

ABSTRACT

Sidik Permana Putra, 24020121140143. Enhancement of Cell Regeneration in Acute Lung Injury (ALI) Models through Co-culture and Secretome Therapy Derived from Human Wharton's Jelly Mesenchymal Stem Cells (hWJ-MSC).

Acute Lung Injury (ALI) is characterized by massive damage to alveolar epithelial integrity, necessitating regenerative strategies based on stem cell. The absence of specific pharmacological therapies capable of regenerating the alveolar epithelium without systemic side effects remains a major challenge in ALI management, as current conventional treatments are merely supportive and carry risks of resistance and long-term hormonal imbalances. This study aims to evaluate the effectiveness of cell-based therapy (co-culture) and cell-free therapy (secretome) derived from human Wharton's Jelly Mesenchymal Stem Cells (hWJ-MSCs) on the regeneration of A549 cells as an ALI model. The ALI model was induced through mechanical injury using a scratch assay and chemical induction with lipopolysaccharide (LPS) from *Escherichia coli* at a concentration of 100 µg/mL. The measured parameters included the percentage of wound closure over a 9-day observation period, total protein concentration assessed by the bicinchoninic acid (BCA) assay, protein profile analysis using (SDS-PAGE), and cytokine levels measured by Cytometric Bead Array (CBA). This study specifically presents a direct comparative analysis between cell-based therapy using the co-culture method and cell-free therapy using secretome, revealing differences in regenerative responses in the A549 cell-based ALI model. The results demonstrated that secretome-based therapy groups (P5–P8) exhibited a faster regenerative response during the early phase (day 1), attributed to their ready-to-use properties. Based on statistical analysis using two-way ANOVA followed by Tukey's post hoc test, cell-based therapy, particularly spheroid co-culture (P3), showed the highest efficacy at the end of the observation period (day 9), achieving a wound closure percentage of 73.75%. This outcome was significantly different compared to the negative control ($p = 0.046$; $p < 0.05$) and highly significant compared to the positive control dexamethasone 100 ng/mL ($p < 0.001$). Furthermore, BCA and SDS-PAGE analyses confirmed that the spheroid co-culture group facilitated more efficient utilization of bioactive molecules through dynamic intercellular communication. In conclusion, hWJ-MSC therapy using a spheroid co-culture approach represents the most effective strategy for promoting A549 cell regeneration in an *in vitro* ALI model.

Keywords: *Acute Lung Injury (ALI), hWJ-MSC, co-culture, spheroid.*