

# The effects of cell–matrix interactions on encapsulated $\beta$ -cell function within hydrogels functionalized with matrix-derived adhesive peptides

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## Abstract

The influence of matrix-derived adhesive peptide sequences on encapsulated  $\beta$ -cell survival and glucose-stimulated insulin release was explored by covalently incorporating synthetic peptide sequences within a model encapsulation environment. Photopolymerized poly(ethylene glycol) (PEG) hydrogels were functionalized via the addition of acrylate-PEG-peptide conjugates to the polymer precursor solution prior to  $\beta$ -cell photoencapsulation. Individual MIN6  $\beta$ -cells were encapsulated in the presence of the laminin-derived recognition sequences, IKLLI, IKVAV, LRE, PDSGR, RGD, and YIGSR, and the collagen type I sequence, DGEA. In the absence of cell–cell and cell–matrix contacts, encapsulated MIN6  $\beta$ -cell survival diminishes within one week; however, in PEG hydrogel derivatives including the laminin sequences IKLLI and IKVAV, encapsulated  $\beta$ -cells exhibit preserved viability, reduced apoptosis, and increased insulin secretion. Interactions with the laminin sequences LRE, PDSGR, RGD, and YIGSR contribute to improved viability, but insulin release from these samples was not statistically greater than that from controls. MIN6  $\beta$ -cells were also encapsulated with various concentrations of IKLLI and IKVAV (0.05–5.0 mM), individually, and the peptide combinations IKLLI–IKVAV, IKVAV–YIGSR, and PDSGR–YIGSR to explore synergistic effects. The presented results give evidence that synthetic peptide epitopes may be useful in the design of an islet encapsulation environment that promotes cell survival and function via targeted cell–matrix interactions.

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## 1. Introduction

Encapsulation of pancreatic islets within a semi-permeable barrier is a potential transplantation therapy independent of immune suppression for type 1 diabetes. Encapsulation barrier materials are designed to allow the passage of low molecular weight nutrients and waste, as well as small proteins (e.g., insulin), while simultaneously preventing cell–cell contact between host immune cells and encapsulated insulin-producing cells and the penetration of large immune cell-secreted antibodies. In addition to the protection of encapsulated cells, encapsulation barriers must elicit a minimal host response to avoid sequestration of insulin-producing cells within a fibrous capsule upon

transplantation. Several inert barrier materials have been developed toward this application [1]. However, homogeneous, inert barriers provide little cell–matrix support to encapsulated cells, and in recent years, in vitro islet research has produced extensive evidence that cell–matrix interactions contribute to improved islet survival and function.

The goal of this work was to investigate cell–matrix interactions via adhesive peptide sequences covalently incorporated into a three-dimensional hydrogel encapsulation model. Adhesive peptide sequences offer biological matrix functionality through small molecules that are readily incorporated into synthetic hydrogel cell carriers through covalent linkers. Covalent attachment provides a stable presence of biological moieties within the encapsulation environment, while the small size of these molecules, relative to whole matrix proteins, limits interference with

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the precisely designed diffusion properties of encapsulation membranes.

Studies of islet cell–matrix interactions have employed whole islets and individual  $\beta$ -cells on both two-dimensional surfaces and in three-dimensional matrices. As purified matrix proteins and as components of cell-secreted matrices, collagen type I [2,3], collagen type IV [3,4], and laminin [3,5,6] have all been shown to improve  $\beta$ -cell survival and function. Interactions between  $\beta$ -cells and these matrix proteins are mediated by integrins and laminin-specific binding proteins on the cell surface. Often cellular receptors recognize and bind to short peptide sequences within a given matrix protein. Adhesive peptide sequences within many ECM proteins have been discovered via competitive adhesion assays and mutagenesis experiments [7]. The most extensively studied adhesive recognition sequence is the tri-peptide, RGD, originally identified within fibronectin, but also found in laminin, collagen type I, fibrinogen, vitronectin, and others [8]. Recent studies of RGD and isolated islets in solution found that RGD binding reduced apoptosis in cultured islets [9] and that incubation with RGD prior to seeding on collagen substrates reduced the adhesion of islets, indicating integrin-mediated interactions [2]. In addition to RGD, known laminin adhesion sequences include IKLLI, IKVAV, LRE, PDSGR, and YIGSR [7,10]. These sequences, as well as the collagen type I sequence DGEA, were presented to encapsulated  $\beta$ -cells via covalent incorporation into PEG hydrogels.

Covalent immobilization of adhesive peptide sequences within synthetic hydrogel networks has become an established method for providing matrix interactions to cells seeded on hydrogel surfaces and cells encapsulated within hydrogels. RGD-containing peptides tethered to monoacrylated poly(ethylene glycol) (PEG) molecules have been copolymerized into PEG gels to promote the attachment of fibroblasts [11,12], aortic smooth muscle cells [13], osteoblasts [14,15], and mesenchymal stem cells [16] for various tissue engineering applications. Using this peptide incorporation technique, PEG hydrogels were synthesized containing peptide tethers of IKLLI, IKVAV, LRE, PDSGR, YIGSR, RGD, DGEA, and combinations thereof. Individual MIN6  $\beta$ -cells were encapsulated in peptide-containing hydrogels, and cell survival, apoptosis, and glucose-stimulated insulin secretion were observed with culture time. In previous reports, MIN6  $\beta$ -cells have served as a useful model for primary insulin-producing cells in the screening of various cell–cell and cell–matrix interactions [17–19]. Previous research has also demonstrated that dispersed MIN6  $\beta$ -cells encapsulated in PEG hydrogels without cell–cell or cell–matrix contact achieve low survival rates within one week [20], making this pairing of MIN6  $\beta$ -cells and PEG hydrogels a useful screening tool for identifying cell–matrix interactions capable of preserving survival and ultimately  $\beta$ -cell function. While the use of peptides for functional cell activation has been applied in the engineering of several tissues, previous reports of

interactions between insulin-producing cells and peptide sequences are limited to the presentation of RGD to  $\beta$ -cells in two-dimensional culture [2,9]. The presented research expands the number of peptides studied in contact with insulin-producing cells within a three-dimensional model encapsulation environment.

