BIOFABRICATION OF HUMAN ADIPOSE STEM CELL SPHEROIDS FOR OSTEOGENIC DIFFERENTIATION ASSAY

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Resumo:
New bone formation may be desirable in a variety of clinical settings, such as osteoporosis, skeletal deformities, and in nonunion bone fractures. Alternatively, adult stem cells derived from adipose tissue (Adipose Stem Cells - ASCs) represent a promising cell source for new bone formation via cell-based tissue engineering, because of its easy accessibility and abundance. In this context, a novel approach known as modular tissue engineering focuses on fabricating tissues using spheroids as building blocks with specific microarchitectural features and using these modular units to engineer biological tissues from the bottom up approach. The objective of our study is to induce ASCs towards the osteogenic lineage in a three-dimensional cell culture system. Human lipoaspirate samples were obtained according to local research ethics committee and ASCs were isolated by the mechanical dissociation method established by our group. Cells will be seed in the micromolded resections of agarose hydrogel, at where each spheroid is formed per resection. The spheroids will be maintained in vitro for three weeks in an osteogenic induction medium containing -glycerophosphate, dexamethasone, ascorbic acid and human recombinant BMP-2. Initially, morpho-quantitative analysis of the induced spheroids will be done which includes aggregate diameter estimation and structural analysis. Histological analysis will be carried out using alizarin red staining for extracellular mineralization. Simultaneously, immunohistochemistry analyzes will be done using specific antibodies for extracellular matrix components of bone tissue as osteopontin, osteonectin and collagen type I. The surface morphology of the induced spheroids will be done using scanning electron microscope (SEM) as well as calcium x-ray spectrum. We expected to establish an in vitro osteogenesis model from human ASCs spheroids for bone tissue engineering.