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Development of Bone-Like Tissue Graft in Vitro using Multilayer Cell Sheet Technology

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Abstract:

The use of stem/osteoprogenitor cells, scaffolds and growth factors has been the gold standard in bone tissue engineering. However, many disadvantages have limited their efficacy in clinical therapy. This study aims to use multi-layered cell sheet (MLCS) technology and human dental pulp stem cells (DPSCs) to regenerate bone-like tissue grafts for bone augmentation.

Human DPSCs were cultured on temperature-responsive culture dishes. The cultured cells were harvested as an intact single cell sheet by temperature reduction and MLCSs were assembled from those monolayer cell sheets. Then, the MLCSs were continuously cultured in osteogenic medium for further two weeks to allow more extracellular matrix production and mineralisation. The formation of mineralised 3D MLSCs, a bone-like tissue constructs, was confirmed by histological, immunohistochemical assessment and imaging analysis.

After two weeks of continuously culture in osteogenic medium in vitro, hematoxylin/eosin staining and SEM confirmed the formation of MLCSs 3D structure in vitro. Live/dead fluorescent labelling and confocal microscope showed that the majority of hDPSCs stayed viable within the construct , The results of Sirius Red staining and birefringence microscope indicated the enhanced deposition of collagen matrix between the cell layers. Formation of the bone-like tissue within the MLCSs was confirmed by positive staining for human specific type I collagen and Von Kossa.

In conclusion, this study indicated that the additional two weeks culture of HDPSCs MLCS in osteogenic medium is essential for enhancing type I collagen production and extracellular matrix mineralisation to form bone-like tissues as 'bone grafts' for clinical bone augmentation.

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