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In vitro differentiation of chorionic mesenchymal stem cells for applications in biomedical engineering

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Abstract: Mesenchymal stem cells (MSCs) are multipotent stromal cells with significant regenerative potential. This study evaluates the differentiation capacity of chorionic mesenchymal stem cells (CMSCs) into adipogenic, osteogenic, and chondrogenic lineages under in vitro conditions. Experimental results confirmed successful lineage specification using histological staining methods. Adipogenic differentiation was characterized by lipid accumulation, osteogenic differentiation by mineralized matrix formation, and chondrogenic differentiation by extracellular matrix production. The findings highlight the applicability of CMSCs in regenerative medicine and tissue engineering, particularly in scaffold-based systems and advanced therapeutic strategies. These findings contribute to the growing body of knowledge on MSC-based applications in biomedical engineering.

1 Introduction

Mesenchymal stem cells (MSCs) are multipotent stromal cells characterized by their ability to self-renew and differentiate into multiple cell lineages, including adipocytes, osteocytes, and chondrocytes [1]. Due to these properties, MSCs have become a key focus in regenerative medicine and tissue engineering, where they are utilized for the repair and regeneration of damaged tissues [2]. MSCs can be isolated from various sources, such as bone marrow, adipose tissue, and perinatal tissues. Among these, chorionic mesenchymal stem cells (CMSCs) represent a promising alternative due to their accessibility, high proliferative capacity, and reduced invasiveness of collection procedures [3]. In addition, perinatal tissues raise fewer ethical concerns compared to embryonic sources, further supporting their use in biomedical applications. Recent studies have emphasized the critical role of microenvironmental factors in regulating stem cell behaviour. Parameters such as biochemical signaling, extracellular matrix composition, and mechanical properties (e.g., substrate stiffness) significantly influence differentiation pathways and lineage specification [4,5]. These findings are particularly relevant for biomedical engineering, where scaffold design and biomaterial properties are created to guide cell fate and enhance tissue regeneration. Despite extensive research on MSCs, the differentiation potential of CMSCs under standardized in vitro conditions remains an important area of investigation, particularly in the context of their applicability in biomedical engineering systems. Understanding their behavior after initial passages is essential for ensuring reproducibility and reliability in experimental and clinical applications. Therefore, the aim of this study is to evaluate the in vitro differentiation potential of chorionic mesenchymal stem cells into adipogenic, osteogenic, and chondrogenic lineages and to assess their suitability for applications in biomedical engineering, particularly in tissue engineering and regenerative medicine.

2 Materials and Methods

Chorionic mesenchymal stem cells (CMSCs) were isolated from chorionic tissue and cultured under standard in vitro conditions. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2 in a precisely defined complete culture medium consisting of a mixture of DMEM and α-MEM *(Thermo Fisher Scientific, Gibco, California, USA*) in a ratio of 1:1, supplemented with 10% fetal bovine (*Thermo Fisher Scientific, Gibco, California, USA*) serum and 1% antibiotic-antimycotic solution (*Thermo Fisher Scientific, Gibco, California, USA*). After reaching approximately 80–90% confluence, cells were passaged and subsequently used for differentiation experiments. Adipogenic differentiation was induced using a culture medium supplemented with insulin, dexamethasone, and other adipogenic factors. The differentiation process was monitored over a period of 14–21 days. The presence of intracellular lipid droplets was

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evaluated using Oil Red O staining, which specifically binds to neutral lipids. Osteogenic differentiation was performed using osteoinductive medium containing β-glycerophosphate, ascorbic acid, and dexamethasone. Mineralization of the extracellular matrix was assessed using Von Kossa staining, which detects calcium phosphate deposits. Chondrogenic differentiation was induced using a specialized medium promoting the synthesis of cartilage-specific extracellular matrix components. Glycosaminoglycan production was confirmed using Alcian blue staining. All experiments were conducted under sterile laboratory conditions. Morphological changes were monitored using light microscopy, and differentiation was evaluated qualitatively based on staining intensity and cell morphology, in accordance with established protocols [6,7], as well as studies focused on MSC applications in biomedical engineering [8-10].

