Synergistic interplay between human BMSCs and HUVECs in 3D spheroids laden in gelatin/hyaluronan hydrogels for simultaneously enhancing osteogenesis and vascularization

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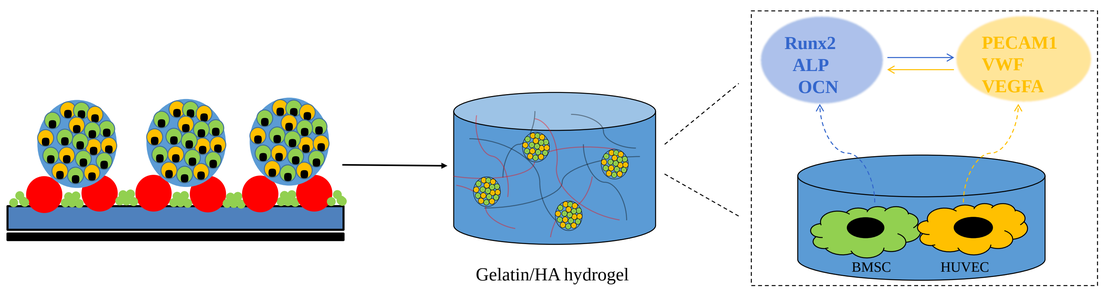
Introduction:

Vascularization is the major obstacle in bone regeneration, particularly in large bone defects. An effective strategy is the conjugation of vascular endothelial cells with osteoblasts for repairing bone defects. Recently, biomaterials were designed to promote bone formation and angiogenesis by enhancing the interaction between the two types of cells and the secretion of cytokines in co-culture. However, there are few platforms for investigating the interaction of osteoblasts and vascular endothelial cells in a three-dimensional (3D) environment.

Methods:



Here, we used silicon (Si) and polyethylene (PS) particles with different sizes to self-assemble into a like 3D structure with monolayer ordered topology surface, called Colloidal Self Assembled Patterns (cSAPs), on which human bone marrow stem cells/ human umbilical vein endothelial cells (hBMSCs/hUVECs) co-spheroids was rapidly formed within 48 h in vitro. We monitored the co-spheroids-forming process and evaluated cellular behavior for cell adhesion and migration. Then, the hBMSCs/hUVECs co-spheroids were embedded in a visible-light cross-linked gelatin/hyaluronan hydrogel to investigate the synergistic interactions for differentiation of osteogenic and angiogenesis.

Results:

