Abstract

Secretome is a bioactive substance secreted by MSCs in a conditioned media, which consists of growth factors, other substances that are able to provide a biological response by undergoing signal transduction. A secretome-based therapy has gained attention as a novel cosmetic active ingredient for its antioxidant and wound healing properties. To overcome the stability issue, freeze-drying technique was used to preserve secretome from degradation, into a convenient, ready-made preparation for subsequent formulation, storage, and transport. As the freezing step is the first critical step in the freeze-drying, ice crystal morphology is affecting the quality of the end product. This research was aimed to observe the effect of the freezing time parameter toward the secretome protein stability which can be evaluated from the secretome potency on the 3T3 fibroblast cell, through the oxidative stress assay and *in-vitro* scratch assay. Prior to the potency evaluations, total protein content quantification and cytocompatibility test were done to determine the secretome concentrations and the safe concentrations of secretome, resveratrol, and H_2O_2 toward the cells, respectively. From the oxidative stress assay and *in-vitro* scratch assay, the FD secretome from 24 hours of freezing time was found to have the highest cytoprotective properties against H_2O_2 and induce the fastest wound healing activity. Meanwhile, the non-FD secretome was found to have no cytoprotective and cell proliferation and migration influence toward the fibroblast cells. Therefore, it can be concluded that freeze-drying with sufficient freezing time is crucial in preserving secretome from instability.

Keywords: *Umbilical cord-Mesenchymal stem cell (UC-MSC), Secretome, Freeze-drying, Oxidative stress assay, In-vitro scratch assay*