

PLANT-DERIVED NANOVESICLES DISPLAY WOUND HEALING PROPERTIES

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The process of wound healing is complex and often unresponsive to traditional treatment approaches, placing a considerable burden on patients [1]. Chronic nonhealing wounds are characterized by a reduction in the production of growth factors and chemokines, as well as impaired fibroblast proliferation, migration, and inflammatory responses [2]. Nanotechnology has emerged as a groundbreaking field, reshaping various aspects of medical science. Nanovesicles, which are present in various cell types, have bilayer membranes and possess unique compositions of nucleic acids, proteins, secondary metabolites, and lipids [3]. Such properties hold promise as therapeutic agents, and one of their versatile applications lies in the realm of wound healing. Plant-derived nanovesicles, known for their excellent compatibility with human body and penetration into skin cells, exhibit potential for enhancing wound healing [4]. Given the established efficacy of certain plant-derived nanovesicles in promoting wound closure, it becomes essential to identify specific sources of these vesicles and investigate their capacity for alleviating *in vitro* wound healing.

Commercial keratinocyte (HaCaT) and human skin fibroblast (BJ-5ta) cell lines were used in the study. Plant nanovesicles were isolated from lyophilized plant powder using polymeric precipitation method. The Bradford method was applied to characterize the obtained nanovesicles according to the total protein content, Trizol reagent was used to determine the amount of RNA, and nanoparticle analysis was performed (Nanosight S300). The effects of nanovesicles were evaluated by seeding keratinocytes and fibroblasts in 96-well plates, incubating these cells with nanovesicles for 48 hours and measuring proliferation with PrestoBlue™ reagent. *In vitro* wound studies were also performed using commercial skin cells to determine the effect of isolated nanovesicles on wound healing.

Following the extraction of nanovesicles derived from various plants (nettle, black currant berry, blueberry, rose, lion's mane mushroom, and lingonberry) and nanoparticle tracking analysis, it was observed that the size of these vesicles fell within the 50-250 nm range, consistent with existing literature descriptions. The isolated nanovesicles were found to contain substantial quantities of RNA and proteins. Cell viability was influenced by nanovesicle concentration, with higher concentrations leading to decreased viability, while lower concentrations had no negative impact on the cells. *In vitro* scratch test experiments revealed that nettle, blackcurrant, and blueberry nanovesicles at lower concentrations accelerated wound closure rates in both keratinocyte and fibroblast cell cultures. However, it is vital to be cautious regarding nanoparticle dosage, as higher concentrations may exert adverse effects on skin cells.

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