Enhanced Rat Islet Function and Survival *In Vitro* Using a Biomimetic Self-Assembled Nanomatrix Gel

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Peptide amphiphile (PA) is a peptide-based biomaterial that can self-assemble into a nanostructured gel-like scaffold, mimicking the chemical and biological complexity of natural extracellular matrix. To evaluate the capacity of the PA scaffold to improve islet function and survival *in vitro*, rat islets were cultured in three different groups—(1) bare group: isolated rat islets cultured in a 12-well nontissue culture-treated plate; (2) insert group: isolated rat islets cultured in modified insert chambers; (3) nanomatrix group: isolated rat islets encapsulated within the PA nanomatrix gel and cultured in modified insert chambers. Over 14 days, both the bare and insert groups showed a marked decrease in insulin secretion, whereas the nanomatrix group maintained glucose-stimulated insulin secretion. Moreover, entire islets in the nanomatrix gel stained positive for dithizone up to 14 days, indicating better maintained glucose-stimulated insulin production. Fluorescein diacetate/propidium iodide staining results also verified necrosis in the bare and insert groups after 7 days, whereas the PA nanomatrix gel maintained islet viability after 14 days. Thus, these results demonstrate the potential of PAs as an intermediary scaffold for increasing the efficacy of pancreatic islet transplantation.

Introduction

THE ONGOING INVESTIGATION of treatment options for human type I diabetes mellitus requires the continual development of innovative strategies that more effectively restore long-term physiological function, while still maintaining a simple approach with minimal invasiveness. In the past decade, islet engraftment has been heavily investigated as one such promising treatment for type I diabetic patients, offering a less invasive alternative to full pancreas replacement.¹ In spite of the numerous efforts to improve islet engraftment, clinical efficacy is still lacking because the primary focus has been on avoiding host immune response via the development of semipermeable and biocompatible membranes, while relatively ignoring the potential benefits of a more biomimetic engraftment material.² The need for improving the biomimetic character of islet scaffolds is supported by recent literature demonstrating that substantial β -cell loss during the peri-transplant period is detrimental to the efficacy of islet transplantation.³ Specifically, the loss of β -cell function during islet isolation is believed to occur from the destruction of the native islet microenvironment, thereby triggering islet death.⁴

In addition, the disruption of islet–extracellular matrix (ECM) interactions exposes the islets to a variety of cellular stresses that further contribute to loss of biological functions. Therefore, biomimetic materials that create more ECM-mimicking environments are needed to better maintain islet function and survival during the intermediate stage between implantation and fully restored host integration.

The importance of islet-ECM interactions is well documented by a number of studies that demonstrate improved islet survival and function facilitated through the use of scaffolds containing isolated ECM proteins. For example, Nagata *et al.* demonstrated that a mixture of different types of collagen increased both rat islet β -cell viability and glucose-stimulated insulin secretion.⁵ Human pancreatic β -cells grown on a bovine corneal endothelial cell matrix have also been found to maintain glucose-stimulated insulin secretion.⁶ Additionally, laminin-5-rich ECM substrates derived from rat bladder carcinoma cells have been shown to enhance the spreading of rat islet β -cells and improve glucose-stimulated insulin secretion.⁷ Thus, many research groups have begun incorporating ECM proteins into synthetic biomaterials for the encapsulation of islets or β -cells. To this effect, collagen IV absorbed in a

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poly(lactide-co-glycolide) scaffold showed enhanced function of transplanted islets.⁸ Similarly, MIN6 β -cells showed reduced apoptosis and maintained insulin secretion when cultured in poly(ethylene glycol) (PEG) hydrogels containing ECM proteins compared to control hydrogels alone.⁹

However, for clinical applications, the use of ECM proteins has some potential problems, such as undesirable immune responses, higher infection risks, variability in biological sources, and increased costs.¹⁰ To overcome these limitations, small peptide sequences derived from ECM proteins have been employed to modify the different types of polymers used for islet engraftment. For example, the growth and function of MIN6 cells were enhanced when encapsulated in either a PEG- β -based thermo-reversible gel conjugated with Gly-Arg-Gly-Asp-Ser (GRGDS) or photopolymerized PEG hydrogels modified with various laminin-derived peptides or type I collagen-derived peptides.^{11,12} Despite the benefits of the isolated ECM peptides, limitations of encapsulating biomaterials still need to be given consideration. In particular, the entrapment of cells in photopolymerized biomaterials can lead to many potential problems after implantation, such as the formation of fibrotic processes, poor degradation of the scaffold, and local and/or systemic toxicity.¹³ Differing compositions and concentrations of alginate have also been found to affect the cellular overgrowth of implanted capsules, as metabolic barriers to nutrient diffusion can form around the implant if nonoptimal levels of the material are used, despite the established biocompatibility of alginate.¹⁴

