Investigating the effect of exosomes extracted from saffron petals on scratch repair in fibroblast cells

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Introduction

Fibroblasts, as the principal cells of connective tissue, play a critical role in maintaining tissue integrity, facilitating wound healing, and regulating cellular responses to oxidative stress. They are responsible for producing extracellular matrix components and various growth factors, and due to their sensitivity to environmental changes—particularly oxidative damage—they serve as an important model for evaluating the biological effects and safety of natural compounds. Since many plant-derived products contain antioxidant and bioactive molecules, assessing their interaction with fibroblasts can provide valuable insight into their biocompatibility and therapeutic potential. Saffron petals, known to be rich in antioxidant compounds, represent a promising source for isolating plant-derived extracellular vesicles capable of carrying functional biomolecules to target cells. In this context, the present study aims to explore the characteristics and biological influence of these vesicles on fibroblast cells, providing a foundation for understanding their potential application as natural protective or bio-enhancing agents.

Methods

Fresh saffron petals were washed, homogenized in phosphate-buffered saline (PBS), and subjected to sequential centrifugation to remove debris and isolate extracellular vesicles. The obtained vesicles were resuspended in PBS for further analysis. Their morphology was examined using field emission scanning electron microscopy (FE-SEM), while particle size distribution and zeta potential were measured by dynamic light scattering (DLS). The antioxidant activity of the vesicles was evaluated using the FRAP assay.

For cellular studies, L929 fibroblast cells were cultured under standard conditions (37 °C, 5% CO_2). Cell viability after treatment with different concentrations of saffron petal–derived extracellular vesicles was assessed using the MTT assay. In addition, a scratch wound healing assay was performed to evaluate the effect of the vesicles on fibroblast cell migration. Images were recorded at specific time points and wound closure was analyzed.

Results

The isolated extracellular vesicles obtained from saffron petals showed a uniform nanoscale morphology under FE-SEM imaging, appearing as spherical particles with smooth surfaces. DLS analysis confirmed a narrow size distribution consistent with typical plant-derived vesicles, and the zeta potential measurements indicated a negative surface charge supporting their colloidal stability. The vesicles also exhibited measurable antioxidant activity in the FRAP assay, reflecting their ability to reduce ferric ions.In cellular assays, L929 fibroblast cells maintained high viability at lower concentrations of the vesicles, suggesting good biocompatibility, while higher doses led to a moderate decrease in viability. The scratch wound healing assay demonstrated that treatment with saffron petal–derived vesicles supported fibroblast migration, as evidenced by enhanced wound closure compared with untreated controls over the observed time intervals.

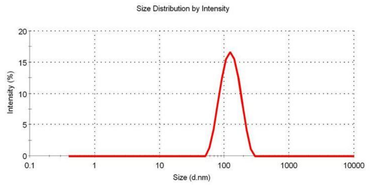

Figure 1. DLS

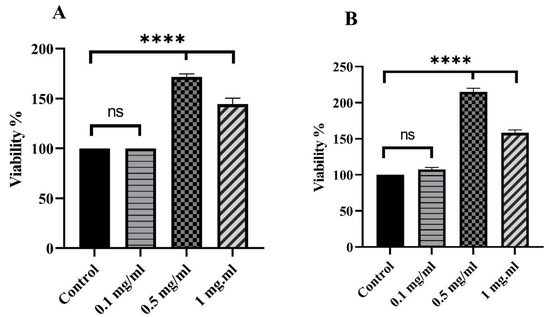

Figure 2. Evaluation of cell viability of L929 cells after treatment with saffron petal-derived EVs at different concentrations (0.1, 0.5, and 1 mg/ml) using the MTT assay at 48 hours (A) and 72 hours (B). The results showed a significant increase in cell viability at 0.5 mg/ml, with a slight decrease at 1 mg/ml, yet remaining significantly higher than the control group.

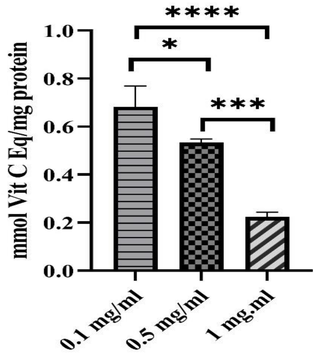

Figure 3. FRAP assay results showing the antioxidant capacity of saffron petal EVs at different concentrations (0.1, 0.5, and 1 mg/ml). The antioxidant activity is expressed as mmol Vitamin C equivalent per mg protein. The highest antioxidant potential was observed at 0.1 mg/ml, with statistically significant differences among the tested concentrations. *p < 0.05, ***p < 0.001, ****p < 0.0001.

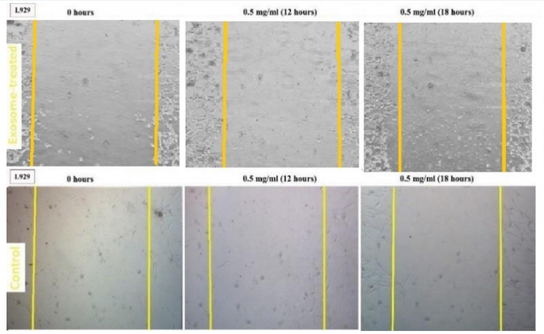

Figure 4. Scratch assay evaluating the effect of saffron petal EVs (0.5 mg/ml) on cell migration at 0, 12, and 18 hours. Wound closure percentages after 18 hours were approximately 30.65±0.64% for L929 fibroblasts

Conclusions

In this study, extracellular vesicles derived from saffron petals demonstrated stable nanoscale characteristics and notable antioxidant activity. Their evaluation on L929 fibroblast cells showed that these vesicles exhibit good biocompatibility at lower concentrations, while higher doses caused only a moderate reduction in cell viability, indicating that their biological effects are dose-dependent. Moreover, the scratch assay revealed that the vesicles enhanced fibroblast migration, suggesting a supportive role in tissue repair processes. Overall, these findings indicate that saffron petal–derived vesicles may serve as natural, biocompatible agents capable of promoting fibroblast function and hold promise for future applications in regenerative and protective therapies.