## Evaluation of human amnion denuded derived mesenchymal stem cell on 3D porous hydroxyapatite composite scaffolds for osteogenic differentiation: Prolonged in vitro study

## ABSTRACT

Recent studies using composite porous scaffolds have globally demonstrated the biocompatibility of mesenchymal stem cell sources. It is especially important in hard tissue bioengineering due to its desirable features and stemness effectivity. This study has emphasized primarily the evaluation of the morphological aspects of human amnion-denuded derived mesenchymal stem cells (hAMMSC) in combination with the fabrication of threedimensional (3D) porous hydroxyapatite (Hap) composite scaffolds for bone tissue engineering based on the principal component of bone mineralization. We presented a novel combination of hAMMSC and nano-crystalline powder hydroxyapatite/bioactive glass (Hap/BG) composite scaffolds fabricated through the hydrothermal method via a novel formulation method from a previous study of stem cell in vitro prolonged culture. The 3D porous scaffold, 6 mm in size, was fabricated from 70 nm nanocrystalline powder and interacted with hAMMSC cultured in osteo-inductive conditions for 30 days. The characterization of 3D porous scaffolds was analyzed via Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) to explore the particle structure, morphology, bioactivity, and porosity of the composite powder and scaffolds in contact with hAMMSCs culture. Furthermore, biocompatibility was assessed using the PrestoBlueTM viability assay and scanning electron microscopy observation (SEM), which revealed the attachment, morphology, and spreading of hAMMSCs on the 3D porous scaffolds of Hap/BG by showing spindle-like morphology and forming the spreading filopodia bridge-like structure within the scaffolds. Subsequently, the secondary objective is the characterization of human AMMSC differentiation capacity assessed by early osteoblast differentiation and mineralization behaviour investigated by early detected assay via alkaline phosphatase activity (ALP) activity marker and energy dispersive X-ray (EDX) analysis simultaneously of the mineralization within bone matrix elements. It was comparatively evaluated via ALP and EDX for calcium quantification elements. Immunomodulatory properties were analyzed via ELISA kits. In conclusion, our findings indicate that 3D porous scaffolds made of Hap/BG composites could be great candidates for future regenerative medicine applications due to their support towards stem cell growth and enhancement of bone cell capacity.