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Article

Isolation, Expansion, and Characterization of Placenta Originated *Decidua Basalis*-Derived Mesenchymal Stromal Cells

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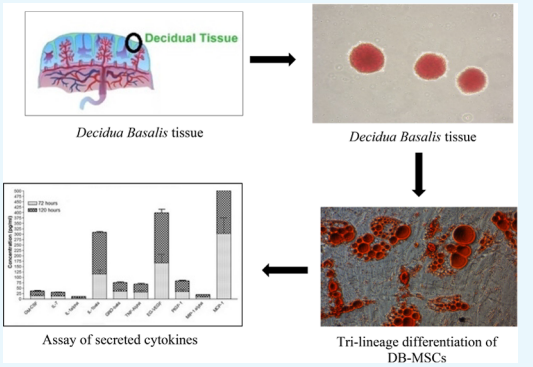
ABSTRACT: Mesenchymal stromal cells (MSCs) were isolated from *Decidua Basalis* (DB) and studied for their final cellular product measures, such as safety, purity, quality, quantity, and integrity that are ascribed as cellular products. This research aimed to isolate MSCs for expansion under the clinical scale level with potency, secretion of cytokines, growth factors secreted by DB-MSCs, and their role in wound healing. Placentas isolated from DB were expanded up to the 10th passage, and their characteristics were assessed by phenotypic characterization using a flow cytometer and analyzed for trilineage differentiation by cytochemical staining. Growth factors (GF), interleukins (IL), chemokines, and tissue inhibitors of metalloproteinases (TIMP) were measured with enzyme-linked immunosorbent assays. The harvested cells from the placenta yield 1.63−2.45 × 10^4cells/cm^2 at P(0), 3.66−5.31 × 10^4cells/cm^2 at P(1), 4.01−5.47 × 10^4cells/cm^2 at P(2), and 3.94−5.60 × 10^4cells/cm^2 at P(10) accordingly; up to 4.74 × 10^9 P(2) DB-MSCs were harvested within 9−11 days. The viability of the freshly harvested cells was greater than 90% in all cases. It is able to differentiate into chondrocytes, adipocytes, and osteogenic cells, proving their ability to differentiate into a trilineage. Thus, this study put an insight into a secure and conventional approach toward their ability to differentiate into multiple lineages and secrete factors related to immune regulation, making DB-MSCs a potential source in various therapeutic applications.



INTRODUCTION

Wound healing is accomplished through cellular homeostasis, inflammation, proliferation, and tissue remodeling by total physical and functional regeneration of injured tissue.[1−3] Furthermore, these metabolic processes are controlled by extracellular signaling pathways such as cytokines, growth factors, and membrane receptors.[4,5] However, wound curing is a usual biological process; chronic wound treatment frequently necessitates therapeutic intervention to provide a biochemical environment that promotes normal healing. To treat and control the complicated pathophysiology of chronic wounds, many therapeutic modalities are already available.[6] Among those, biophysical practices like electro-physical stimulation, recombinant growth factor therapy, platelet rich plasma treatment, and stromal cell based treatment are very common.[7,8] Particularly, the wound healing process is a multimodal strategy that stimulate signaling response; then, the replacing growth factors or targeting particular processes like angiogenesis or proliferation are more likely to provide therapeutic advantages.

Placental membranes, the earliest known biomaterials utilized for wound healing, are one such technique. Placental membranes have been shown to have good clinical effectiveness as well as a minimal treatment cost.[9] Extra-embryonic tissue gives rise to placental membranes. A fetal component (the chorionic plate) and a maternal component (the *Decidua Basalis*) make up this tissue.[10−12] Human-derived *Decidua Basalis* has been found to help heal chronic wounds in riffle clinical trials and recent *in vitro* investigations due to their capacity to produce cytokines and impact on cell propagation, angiogenesis, and exodus.

Here, we optimized the utilization of *Decidua Basalis*-derived mesenchymal stromal cells (DB-MSCs) for wound healing and therapeutic purposes, which, on the other hand, is dependent on their subsequent large-scale *in vitro* growth. To address clinical demand and biological research demands, a rapid and effective methodology for producing large amounts of DB-

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