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## Isolation and Expansion of Human Periosteum-Derived Mesenchymal Stem Cells in Defined Serum-Free Culture Medium

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Abstract: Introduction: Mesenchymal stem cells (MSCs) have the capacity to be differentiated into several cell lineages and are a promising source for cell therapy and tissue engineering. However, currently most MSCs culturing protocols use media supplemented with fetal bovine serum (FBS), which limits their application in clinic due to the possibility of zoonotic infections, contamination and immunological reactions. Consequently, formulating effective serum free culture medium becomes one of the important problems in contemporary cell biotechnology. Objectives: The aim of this study was to define an optimal serum-free medium for culturing of periosteum derived MSCs. Materials and methods: The MSCs were extracted from human periosteum and transferred to the culture flasks pretreated with CELLstart™. Immunophenotypic characterization, proliferation and in vitro differentiation of cells grown on STEM PRO® MSC SFM were compared to the cells cultured in the standard FBS containing media. Chromosome analysis and flow cytometry were also performed. Results: We have shown that cells were grown on STEM PRO® MSC SFM retained all the morphological, immunophenotypic (CD73, CD90, CD105, vimentin and Stro-1) and cell differentiation characteristics specific to MSCs. Chromosome analysis indicated no anomalies in the chromosome structure. Flow cytometry showed a high expression of cell adhesion molecules CD44 (98,8%), CD90 (97,4%), CD105 (99,1%). In addition, we have shown that cell is grown on STEM PRO® MSC SFM have higher proliferation capacity compared to cell expanded on standard FBS containing the medium. Conclusion: We have shown that STEM PRO® MSC SFM is optimal for culturing periosteum derived human MSCs which subsequently can be safely used in cell therapy.

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