Evaluation of cellular wound healing using Flowcytometry

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Abstract

Wound healing is a well- orchestrated complex cellular process and it involves the spatial and temporal synchronization of different cell types with unique roles in the phases of hemostasis, inflammation, proliferation, and Extra cellular matrix remodeling. Flowcytometry is one of the well -advanced single cell technologies that is utilized to uncover phenotypic and functional heterogeneity within a complex process like wound healing. Platelets and fibroblasts are the major cell types involved in wound healing process. In the present study, we focused to analyse the effect of MSCs secretome and activated platelet rich plasma on impaired wound healing in-vitro models using flow cytometry. Adipose tissue derived MSCs (ADMSCs) and PRP was isolated from healthy rabbits (Newzeland White, 2kg, 6 months) after obtaining Institutional Animal Ethics Committee clearance (SCT/IAEC-439/July/2022/113). Impaired wound healing studies were done in hypoxia and hyperglycemia induced cellular models. Hypoxia was induced by incubating fibroblasts in 600µm H_2O_2 for 2hrs. Cytoprotective effect of MSC secretome on hypoxia induced fibroblasts were evaluated by apoptosis assay and confirmed by Live/dead assay. The effect of MSC secretome on macrophage polarization was evaluated using M1 and M2 macrophage surface markers CD163 and CD80 by flowcytometry. Clinical relevance of hyperglycemia on platelet activation was studied using P selectin marker. We observed that MSC secretome treatment significantly increased live cells in live/dead assay and induced macrophage polarization. Our results showed that hyperglycemia induces hyperactivation in platelets that may lead to prolonged inflammatory phase in diabetic patients. Further in vivo evaluation of MSc secretome on impaired wound healing need to be performed in diabetic models as future prospectives.