Investigating the Therapeutic Potential of Pooled Human Bone Marrow Derived Mesenchymal Stromal Cell-Conditioned Medium for Diabetic Foot Ulcers: An *In Vitro* Study

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Abstract

Diabetic foot ulcers (DFUs) are severe complications of diabetes and are driven by impaired wound healing, poor vascularization, and chronic inflammation. Mesenchymal stromal cell (MSC)-derived secretomes have emerged as promising cell-free therapeutic alternatives because of their regenerative and immunomodulatory properties. This study investigated the therapeutic potential of pooled human bone marrow-derived mesenchymal stromal cell (BM-MSC) conditioned medium (CM) as a cell-free approach for DFU treatment. Pooled human BM-MSCs were isolated and characterized according to the ISCT criteria. The expression of characteristic surface markers and their trilineage differentiation potential were confirmed. Conditioned medium was collected and concentrated using tangential flow filtration to obtain concentrated conditioned medium (CCM). Protein quantification was performed using a Bradford assay, while the levels of key growth factors were measured via ELISA. The biological activity of CCM was evaluated *in vitro* at various concentrations. CCM significantly enhanced cell proliferation and migration, indicating improved wound healing potential. Angiogenic capability was demonstrated through tube formation assays using HUVECs, along with increased endothelial cell proliferation. Furthermore, anti-inflammatory effects were confirmed through macrophage polarization assays, suggesting a shift toward a pro-healing phenotype. These findings highlight CCM as a promising therapeutic strategy for DFUs. Future work will focus on formulation development incorporating CCM with suitable excipients, followed by physicochemical characterization and evaluation of safety and efficacy in diabetic mouse models. This approach may offer a scalable and clinically translatable alternative to cell-based therapies.