As a promising solution to overcome these drawbacks, peptide amphiphile (PA) nanomatrix gels would offer improved efficacy in pancreatic islet engraftment. The use of the PA nanomatrix gel as a transplant intermediary for islets is potentially advantageous because they meet the essential design criteria for synthetically recapitulating the ECM: rapid gel-like 3D network formation by self-assembly, versatility to incorporate various cell adhesive moieties, and cell-mediated degradable sites (matrix metalloproteinase-2 [MMP-2]) for progressive scaffold degradation and eventual replacement by host-ECM.¹⁵ Structurally, the PA consists of a hydrophilic functional peptide sequence attached to a hydrophobic alkyl tail, and the internal peptide structure can be adapted to mimic the characteristic properties of the natural ECM.^{16–20} Further, PAs self-assemble into long cylindrical structures that are 8–10 nm in diameter with a length up to several microns in length, and the self-assembly process is initiated by lowering the pH or adding multivalent ions, providing biocompatible means to encapsulate islets for engraftment.^{16,17}

In this study, isolated rodent islets were incorporated into the PA nanomatrix gel containing a cell-adhesive ligand isolated from ECM proteins, arginine-glycine-aspartic acid (RGD), as well as an MMP-2 sensitive sequence. The interactions between islets and ECM are known to be important for β -cell viability and function, especially integrin signaling via RGD, which has been shown to decrease apoptosis of islets.²¹ Moreover, the MMP-2 enzyme is activated during rat pancreatic development, enabling cell migration of pancreatic endocrine cells throughout the ECM during islet morphogenesis.²² Thus, it is believed that the PA nanomatrix gel will facilitate progressive degradation and replacement by host ECM after transplantation *in vivo*. Importantly, the PA forms a rapid, viscoelastic 3D microenvironment without any organic

solvents or chemicals, providing the islets with a protective and nurturing environment to promote islet survival and function. Hence, we hypothesize that the PA nanomatrix gel will provide an ECM-mimicking microenvironment that imitates the native ECM microenvironment between islets and ECM, thereby improving islet survival and function. To evaluate this hypothesis, rat islets encapsulated within the PA nanomatrix gel were studied over 14 days of cultivation.

Since traditional islet culturing methods cause variability in the results due to physical loss during cultivation, we have also developed a new *in vitro* culture method consisting of a modified insert chamber with a 5 μ m nylon mesh sheet. This system allows long-term cultivation with a minimal loss of islets. Thus, we were able to assess the islet survival and function more consistently and precisely. Overall, our results demonstrated that islets encapsulated in the PA nanomatrix gel maintained their function with increased glucose-stimulated insulin secretion throughout the 14 days compared to the other groups. This demonstrates the potential of the PA nanomatrix gel to restore islet-ECM interactions and provide more precise evaluations of islet responses using the devised culture method. Therefore, this study proposes an innovative strategy to tackle some of limitations facing the clinical practice of islet transplantation.

Materials and Methods

Materials and animals

CMRL-1066 tissue culture medium, L-glutamine, penicillin, and streptomycin were purchased from Gibco (Grand Island, NY). Fetal bovine serum was obtained from Hyclone (Logan, UT). Male Sprague-Dawley rats (250–300 g) were purchased from Harlan Laboratories (Indianapolis, IN). Collagenase type XI was purchased from Sigma Chemical Co. (St. Louis, MO). Rat insulin ELISA kit was obtained from Crystal Chem Inc. (Downers Grove, IL). All other reagents were purchased from commercially available sources.

Islet isolation and culture

Islets were isolated from male Sprague-Dawley rats (250–300 g) by collagenase digestion as described previously.²³ After digestion, islets were isolated by density gradient purification and individually hand picked under a dissection microscope. Fifty hand-picked islets were used per sample for each condition group. All islet samples were cultured at 37°C in an atmosphere of 95% air and 5% CO₂ in CMRL-1066 tissue culture medium supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin.

