ELMINA 2024

THIRD INTERNATIONAL CONFERENCE ON ELECTRON MICROSCOPY OF NANOSTRUCTURES

ТРЕЋА МЕЂУНАРОДНА КОНФЕРЕНЦИЈА О ЕЛЕКТРОНСКОЈ МИКРОСКОПИЈИ НАНОСТРУКТУРА

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THIRD INTERNATIONAL CONFERENCE
ON ELECTRON MICROSCOPY OF NANOSTRUCTURES

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ORGANIZERS AND GENERAL INFORMATION

SERBIAN ACADEMY OF SCIENCES AND ARTS
Knez Mihailova 35, 11000 Belgrade, Serbia
Phone: +381 11 2027200
https://www.sanu.ac.rs/en/

FACULTY OF TECHNOLOGY AND METALLURGY, UNIVERSITY OF BELGRADE
Karnegijeva 4, 11000 Belgrade, Serbia
Phone: +381 11 3370425
https://www.tmf.bg.ac.rs/en/

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The conference will be held September 9th-13th, 2024 at the Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000 Belgrade, Serbia, beginning at 8:30 AM on September 9th, in the Solemn hall.

Visualization of Extracellular Vesicles Derived from Dental Mesenchymal Stem/Stromal Cells Using SEM and Immunogold TEM Analyses

Marija Milivojević[1], Maja Kosanović[2], Sergej Tomić[2], Marina Bekić[2], Miodrag Ćolić[3], and Đorđe Janaćković[4]

1 Innovation Center of The Faculty of Technology and Metallurgy Ltd., Belgrade, Serbia

2 Institute for the Application of Nuclear Energy, INEP, Belgrade, Serbia

3 Serbian Academy of Sciences, Belgrade, Serbia

4 Faculty of Technology and Metallurgy, Belgrade University, Belgrade, Serbia

Extracellular vesicles (EVs) derived from mesenchymal stem/stromal cells (MSCs) have emerged as promising therapeutic agents in regenerative medicine. EVs are nanosized, membrane-limited vesicles containing protein and RNA cargo, released by various cell types for intercellular communication. It has been shown that EVs derived from MSCs play a significant role in the tissue regeneration process [1]. Dental MSCs such as human stem cells from the dental pulp of deciduous teeth (SHED cells) are easily accessible MSCs with high proliferative capacity [2]. The objective of this study was to isolate and characterize EVs derived from SHED, to explore their potential utility in regenerative medicine applications.

SHED cells were isolated from deciduous teeth from three donors, and their morphology and osteogenic potential were assessed through optical microscopy and Alizarin Red staining of cells cultured in osteogenic cell media for 21 days. SHED cells were then cultured in EVs depleted cell culture media and EVs were isolated from conditioned cell media by differential ultracentrifugation. The isolated EVs pellets underwent further characterization, including Western Blot for the detection of CD63 surface marker, Nanoparticle Tracking Analysis (NTA) for the size distribution, Bicinchoninic acid assay (BCA) to determine protein concentration and electron microscopy analyses for visualization. For the scanning electron microscopy (SEM) analysis EVs were fixed with glutaraldehyde, placed on aluminum stubs, and gold-sputterd for analysis. For immuno-gold transmission electron microscopy (TEM) analysis, samples were fixed on carbon-coated copper TEM grids, followed by immunoreactive binding of streptavidin-gold nanoparticles (10 nm) to the CD63-positive population of EVs.

Optical microscopy unveiled typical MSC morphology, exhibiting elongated spindle, cuboidal, and polygonal shapes consistent with 2D cell culture isolates. Alizarin Red staining, observed through optical microscopy, confirmed mineral matrix deposition by cells treated with osteogenic supplementation, indicating their differentiation capacity. Characterization of isolated EVs revealed the presence of the transmembrane protein CD63 by Western blot analysis. Additionally, the median size of the isolated EVs was 156 nm measured via NTA, while BCA analysis determined a total protein concentration of 200 μg/ml. The SEM analysis confirmed the presence of EVs in the isolates (Figure 1), capturing individual particles with spherical morphology and sizes ranging from 70 nm to 250 nm. Immunogold TEM analysis revealed cup-shaped and spheroid morphologies, characteristic of EVs' appearance when using TEM,

as well as positive immunogold-labeling with transmembrane extracellular protein CD63. The presence of gold nanoparticles bound to the majority of EVs indicated a significant number of CD63-positive vesicles.

In conclusion, EVs derived from SHED cells were successfully isolated and characterized. The EVs exhibited characteristics typical for MSC EVs, including CD63 positivity [3], appropriate size distribution, protein content, and morphology [4], [5]. The potential utility of these EVs in regenerative medicine will need to be confirmed by assessing their function [6].

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[6] This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-66/2024-03/200287; 451-03-65/2024-03/200135)

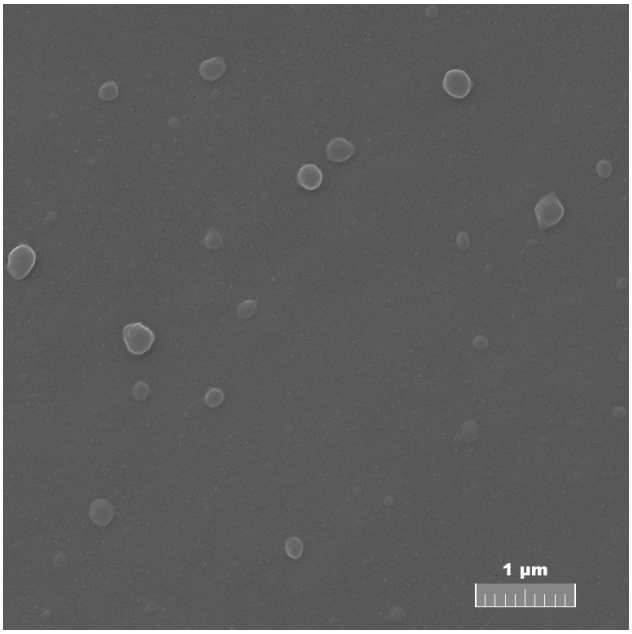

Figure 1. SEM micrograph of EVs.

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