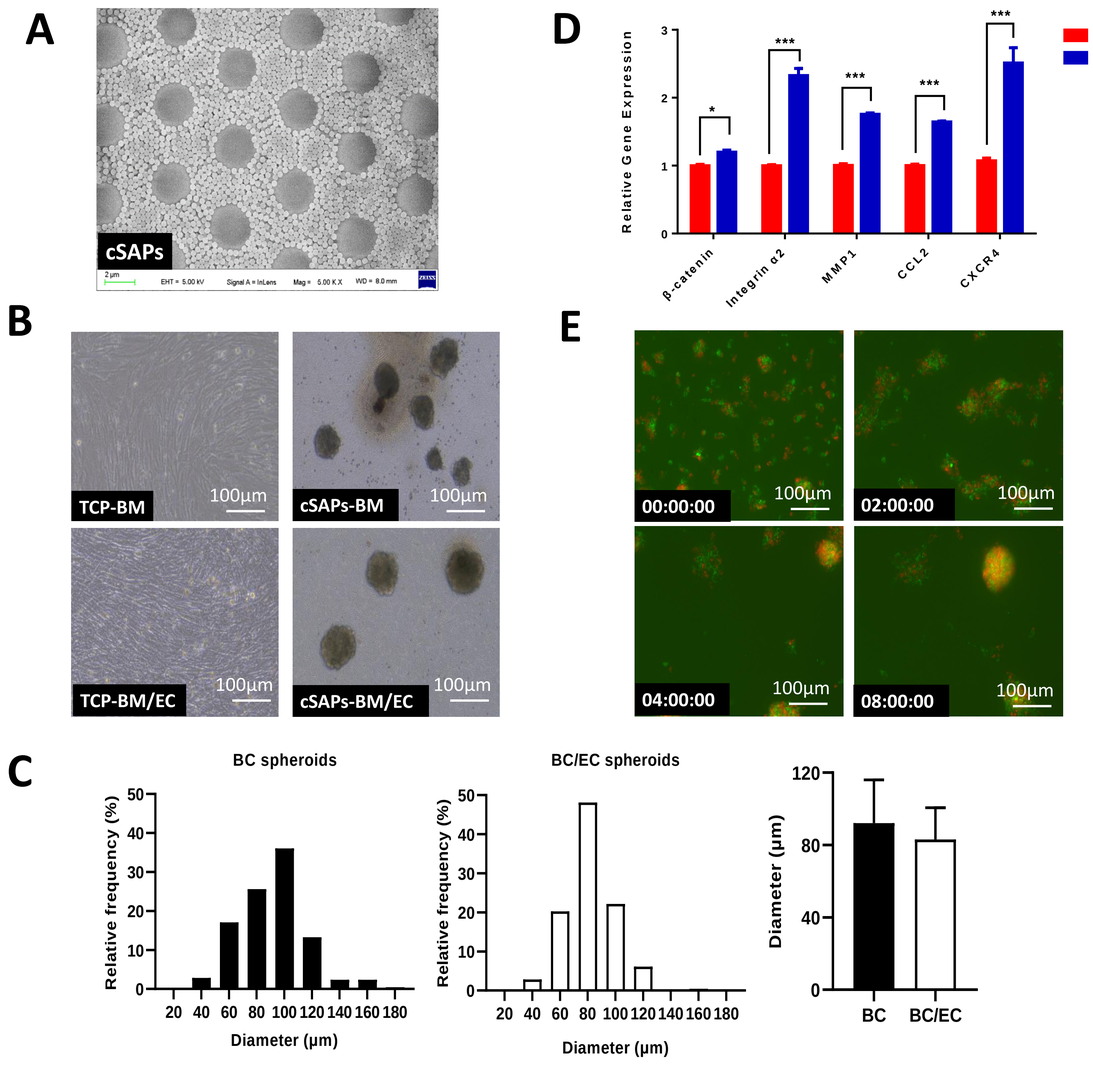


Fig 1. The effect of cSAPs on cellular behavior : A) SEM images of cSAPs; B) Cells were cultured on the surface of cSAPs to form cell spheroids in 48h; C) Size distribution of 3D cell spheres; D) The effect of cSAPs on the expression of adhesion and migration genes of BMSCs; E) Monitoring the process of cell co-spheroids formation.

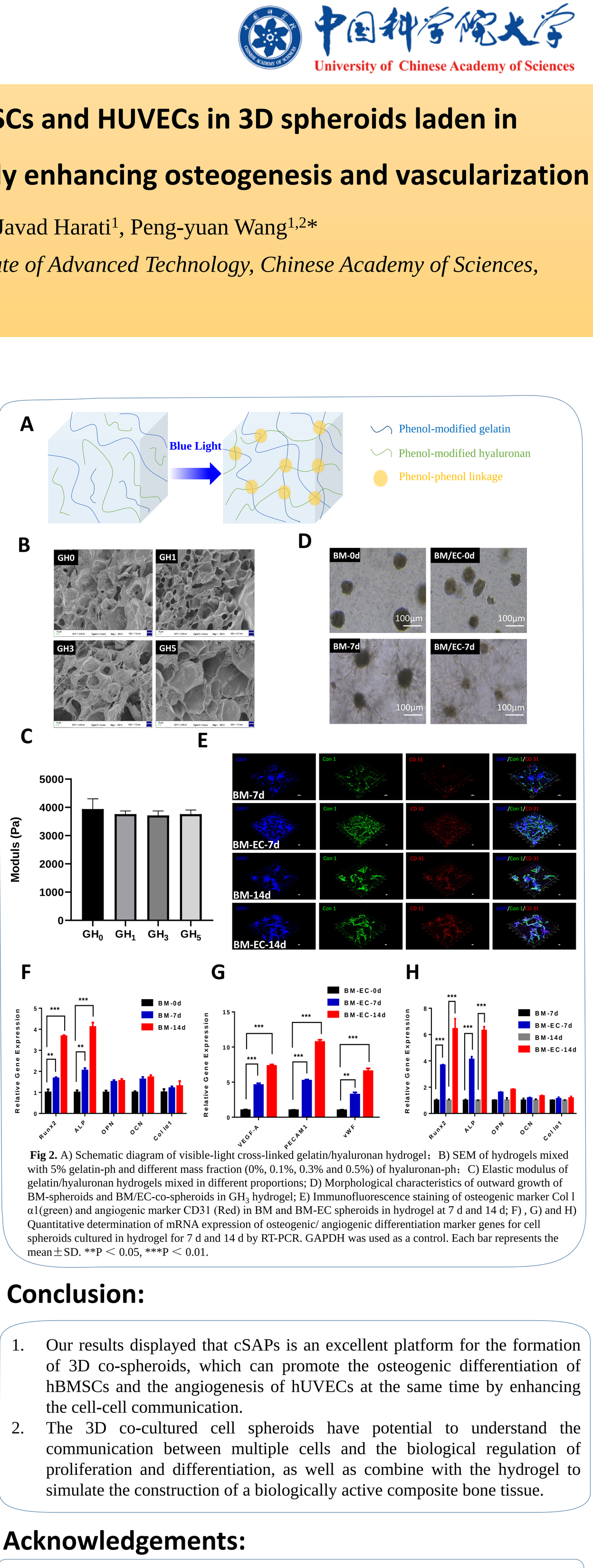


Fig 2. A) Schematic diagram of visible-light cross-linked gelatin/hyaluronan hydrogel； B) SEM of hydrogels mixed with 5% gelatin-ph and different mass fraction (0%, 0.1%, 0.3% and 0.5%) of hyaluronan-ph； C) Elastic modulus of gelatin/hyaluronan hydrogels mixed in different proportions; D) Morphological characteristics of outward growth of BM-spheroids and BM/EC-co-spheroids in GH_3 hydrogel; E) Immunofluorescence staining of osteogenic marker Col Ⅰ α1(green) and angiogenic marker CD31 (Red) in BM and BM-EC spheroids in hydrogel at 7 d and 14 d; F) , G) and H) Quantitative determination of mRNA expression of osteogenic/ angiogenic differentiation marker genes for cell spheroids cultured in hydrogel for 7 d and 14 d by RT-PCR. GAPDH was used as a control. Each bar represents the mean±SD. **P < 0.05, ***P < 0.01.

Conclusion:

1. Our results displayed that cSAPs is an excellent platform for the formation of 3D co-spheroids, which can promote the osteogenic differentiation of hBMSCs and the angiogenesis of hUVECs at the same time by enhancing the cell-cell communication.
2. The 3D co-cultured cell spheroids have potential to understand the communication between multiple cells and the biological regulation of proliferation and differentiation, as well as combine with the hydrogel to simulate the construction of a biologically active composite bone tissue.

Acknowledgements:

This work was supported by Collaborative Innovation Program of Shenzhen (20180921173048123).