Synthesis of PA

Using standard Fmoc-chemistry, the peptide sequence, CH₃(CH₂)₁₄CONH-GTAGLIGQERGDS, was synthesized on an Advanced Chemtech Apex 396 peptide synthesizer as described previously.^{18–20} After synthesis, the peptide was alkylated at the N-termini with palmitic acid by a manual coupling reaction. Alkylation was performed for 24 h at room temperature in a mixture of *o*-benzotriazole-*N,N,N',N'*-tetramethyluroniumhexafluorophosphate, diisopropylethylamine, and dimethylformamide. Cleavage and deprotection were performed with a mixture of trifluoroacetic acid,

deionized water, triisopropylsilane, and anisole (40:1:1) for 3 h at room temperature. The PA solution was precipitated in cold ether after removing excess trifluoroacetic acid and lyophilized. Matrix-assisted laser desorption ionization time of flight mass spectrometry was used for PA characterization.

Islet encapsulation within PA self-assembled nanomatrix gel

PA stock solution (2% weight/volume) was prepared and buffered to neutral pH (~ 7) with NaOH. Self-assembly of the PA with rat islets was induced by combining 50 μL of PA solution with 50 μL of complete CMRL-1066 medium and 15 μL 0.1 M CaCl_2 in 12-well silicone flexiPERM cell-culture chambers (Sigma-Aldrich, St. Louis, MO) attached to glass coverslips. The molar ratio between PA and calcium ion ($\text{Mr} = \text{Ca}^{2+}/\text{PA}$) was held constant at $\text{Mr} = 2$, as previously described.¹⁷ The PA self-assembled nanomatrix gel was formed as a sphere-shaped hydrogel with an approximate 7.2 mm diameter. After encapsulating, the PA self-assembled nanomatrix gel containing 50 hand-picked rat islets (nanomatrix group) was transferred into the fabricated insert with 5 μm nylon mesh and cultured in a 12-well nontissue culture-treated plate (Corning Costar, Corning, NY) (Fig. 1A).

Glucose-stimulated insulin secretion

Glucose-stimulated insulin secretion was assessed at 3, 7, and 14 days after encapsulation. To eliminate any residual

insulin, all samples were preincubated for 1 h in low-glucose Krebs-Ringer bicarbonate buffer (low-glucose KRB) (25 mM HEPES, 115 mM NaCl, 24 mM NaHCO_3 , 5 mM KCl, 1 mM MgCl_2 , 2.5 mM CaCl_2 , and 0.1% bovine serum albumin, and 3 mM D-glucose, pH 7.4). Then, each sample was placed in 1 mL low-glucose KRB for 1 h, followed by incubation in 1 mL high-glucose KRB (20 mM D-glucose) for 1 h. The supernatant was withdrawn, and insulin was measured by the ELISA method. To normalize the secreted insulin data, a fluorometric PicoGreen DNA kit (Molecular Probes, Eugene, OR) was used to measure DNA content of each sample using a microplate fluorescent reader (Synergy HT; BIO-TEK Instrument, Winooski, VT).¹⁷

Islet viability assessment

Islet cell viability in each group was assessed by microscopic examination using fluorescein diacetate/propidium iodide (FDA/PI) staining at 3, 7, and 14 days after encapsulation. A FDA stock solution was prepared by dissolving 10 mg FDA into 2 mL of acetone. The FDA stock solution was stored at -20°C . When in use, 10 μL of FDA stock solution was diluted with 990 μL of phosphate-buffered saline (PBS). PI (1 mg/mL; Invitrogen, Eugene, OR) was prepared each time to be used immediately, as 50 μL of solution was diluted with 450 μL of PBS. For viability staining, each sample was immersed in a mixture of 2 mL PBS, 10 μL of diluted PI, and 20 μL of diluted FDA. Stained islets were observed with a Nikon TE2000-S fluorescence microscope (Nikon Inc., Tokyo, Japan) equipped with a Nikon high-pressure mercury arc lamp. Fluorescein dyes that deacetylated from FDA through nonspecific esterases in the cytoplasm of cells were shown under a fluorescent blue filter, whereas PI dyes, staining nucleic acids of dead cells, were viewed under a fluorescent green filter.

