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P01-071

3D Bioprinting of Insulin -Producing Cell Aggregates- Derived from Human Pluripotent Stem Cells with Pancreatic Tissue-Derived Bioink

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Native pancreatic islets are clustered with diverse endocrine cells as functional units and surrounded by microvasculature networks communicating with neighboring cells for releasing hormones in response to glucose stimulation. 3D bioprinting technology has emerged as an promising strategy for the fabrication of engineered pancreatic tissue constructs because it enables implementation of complex tissue microarchitecture by precise positioning of multiple cells within functional bioink. In this study, we fabricated a pancreatic tissue construct through 3D aggregate bioprinting method using tissue-derived bioink with stem cell-derived insulin producing cells (IPC) to replicate the structural and functional features of native human pancreatic tissue. Selection of suitable bioink is a first step towards building functional 3D pancreatic tissue constructs. In previous study, we suggested pancreatic tissue derived-decellularized extracellular matrix (pdECM) as a attractive printable material to recapitulate native pancreatic cell niche. Here, we investigated representative constituents of pdECM bioink through proteomic analysis to evaluate that pdECM bioink can provide sufficient microenviromental cues. Additionally, functional gene classification regarding matrix-mediated characteristics were observed via gene ontology (GO) analysis. Collagen type VI was most abundant protein in pdECM bioink and other crucial ECM proteins for cell-matrix interactions were also enriched compared to the collagen bioink. Furthermore, we differentiated human embryonic stem cells (hESC) into IPC via four-stage protocol to generate functional human pancreatic cells. The differentiated cells we obtained at each stage were characterized by gene expression profile and flow cytometry analysis. Key markers of beta cells including PDX1, NKX6.1, and insulin were highly expressed after stage 3. After generation of IPC, printing conditions regarding the size of IPC aggregates were optimized for mimicking native pancreatic tissue geometry. In addition, we demonstrated interaction capacity between IPC aggregates with co-culture condition using human umbilical vein endothelial cells (HUVEC) and human mesenchymal stem cells (hMSC). Immunofluorescent staining results of printed constructs revealed that rapid induction of IPC aggregates networks can occur in tri-culture condition. The developed 3D human pancreatic tissue constructs will be able to broaden the application of in vitro disease models of diabetes and transplantable constructs for in vivo study.

Keywords
3D Bioprinting, Decellularized extracellular matrix, Human pluripotent stem cells, Insulin-producing cells, Cell aggregates

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P01-072

Induction of Designed Micro-vascular Network with 3D Bioprinting

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One of the challenging issues in tissue engineering is a building of functional vascular network systems inside an artificial tissue. Without proper vascular networks, enough oxygen and nutrients cannot be provided to the cells in the artificial tissue. Therefore, the cells cannot maintain their viability and functionality during tissue growth and maturation without proper vascular networks. Also, the structure of vascular networks has a significant role in living tissues to improve the functionality of the cells. To fabricate a functional and well-organized vascular structure, researches using 3D bioprinting are actively studied due to its superior ability to produce complex structures with variable materials in desired patterns. Here, we propose the novel method for the fabrication of complex vascular networks using 3D bioprinting technology. The vascular channels were formed by direct printing of hydrogels containing endothelial cells and fibroblasts were placed at a distance to induce the formation of micro-vessels from the vascular channels. To control the angiogenesis pattern, different concentrations of fibrin gel were patterned to differentiate the diffusion rate of angiogenic factors and matrix stiffness within the structure. Using this method, it was possible to induce the desired structure of vascular network.

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Abstract

Native pancreatic islets are clustered with diverse endocrine cells as functional units and surrounded by microvasculature networks communicating with neighboring cells for releasing hormones in response to glucose stimulation. 3D bioprinting technology has emerged as an promising strategy for the fabrication of engineered pancreatic tissue constructs because it enables implementation of complex tissue microarchitecture by precise positioning of multiple cells within functional bioink. In this study, we fabricated a pancreatic tissue construct through 3D aggregate bioprinting method using tissue-derived bioink with stem cell-derived insulin producing cells (IPC) to replicate the structural and functional features of native human pancreatic tissue. Selection of suitable bioink is a first step towards building functional 3D pancreatic tissue constructs. In previous study, we suggested pancreatic tissue derived-decellularized extracellular matrix (pdECM) as a attractive printable material to recapitulate native pancreatic cell niche. Here, we investigated representative constituents of pdECM bioink through proteomic analysis to evaluate that pdECM bioink can provide sufficient microenviromental cues. Additionally, functional gene classification regarding matrix-mediated characteristics were observed via gene ontology (GO) analysis. Collagen type VI was most abundant protein in pdECM bioink and other crucial ECM proteins for cell-matrix interactions were also enriched compared to the collagen bioink. Furthermore, we differentiated human embryonic stem cells (hESC) into IPC via four-stage protocol to generate functional human pancreatic cells. The differentiated cells we obtained at each stage were characterized by gene expression profile and flow cytometry analysis. Key markers of beta cells including PDX1, NKX6.1, and insulin were highly expressed after stage 3. After generation of IPC, printing conditions regarding the size of IPC aggregates were optimized for mimicking native pancreatic tissue geometry. In addition, we demonstrated interaction capacity between IPC aggregates with co-culture condition using human umbilical vein endothelial cells (HUVEC) and human mesenchymal stem cells (hMSC). Immunofluorescent staining results of printed constructs revealed that rapid induction of IPC aggregates networks can occur in tri-culture condition. The developed 3D human pancreatic tissue constructs will be able to broaden the application of in vitro disease models of diabetes and transplantable constructs for in vivo study.

Conclusion

- Proteomic analysis showed that collagen type VI was most abundant protein in pdECM bioink and other crucial ECM proteins for cell-matrix interactions were also enriched compared to that of the collagen bioink.
- Representative FACS plots revealed that S4 cells expressed key beta-transcription factors such as PDX1, NKX6.1 and insulin. 3D bioprinted IPC aggregates, which were co-cultured with HUVEC and MSC, rapidly induced cell networks between aggregates.
- The developed 3D human pancreatic tissue constructs will be able to broaden the application of in vitro disease models of diabetes.

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