Enhanced Islet Function and Survival of Rat Islets in a Biomimetic Self-assembled Nanomatrix Gel

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Statement of Purpose

Pancreatic islet transplantation (PIT) has been given increasing attention as an alternative treatment for insulin-dependent diabetes mellitus, but a few limitations to its success in clinical trials have been identified. In particular, the substantial loss of islets in the culture period before transplantation is reported as one of primary causes of islet graft failure because the destruction of ECM around islets causes reduced beta-cell function as well as reduced survival.[1] Since interactions between islet cells and extracellular matrix (ECM) regulate survival, proliferation, and insulin secretion, several encapsulating materials have been developed.[2] However, ECM-derived materials and synthetic materials have been used with limited success due to the intrinsic limitations of each biomaterial. To enhance the efficacy of PIT, there is an imperative need to develop an ECM mimicking material capable of providing the islet with protecting and nurturing microenvironment. Therefore, we have developed a self-assembled peptide amphiphile (PA) nanomatrix gel that is inscribed with adhesive ligands as well as enzyme-mediated degradable sites to restore islet-ECM interactions. Viability and glucose stimulated insulin secretion were evaluated over a 14 day time period in three different culture conditions.

Methods

Islets isolated from male Spargue-Dawley rats were cultured in three different conditions (Table 1): To minimize an unexpected loss of islet mass during the culture period, a mesh insert culture method was also devised, in which 5 ㎛ nylon mesh was placed into an insert to retain intact rat islets over the culture period. Rat islets were also encapsulated within PA nanomatrix gels. Glucose stimulated insulin secretion was studied for 14 days. Islet viability was assessed with a fluorescein diacetate/propidium iodide (FDA/PI) staining method, and insulin production in islets was assessed with a dithizone (DTZ) staining method. To assess morphological changes, the remaining islets were observed during cultivation under a light microscope.

Table 1. Experimental groups that were defined to culture

Group	Descriptions
Bare	Cultured in a 12 well non-tissue culture treated plate
Insert	Cultured in the modified insert chambers
Nanomatrix	Encapsulated within PA nanomatrix gels in the modfied insert chambers

Results

In the clinical practice, an unexpected loss of islet mass occurs during the culture period before transplantation.[3] To minimize the loss of islets in this study, we developed an insert chamber in which a 5 ㎛ nylon mesh sheet was placed into a commercial insert chamber to examine islet function with a minimal loss of islets. In terms of islet function, there was a marked decrease in glucose-stimulated insulin secretion in both the bare and the insert group while islets of the nanomatrix group maintained their function even after 14 days (Figure 1). Entire islets in the nanomatrix gel still stained with DTZ even after 14 days whereas in both the bare and the insert group, islets were only lightly stained meaning that islets in the nanomatrix group better maintained glucose-stimulated insulin secretion. Using FDA/PI staining, it was observed that after 7 days cultivation islets in the bare and the isert group had became necrotic, whereas islets within the PA nanomatrix gel maintained their viability. Moreover, we found that during the 14 day cultivation period most islets were retained in the nanomatrix group with good integrity whereas only a few islets remained in the bare and the insert group with fragments.

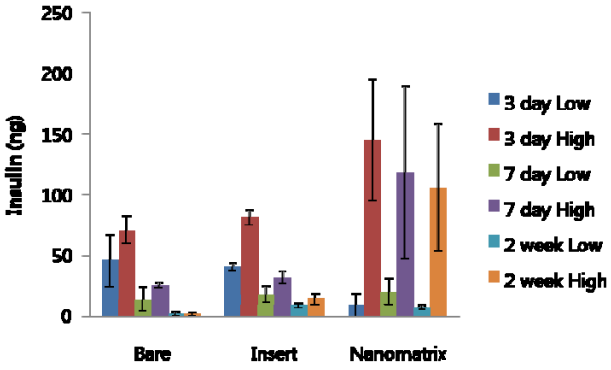

Fig. 1. Glucose-stimulated insulin secretion for 14 days of cultivations ($p<0.05$) ($n=4$).

Conclusion

After a 14 day culture period, rat islets embedded in the PA nanomatrix gel showed significantly enhanced viability and glucose-stimulated insulin secretion response compared to both the bare and the insert groups. These results demonstrated that the self-assembled PA nanomatrix gel has a great potential for pancreatic islet transplantation because they may provide an islet-ECM mimicking microenvironment. Currently, in-vivo studies using diabetic mice are under investigation.

References

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Acknowledgments

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