3 Results

During in vitro cultivation, CMSCs exhibited typical spindle-shaped fibroblast-like morphology (Figure 1). Cells adhered to the culture surface within 24 hours and formed a homogeneous monolayer after approximately 7–10 days of cultivation.

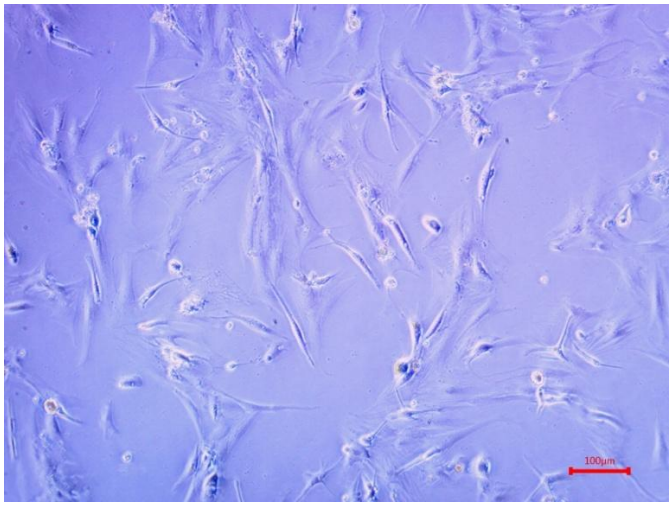


Figure 1 Microscopic image of CMSCs displaying typical spindle-shaped fibroblast-like morphology and adherent growth pattern. Cells are well distributed across the culture surface with elongated cytoplasmic extensions, indicating healthy morphology and active proliferation. Scale bar: 100 µm.

Following adipogenic induction (Figure 2), visible intracellular lipid droplets were observed after approximately 10–14 days. The intensity of Oil Red O staining increased over time, indicating progressive lipid accumulation and successful adipogenic differentiation. The proportion of positively stained cells increased throughout the cultivation period, suggesting stable differentiation efficiency.



Figure 2 Microscopic image of adipogenic differentiation of CMSCs showing intracellular accumulation of lipid droplets stained red, indicating successful adipogenic lineage differentiation. Cells exhibit elongated morphology with scattered lipid-rich regions characteristic of adipocyte formation.

Osteogenic differentiation resulted in the formation of mineralized nodules, which became evident after approximately 14–21 days. *Von Kossa* staining confirmed the presence of calcium deposits, indicating extracellular matrix mineralization and osteoblastic lineage commitment.

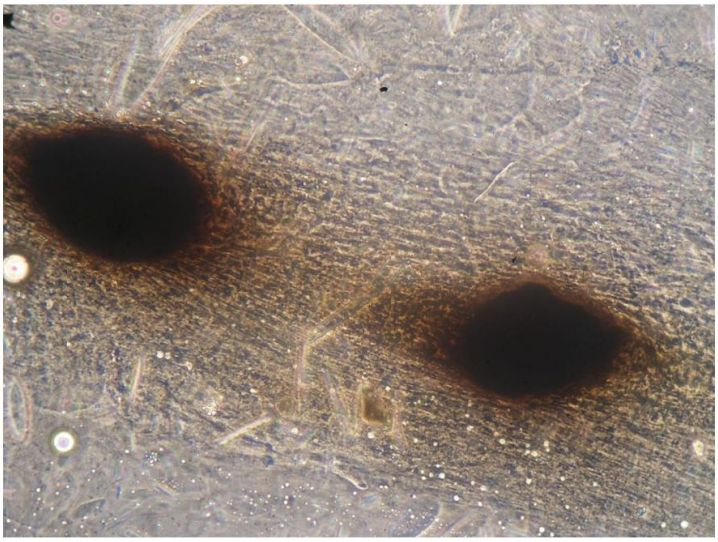


Figure 3 Microscopic image of osteogenic differentiation of CMSCs demonstrating formation of mineralized extracellular matrix deposits visible as dark-stained nodular areas. Surrounding cells exhibit elongated morphology and dense growth pattern, confirming successful osteogenic lineage differentiation and matrix mineralization.

Chondrogenic differentiation (Figure 4) was characterized by the production of glycosaminoglycan-rich extracellular matrix. Alcian blue staining revealed an increasing intensity of extracellular matrix deposition over time. Cells gradually adopted a rounded morphology typical of chondrocytes.

