GelMA-encapsulated of limbal mesenchymal stem cells and exosomes for corneal inflammation modulation in ocular surface chemical injuries

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Aims / Purpose:
The aim of this study is to investigate the effect of limbal mesenchymal stem cells (LMSCs) and their derived exosomes, when encapsulated with GelMA, on corneal regeneration and inflammation in ocular surface chemical injuries (OSCI).

Methods:
Human limbal mesenchymal stem cells (hLMSCs) and their exosomes were first isolated and characterized. A rabbit ocular surface chemical injury (OSCI) model was then created using NaOH. The hLMSCs and hLMSCs-exosomes were encapsulated in GelMA and crosslinked under visible light. Treatments were applied for 7, 21, and 60 days. At the end of these periods, samples were collected from the corneal surface via impression cytology. Expression changes in MUC1, CK19, IFN-ɣ, IL-6, CCL2, IL-1β, CXCL-8, and MMP-9 genes were analyzed by Real-Time PCR, while HLA-DR, ICAM-1, CD8, CD31, and CD68 were examined using flow cytometry.

Results:
In the OSCI model, an increase in anti-inflammatory mediator gene expression levels ($p<0.05$) was observed in hLMSCs and hLMSC-exo treatment groups compared to control groups, while a decrease in inflammation marker gene expression levels was observed. Similarly, flow cytometry analysis revealed a decrease in inflammation severity and activation ($p<0.05$), and it was observed to regulate the cellular response.



Figure 1. A) Characterization of hLMSCs at Passage 3 and Differentiation Analysis: Microscopic images of isolated hLMSCs at passage 3 following adipogenic, osteogenic, and chondrogenic differentiation. (A) Phase-contrast microscopic appearance of neutral lipid droplets in hLMSCs differentiated into the adipogenic lineage on day 21. (B) Oil Red O (ORO, red) staining of neutral lipid droplets after adipogenic differentiation. (C) Immunofluorescence characterization of adiponectin (red) following adipogenic differentiation. (D) Phase-contrast microscopic appearance of mineral nodules in hLMSCs differentiated into the osteogenic lineage on day 28. (E) Alizarin Red S (ARS, red) staining of mineral nodules after osteogenic differentiation. (F) Immunofluorescence characterization of osteocalcin (green) following osteogenic differentiation. (G) Alcian Blue (AB) staining to demonstrate glycosaminoglycan formation after chondrogenic differentiation. (H) Safranin O (SO) staining to demonstrate glycosaminoglycan formation after chondrogenic differentiation. Scale bars: (A, B, C) 50 µm; (D, E, F, G, H) 100 µm. (I) TEM image of exosomes derived from iLMSCs. Exosomes were observed to range in size between 50–150 nm and exhibit a double-membrane structure (Scale bar: 200 nm). **Immunophenotypic analysis of hLMSCs performed by flow cytometry:** hLMSCs were positive for markers CD90 (99.89%), CD73 (94.49%), CD105 (28.84%), CD44 (99.83%), and CD29 (99.62%); and negative for CD34 (0.00%), CD45 (0.00%), CD69 (0.36%), CD25 (0.00%), CD19 (0.05%), CD14 (6.38%), HLA-ABC (0.28%), and HLA-DR (0.00%). In the histograms, target antibodies are shown in blue, and isotype antibodies (negative control) are shown in purple.
B) Immunophenotypic Analysis of hLMSC-derived Exosomes: Flow cytometric analysis of exosome-specific surface markers and MSC-specific markers. Exosomes were found to be positive for CD81 (96.27%), CD9 (23.65%), CD90 (9.78%), CD73 (7.12%), and CD105 (9.12%); and negative for CD34 (0.15%) and CD45 (0.09%). The "Dot Plots" column shows the size distribution of the exosomes. The "Singlets" column demonstrates the specification of the gate selected for appropriately sized exosomes within the dot plots. The "Histograms" column shows the fluorescence intensity measured by the device for surface markers compared to the isotype control. In the histograms, target antibodies are shown in blue, and isotype antibodies (negative control) are shown in purple.
C) Gene Expression Analysis in Impression Cytology Samples (RT-PCR): Time-dependent graphs illustrating the effects of hLMSC and exosome treatments encapsulated in GelMA hydrogel on days 7, 21, and 60 following NaOH-induced alkali injury. Changes in mRNA levels of corneal surface markers (*MUC1, CK19*), pro-inflammatory cytokines (*IFN-ɣ, IL-6, IL-1β, CXCL-8*), chemokines (*CCL2*), and matrix metalloproteinases (*MMP-9*).
D) Flow Cytometry Analysis of Impression Cytology Samples: Quantitative evaluation of inflammation and immune response parameters in cells collected from the ocular surface. Fluorescence intensities and cell population distributions of antigen presentation (*HLA-DR*), cell adhesion (*ICAM-1*), T-cell infiltration (*CD8*), angiogenesis (*CD31*), and macrophage activation (*CD68*) across treatment groups.
Data are reported as means ± SD (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$; n = 3).

Conclusions:

GelMA-encapsulated hLMSCs and their exosomes effectively suppressed inflammatory signaling targeted immunomodulation that decreases inflammatory activation while promoting corneal regeneration in OSCI.

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