threitol crosslinker) and alginate (slow-gelling crosslinking via $CaCO_3$) hydrogels was evaluated via SEM imaging, and optical imaging and spectroscopy of fluorescently tagged exosome encapsulated hydrogels. THP-1 monocytes were differentiated into M1 macrophages prior to incubation with exosomes (0.125 ug/mL) for 24 hr and activation evaluated via flow cytometry and ELISA (e.g. IL-6, IL-10).

Results: Exosomes were isolated from trophoblast model cell lines JAR and JEG-3, and their size characterized via DLS (**Figure 1**) and TEM imaging, yielding an average of 73.22±9.5nm for JAR vesicles and 37.50±4.4nm for JEG-3 vesicles. Proteomic characterization confirmed multiple exosome markers (CD63, CD81, CD9) and gene ontology enrichment analysis identified numerous proteins involved in immune modulation.

Next, we encapsulated exosomes within hydrogel matrices such as poly(ethylene glycol) (PEG) and alginate. 4-arm 20kDa PEG-maleimide polymers react with free thiols, as present on cysteines, enabling tethering of any cysteine-containing protein. Alginate does not have the potential to conjugate exosomes and was used as a control system for entrapment only. JAR and Jeg3 isolated exosomes were fluorescently tagged and entrapped within PEG and alginate hydrogels and release monitored through spectrophotometry and microscopy (**Figure 2**). Released exosomes were below the detection limit by 1 and 3 days for alginate and PEG, respectively, indicating tethering enhanced retention. Fluorescence above background is detected out to day 14 in PEG hydrogels (**Figure 2B**).

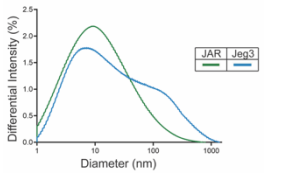

Figure 1. Size characterization of isolated vesicles via DLS.

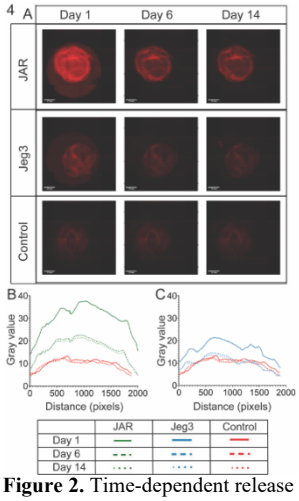

Figure 2. Time-dependent release of labeled exosomes from PEG gels (A), quantified intensity over time for JAR (B) and JEG (C).

Preliminary tests evaluating the impact of the trophoblast secretome on THP-1 monocyte-derived M1 macrophages demonstrated a shift in markers toward an M2 phenotype (e.g. CD163, CD206), indicating a shift toward a tolerogenic phenotype. Ongoing studies are evaluating the impact of isolated trophoblast exosomes and exosome-loaded hydrogels on innate and adaptive immune cells via ELISA and flow cytometry.

Conclusion: Combined tethering and entrapment of exosomes within hydrogels results in longer sustained delivery of tolerogenic trophoblast exosomes than entrapment alone. Ongoing studies are evaluating appropriate loading densities to achieve therapeutic effects in innate and adaptive immune cells in vitro and in vivo.

References:

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