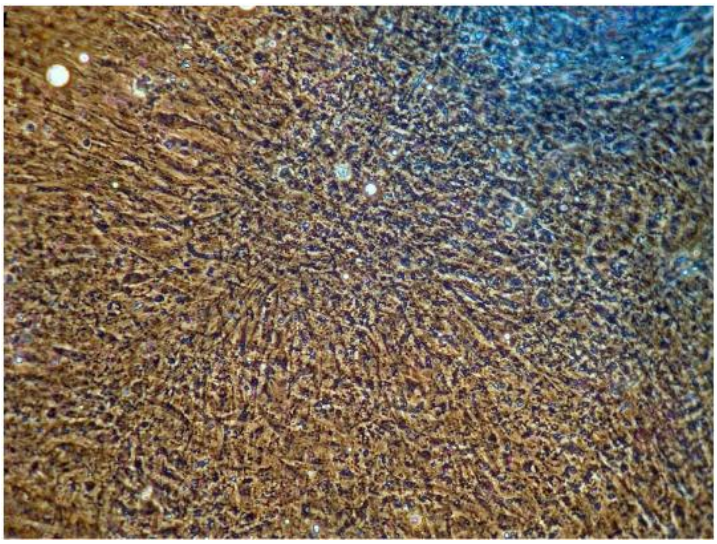


Figure 4 Chondrogenic differentiation of CMSCs stained with Alcian blue. Figure shows a highly confluent cell culture exhibiting dense cellular aggregation and the formation of characteristic chondrogenic nodules. This tight packing and opaque appearance indicate active extracellular matrix (ECM) production and successful transition toward a chondrocyte-like phenotype.

Overall, CMSCs demonstrated stable and reproducible differentiation into all three lineages after the first passage. These findings are consistent with previously reported characteristics of mesenchymal stem cells derived from perinatal tissues [3,8]. No significant morphological abnormalities were observed during differentiation.

4 Discussion

The results of this study confirm that chorionic mesenchymal stem cells retain their multipotent differentiation capacity under standard in vitro conditions. The observed morphological changes and staining patterns correspond with well-established markers of adipogenic, osteogenic, and chondrogenic differentiation [1,3]. The progressive increase in

staining intensity during differentiation suggests a time-dependent maturation process, which is consistent with findings reported in previous studies [4]. These results indicate that CMSCs respond effectively to biochemical stimuli and maintain stable differentiation potential after initial passages. From the perspective of biomedical engineering, these findings are particularly significant. The ability of CMSCs to differentiate into multiple lineages makes them suitable candidates for scaffold-based tissue engineering applications. Previous studies have demonstrated that the combination of MSCs with biomaterials and three-dimensional scaffolds enhances differentiation efficiency and improves tissue regeneration outcomes [5,9].

In comparison with bone marrow-derived MSCs, CMSCs offer several advantages, including less invasive harvesting procedures and higher proliferative capacity. Similar observations have been reported in studies by Trebuňová et al. and Bačenková et al., which highlight the potential of perinatal stem cells in regenerative medicine and biomedical applications [8,9]. Furthermore, Čajková et al. emphasized the importance of microenvironmental conditions and biomaterial interactions in directing stem cell behaviour [10]. Despite these advantages, certain limitations must be considered. Variability between donors and sensitivity to culture conditions may influence experimental outcomes. In addition, in vitro models do not fully replicate the complexity of in vivo environments, which may affect the translation of results into clinical practice.

Future research should focus on optimizing culture conditions, integrating advanced biomaterials, and developing standardized protocols to improve reproducibility and facilitate clinical application of CMSCs in biomedical engineering. These findings confirm the robustness of CMSCs as a reliable cell source for biomedical engineering applications [11,12].

5 Conclusions

Chorionic mesenchymal stem cells demonstrate stable and reproducible differentiation into adipogenic, osteogenic, and chondrogenic lineages under in vitro conditions. Their biological properties, including accessibility, high proliferation capacity, and multipotency, make them promising candidates for applications in biomedical engineering and regenerative medicine. The results of this study support the use of CMSCs in tissue engineering systems, particularly in combination with biomaterials and scaffold-based approaches. Further research is required to optimize differentiation protocols and enhance clinical translation.

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