Extracellular vesicles derived from mesenchymal stem/stromal cells derived from dental pulp of exfoliated teeth induce osteogenic differentiation

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

[1]*Inovation 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 and Arts, Belgrade, Serbia*
[4]*Faculty of Technology and Metallurgy, Belgrade, Serbia*

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INTRODUCTION: Large bone defects in the field of orthopedic surgery remains challenging, primarily due to restricted regenerative capacity of bone tissue. The biomimetic bone implants could improve the osteoregeneration process, but the optimal composition for biomimetics remains unknown. Considering the great potential of extracellular vesicles (EVs) derived from mesenchymal stem/stromal cells (MSCs) in regenerative medicine, our aim was to investigate the osteoinductive potential of EVs derived from MSCs from the dental pulp of human exfoliated deciduous teeth (SHEDs) either undifferentiated or those undergone differentiation into osteoblasts (osteoSHED-EVs), for potential use in biomimetic bone implants.

EXPERIMENTAL: SHEDs were isolated from the dental pulp of 5 different donors of deciduous teeth and subjected to characterization by Flow cytometry (FCM). EVs-SHED and EVs-osteoSHED were isolated by sequential ultracentrifugation, and analysed by Western blot, NTA, and electron microscopy. Uptake of PKH67-labelled EVs by SHED was analysed by confocal microscopy and FCM. The effects of EVs on osteogenic differentiation of SHED cells were analysed by monitoring RUNX2 and BMP2 expression by RT-PCR.

RESULTS AND DISCUSSION: SHEDs displayed typical MSC morphology and phenotype, as well as potential to differentiate into osteoblasts as confirmed by FCM, alkaline phosphatase activity, Alizarin red S staining, and gene expression analysis. Both types of EVs were internalized by SHED, as shown by the confocal microscopy and FCM after 4h and 24h incubation, and no significant differences were observed between the two EVs types. Importantly, the osteogenic differentiation of SHEDs cultivated in basal medium was significantly improved in the presence of osteoSHED-EVs, as shown by the upregulation of early osteogenic genes RUNX2 and BMP2.

CONCLUSIONS: These findings significantly improved our understanding on the potential of EVs derived from SHEDs in osteoinduction, laying the groundwork for developing targeted methods that utilize EVs from SHED as biomimetics for effective repair of bone defects.

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*Corresponding author E-mail: mmilivojevic@tmf.bg.ac.rs