Evaluation of insulin-producing β -cells using dithizone staining

To identify insulin-producing β -cells from each group, dithizone (DTZ) staining was used after 3, 7, and 14 days postencapsulation. DTZ forms a red-colored complex when reacted with zinc, indicating positive staining for insulin production. To make the DTZ stock solution, 50 mg of DTZ was dissolved in 5 mL of dimethyl sulfoxide and diluted with 30 mL of PBS. The stock solution was filtered with a 0.45 μm filter. DTZ solution was added to each group for microscopic examination at the predetermined time points.

Statistical analysis

All experiments were performed at least three independent times in quadruplicate. All values were denoted as means $\pm$ standard deviation. Statistical analysis was performed using SPSS 15.0 software (SPSS, Inc., Chicago, IL). One-way analysis of variance was used for statistical comparison. A level $p < 0.05$ was considered to be statistically significant.

Results

Experimental design with a modified insert system

To evaluate the effect of the PA self-assembled nanomatrix gel on isolated rat islet function, three groups were

FIG. 1. Schematic drawing of (A) the modified insert culture system and (B) islet encapsulation within a peptide amphiphile nanomatrix gel. Color images available online at www.liebertonline.com/ten.

designed—(1) bare group: isolated rat islets cultured in a 12-well nontissue culture-treated plate; (2) insert group: isolated rat islets cultured in modified insert chambers; (3) nanomatrix group: isolated rat islets encapsulated within the PA self-assembled nanomatrix gel and cultured in modified insert chambers. In the modified insert chamber design, two key factors were emphasized: (1) maintaining the islet number throughout the culture period because periodic medium change could lead to loss of islets and (2) accurately quantifying the secreted insulin of each experimental group with little variance. From experience using traditional culture methods, we found that most rat islets remained weakly attached to the culture surface, leading to some physical loss of islets when replacing medium over long-term culture. As a result, relative variations in the remaining islets during the cultivation could affect the assessment of islet function. Thus, in this study, we developed a modified insert chamber in which a 5 μ m nylon mesh sheet was placed into a commercial insert chamber to retain free-floating islets, thereby preventing physical loss of islets (Fig. 1A).

Encapsulating rat islets with the PA nanomatrix gel

To encapsulate rat islets, the PA was designed with the following peptide sequence attached to a hydrophobic alkyl tail: $\text{CH}_3(\text{CH}_2)_{14}\text{CONH-GTAGLIGQERGD}$ S. By adding calcium ions to the mixture of PA solution and isolated rat islets suspended in CRML media, we were able to encapsulate the islets under physiological conditions without any organic solvents or chemicals. Thus, the addition of calcium ions triggered self-assembly of the PA into a nanofibrous gel, encapsulating the rat islets within the PA nanomatrix (Fig. 1B).

Assessment of glucose-stimulated insulin secretion

After a period of cultivation, glucose-stimulated insulin secretion responses were measured to evaluate the function of encapsulated rat islets. Over the 14 days of cultivation, both the bare and insert groups showed a marked decrease in insulin secretion, whereas the nanomatrix group maintained glucose-stimulated insulin secretion, even after 14 days (Fig. 2). Additionally, islets in the bare group were not responsive to elevated levels of glucose after 14 days, as most of the islets not only lost their functionality, but were found to be completely missing due to the periodic medium changes. In contrast, the response of β -cells to the high-glucose condition was maintained throughout for the nanomatrix group. Further, over the entire cultivation period, there was a significant statistical difference in glucose-stimulated insulin responses for the nanomatrix group. After 3, 7, and 14 days, the low glucose response values were 8.7 ± 9.6 , 19.9 ± 11.2 , and 7.0 ± 1.9 ng, whereas the high glucose response values were 145.1 ± 49.8 , 118.3 ± 71.0 , and 105.6 ± 52.5 ng, respectively. These results were validated by the stimulation index (SI) data observed. To compare the insulin secretion values between groups, we calculated each average SI obtained by dividing average high glucose response value with average low glucose response value of each group. The average SI values were 16.7, 5.9, and 15.0 for islets in the nanomatrix group after 3, 7, and 14 days, respectively. However, the average SI values over the same time points were 2.0, 1.8, and 1.6 for the insert groups and

FIG. 2. Glucose-stimulated insulin secretion for 14 days of cultivations (*significant differences in insulin release between low-glucose incubation (3 mM) and high-glucose incubation (20 mM), $p < 0.05$) ($n = 4$).

1.6, 1.9, and 1.0 for the bare groups, respectively. Thus, the average SI values after 14 days for the nanomatrix group were approximately sevenfold more than the insert group and almost ninefold greater than the bare group.

