1. **Introduction.** The in vitro pancreatic differentiation of human embryonic stem cells (hESCs) still remains challenging. As revealed by studying mice embryo development, organ development requires strict temporal and spatial control at each stage. The stepwise hESC differentiation approach may mimic a temporal control of organogenesis during embryo development, but fails in imitating a spatial control of the process. In vivo, spatial control is achieved by cell-cell and cell-extracellular matrix (ECM) interaction. ECM forms a three-dimensional (3D) environment and offers niches for cell adhesion, migration, proliferation, and differentiation. It not only provides mechanical support for cells, tissues and organs, but also binds to and stores many growth factors, cytokines, and other signaling molecules for transducing these external signals to regulate cell fate. Thus, it is considerably important to mimic these aspects in vitro differentiation in order to achieve a high degree specification of hESCs into glucose-responsive, insulin-secretion cells for cell-based diabetes therapy. In our previous works, we have successfully developed 3D collagen scaffolds and demonstrated that an islet-like tissue structure could form under 3D environments. The beta-like cells generated using this scaffold produce insulin and c-peptide upon glucose challenge. Based on these fundamental studies, we have recently discovered that the maturity of beta-like cells can be further elevated by blending collagen with ECM components during scaffolding. The combinatorial effect of ECM on hESC pancreatic differentiation was investigated and determined in this work.

2. **Methods and Materials**

   **Feeder-free layer hESC culture.** The hESC line H9 was acquired from Wi-cell Research Institute. Cells were maintained in an undifferentiated state on Matrigel (BD)-coated cell culture plates in an mTeSR1 medium (StemCell Technologies, Vancouver, Canada). The cell culture medium was exchanged daily.

   **Collagen scaffolding and 3D pancreatic differentiation of hESCs.** 3D collage scaffolds were prepared, as described in our previous work. To blend the collagen with ECM proteins during scaffolding, variable amounts of ECM components to a final concentration of 2, 2.5, or 4 ng/ml, respectively. The pH of these ECM protein enhanced collagen solutions were adjusted to 7.4±0.2 by the addition of 1 N NaOH. The cell-containing scaffolds were placed into an incubator and allow cells to grow in scaffolds for 24 h before initiating pancreatic differentiation, as described in.

   **Immunohistochemical staining.** Formalin-fixed paraffin-embedded cell-borne collagen scaffolds were cut into 4-μm thick sections and mounted on glass slides coated with poly-L-lysine. The sections were deparafinized in xylene and rehydrated in 100, 96 and 70 % ethanol for 5 min and rinsed in PBS and them labeled with corresponding fluorescent dye conjugated antibodies for determining pancreatic marker expression in these cells.

   **Quantitative real-time PCR (qRT-PCR).** SYBR Green real time PCR was used to ascertain marker gene expression in differentiated hESCs under 3D environments. Each experiment was carried out in triplicate.

3. **Results**

   Fig. 1 shows the elevated expression of pancreatic markers in 3D differentiated hESCs, suggesting the improvement of beta-like cell maturity within 3D scaffolds.

Reference