



***In vitro* expansion and differentiation of rat pancreatic duct-derived stem cells into insulin secreting cells using a dynamic three-dimensional cell culture system**

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ABSTRACT. In this study, a dynamic three-dimensional cell culture technology was used to expand and differentiate rat pancreatic duct-derived stem cells (PDSCs) into islet-like cell clusters that can secrete insulin. PDSCs were isolated from rat pancreatic tissues by *in situ* collagenase digestion and density gradient centrifugation. Using a dynamic three-dimensional culture technique, the cells were expanded and differentiated into functional islet-like cell clusters, which were characterized by morphological and phenotype analyses. After maintaining 1×10^8 isolated rat PDSCs in a dynamic three-dimensional cell culture for 7 days, 1.5×10^9 cells could be harvested. Passaged PDSCs expressed markers of pancreatic endocrine progenitors, including CD29 (86.17%), CD73 (90.73%), CD90 (84.13%), CD105 (78.28%), and Pdx-1. Following 14 additional days of culture in serum-free medium with nicotinamide, keratinocyte growth factor (KGF), and β fibroblast growth factor (FGF), the cells were differentiated into islet-

like cell clusters (ICCs). The ICC morphology reflected that of fused cell clusters. During the late stage of differentiation, representative clusters were non-adherent and expressed insulin indicated by dithizone (DTZ)-positive staining. Insulin was detected in the extracellular fluid and cytoplasm of ICCs after 14 days of differentiation. Additionally, insulin levels were significantly higher at this time compared with the levels exhibited by PDSCs before differentiation ($P < 0.01$). By using a dynamic three-dimensional cell culture system, PDSCs can be expanded *in vitro* and can differentiate into functional islet-like cell clusters.

Key words: Dynamic three-dimensional culture; Islet β -cells; Pancreatic duct-derived stem cells