FDA/PI staining to determine islet viability

FDA/PI staining was used to assess the viability of the rat islets. After 3 days, islets in all three groups displayed maintained viability (Fig. 3). After 7 days, the cores of the islets in the bare and insert groups developed necrotic cores (dark centers localized with red fluorescence), whereas the islets in the nanomatrix group still retained most of their viability (Fig. 4). After 14 days in the insert or bare groups, there were fewer remaining islets in general, and out of the retained islets, many were fragmented opaque clusters of islets or simply cellular debris lacking in viability. Conversely, the islets in the nanomatrix group still maintained islet integrity and almost all remained viable (Fig. 5). In summary, these FDA/PI staining results represent a marked improvement in maintained islet viability for the nanomatrix group over the entire incubation period compared to the other two groups, which began to display necrosis and reduced viability after 7 days.

DTZ staining to evaluate insulin-producing β -cells

DTZ staining was used to qualitatively assess the function of the islets, appearing as a crimson red-positive stain in the insulin-producing β -cells within the islets.²⁴ After 3 days, all three groups showed positive DTZ staining (Fig. 6A, D, G). After 7 days, the bare and insert groups had significantly reduced positive DTZ staining, whereas the nanomatrix group still maintained high positive staining (Fig. 6B, E, H). After 14 days, the bare group had no intact islets left to stain, and the insert group only retained positive staining in the peripheral areas of a few disintegrated islets (Fig. 6C, F). In contrast, the islets in the nanomatrix group still maintained integrity and DTZ-positive staining throughout the core of the islets (Fig. 6I). Hence, these results indicate that the nanomatrix group maintained function throughout the 14 days.

FIG. 3. Morphology and viability of rat islets in different culture conditions after 3 days of cultivations. (A) Bright-field image of islets in the bare group, (B) fluorescein diacetate/propidium iodide (FDA/PI) staining of islets in the bare group, (C) bright-field image of islets in the insert group, (D) FDA/PI staining of islets in the insert group, (E) bright-field image of islets in the nanomatrix group, and (F) FDA/PI staining of islets in the nanomatrix group. All scale bars indicate 100 µm. Color images available online at www.liebertonline.com/ten.

Discussion

To improve the success of pancreatic islet transplantation for human type I diabetes mellitus, our studies investigated the PA nanomatrix gel as an islet engraftment material that expands on earlier studies primarily focused on the avoidance of the host immune response. Pancreatic islet transplantation has gained lots of attention to treat type I diabetes mellitus. However, about 40%–60% of islet grafts transplanted failed during the peritransplant period.²⁵ One of the main reasons for this failure includes the disruption of the islet–ECM microenvironments during the islet isolation, which leads to reduced islet function and the loss of islet viability.⁴

To create an ECM-mimicking microenvironment, we used a PA nanomatrix gel, which offers inherent biocompatibility, versatility within the internal peptide structure, enzyme-mediated degradability, easily accessible bioactivity, and durability for clinical practice.¹⁹ The PA nanomatrix gel contains RGDS cell-adhesive ligands and enzyme-mediated degradable sites within an ECM-mimicking viscoelastic environment, thereby endowing the needed characteristics for enhanced islet survival and function.

FIG. 4. Morphology and viability of rat islets in different culture conditions after 7 days of cultivations. (A) Bright-field image of islets in the bare group, (B) FDA/PI staining of islets in the bare group, (C) bright-field image of islets in the insert group, (D) FDA/PI staining of islets in the insert group, (E) bright-field image of islets in the nanomatrix group, and (F) FDA/PI staining of islets in the nanomatrix group. All scale bars indicate 100 µm. Color images available online at www.liebertonline.com/ten.

From our investigations, the nanomatrix group demonstrated the best results, maintaining prolonged survival and enhanced function of islets. Over all the experiments, the glucose-stimulated insulin secretion of nanomatrix group was significantly maintained for up to 14 days, whereas the bare and insert groups showed a much lower level of insulin secretion (Fig. 2). Additionally, most of the islets were retained in the nanomatrix gel with positive DTZ staining throughout the 14 days of cultivation, demonstrating that the nanomatrix gel provides the functional support needed to maintain the oxidative capacity for insulin secretion and granule density for prolonged incubation.

For the insert group, however, fewer islets were observed, which had reduced viability and function, whereas the bare group lost most of its islets over the same period and could not be accurately measured. These findings were consistent with the FDA/PI staining results for all three groups. Thus, throughout the entire cultivation period, the nanomatrix group consistently displayed intact islet integrity with enhanced function, whereas fewer islets remained in the bare and insert groups with reduced utility. Moreover, the nanomatrix gels began to degrade as intended after 14 days due to the inclusion of the MMP-2-sensitive sequence. On the basis of the fact that revascularization begins 2–4 days after

FIG. 5. Morphology and viability of rat islets in different culture conditions after 14 days of cultivation. (A) Bright-field image of islets in the bare group, (B) FDA/PI staining of islets in the bare group, (C) bright-field image of islets in the insert group, (D) FDA/PI staining of islets in the insert group, (E) bright-field image of islets in the nanomatrix group, and (F) FDA/PI staining of islets in the nanomatrix group. All scale bars indicate 100 µm. Color images available online at www.liebertonline.com/ten.

islet transplantation and is completed by 10–14 days, these results demonstrate the potential of the nanomatrix gel as a useful intermediary scaffold that bridges the gap between implantation and fully restored host integration.²⁶

In contrast to the traditional islet culturing methods, which lead to variability in the observed results due to

physical loss of islets during cultivation, we devised a modified insert chamber to more accurately measure islet function in this study. Specifically, in traditional culture methods, islets are susceptible to necrotic death within their central cores due to islet size, nonproliferative nature, and the variability of culture conditions.²⁷ Therefore, the devised insert chamber provided a consistent islet culturing method that not only measured islet function more accurately, but also provided a better system for quantifying the viability of the remaining islets over long-term cultivation.

The normalization of islet quantification data is critical to more accurately reflecting the overall islet performance. However, the traditional normalization methods do not account for variations in the viability and number of islets, which can both be altered due to the physical loss that occurs during the cultivation. Thus, traditional methods frequently lead to misinterpretation of the data, especially in long-term studies. Consequently, we normalized the glucose-stimulated insulin secretion values in this study by the amount of DNA per sample to reduce variations and account for the different numbers of remaining islets in each condition. It was found that not only were islets in the nanomatrix group producing significantly more total insulin, but also, when normalized by total islet DNA, the islets in the nanomatrix group showed more insulin per DNA than the bare and insert groups (Fig. 7). In contrast, there were marked decreases in the bare and insert groups when normalized by DNA. Moreover, it is questionable that β -cell proliferation during cultivation affected the normalized islet function data, as cultured β -cells very rarely proliferate under normal culture conditions.^{28,29} Thus, these results indicate that not only were more islets retained in the nanomatrix group, but also the individually remaining islets demonstrated enhanced survival and function per DNA.

In conclusion, this study demonstrates the importance of developing an ECM-mimicking microenvironment for promoting the long-term survival of encapsulated islets *in vitro*. Our findings suggest that the ECM-mimicking PA nanomatrix gel may provide a protective and nurturing microenvironment that enhances islet cell survival, and, most importantly, increases function in the β -cell mass *in vivo*. It is striking that the islets encapsulated in the PA nanomatrix gel maintained normal glucose-stimulated insulin secretion

FIG. 6. Evaluation of insulin-producing β -cells using dithizone staining: (A) after 3 days of cultivation in the bare group, (B) after 7 days of cultivation in the bare group, (C) after 14 days of cultivation in the bare group, (D) after 3 days of cultivation in the insert group, (E) after 7 days of cultivation in the insert group, (F) after 14 days of cultivation in the insert group, (G) after 3 days of cultivation in the nanomatrix group, (H) after 7 days of cultivation in the nanomatrix group, and (I) after 14 days of cultivation in the nanomatrix group. All scale bars indicate 100 µm. Color images available online at www.liebertonline.com/ten.

FIG. 7. Glucose-stimulated insulin secretions normalized by DNA in the (A) bare group, (B) insert group, and (C) nanomatrix group (* $p < 0.05$) ($n = 4$).

throughout the 14 days of *in vitro* culture at a sufficiently high level that could not even be maintained for 3 days in the islets cultured under the free floating conditions of the bare and insert groups. These findings provide experimental evidence that supports the need to continue developing this PA

nanomatrix for islet transplantation, especially for further testing under *in vivo* conditions. Overall, these results demonstrate the potential of the PA nanomatrix gel as an intermediary scaffold for increasing the efficacy of pancreatic islet transplantation.

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Disclosure Statement

No competing financial interests exist.

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