## 2. Materials and methods

### 2.1. PEGDM synthesis, peptide synthesis, and hydrogel preparation

Poly(ethylene glycol) dimethacrylate (PEGDM) was synthesized by reacting linear PEG (Sigma, St. Louis, MO) ( $\bar{M}_n = 10,000$  Da) with methacrylic anhydride (Sigma) at a molar ratio of 1:10 via microwave irradiation [21]. Percent methacrylation was determined using  $^1\text{H}$  NMR, by comparing the area under the integrals for the vinyl resonances ( $\delta = 5.7$  ppm,  $\delta = 6.1$  ppm) to that for the PEG backbone (methylene protons,  $\delta = 4.4$  ppm). Percent methacrylation for all macromers used in this work was greater than 90%.

Hydrogels were formed from a 10 wt% solution of PEGDM in Hanks Balanced Salt Solution (HBSS, Gibco, Carlsbad, CA), with 0.025 wt% of the photoinitiator Darocur 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Ciba-Geigy, Basel, Switzerland) via exposure to 365 nm ultraviolet light at an intensity of  $\sim 7$  mW cm $^{-2}$  for 10 min. These polymerization conditions have been shown to be cytocompatible with a number of cell types, including MIN6 [20,22].

All peptide recognition sequences were synthesized using an Applied Biosystems peptide synthesizer (model 433A). Purified peptides were conjugated to mono-acrylated PEG for covalent incorporation into PEG hydrogels during photopolymerization, as previously described [10,14,15]. Briefly, peptide acrylation was achieved by reacting the N-terminus of each peptide sequence with an *N*-hydroxysuccinimidyl group on one end of mono-acrylated PEG ( $\bar{M}_n = 3400$  Da) (Acr-PEG-NHS, Nektar Therapeutics, Huntsville, AL). This reaction was performed in 0.1 M sodium bicarbonate buffer at pH 8.5 for 2 h at room temperature with a 20% molar excess of peptide. Acrylated peptides were then dialyzed in deionized water overnight using cellulose ester dialysis tubing to remove low molecular weight contaminants and excess, unreacted peptide. The final product was collected by lyophilization. Peptide-containing hydrogels were synthesized by preparing the hydrogel precursor solution as described above with the acrylated peptide dissolved at the desired concentration (0.05–5.0 mM) and the solution polymerized as previously detailed.

### 2.2. MIN6 culture and encapsulation

Murine pancreatic  $\beta$ -cells of the MIN6 cell line were maintained in RPMI 1640 (Gibco) containing 1% penicillin-streptomycin (Gibco), 0.5  $\mu\text{g}/\text{mL}$  fungizone (Gibco), and 10% fetal bovine serum (Gibco). Cells were cultured in 75 cm $^2$  treated tissue culture flasks incubated at 37 °C in humid conditions with 5% CO $_2$ . The culture medium was exchanged every 3 days.

For encapsulation, MIN6  $\beta$ -cells were suspended in peptide-containing PEGDM macromer solution. This suspension was exposed to ultraviolet light as described above. Each hydrogel sample contained approximately 50,000 MIN6  $\beta$ -cells individually suspended throughout hydrogel samples. Post-polymerization, encapsulation samples were immediately placed in culture medium in 6-well tissue culture plates. Samples were cultured on an orbital shaker set at approximately 40 rpm at 37 °C as a precaution to ensure continuous media exchange within hydrogel samples, and the culture medium was changed every 3 days.

### 2.3. Encapsulated $\beta$ -cell viability and apoptosis

A membrane integrity assay, LIVE/DEAD<sup>®</sup>, from Molecular Probes (Eugene, OR) was used to evaluate cell viability. Encapsulation samples were incubated in the LIVE/DEAD<sup>®</sup> staining solution for 10 min at 37 °C, and then rinsed in phosphate buffered saline, pH 7.4, (Gibco) prior to imaging. Live cells are observed when cytoplasmic esterase activity results in the reduction and green fluorescence of calcein AM, and dead cells are identified by the red fluorescence of ethidium homodimer bound to exposed DNA.

Apoptotic cells were identified within encapsulation samples using the Vybrant<sup>®</sup> Apoptosis Assay Kit #3 (Molecular Probes). Hydrogel samples were rinsed with the supplied binding buffer, and incubated in annexin V staining solution for 15 min at room temperature. Samples were rinsed in binding buffer again prior to imaging. Cell-impermeant, FITC-conjugated annexin V binds to phosphatidylserine, usually located on the inner leaflet of the plasma membrane, but translocated to the outer leaflet during the early stages of apoptosis. Necrotic cells were identified by propidium iodide staining to exposed DNA. The compromised cell membrane of necrotic cells allows for annexin V staining of phosphatidylserine as well as propidium iodide binding to DNA, resulting in double staining of necrotic cells. The Vybrant<sup>®</sup> assay does not label non-apoptotic, live cells.

Encapsulated  $\beta$ -cells stained with either fluorescent assay were imaged using a Zeiss LSM 5 Pascal confocal microscope. Image z-stacks, 100–200  $\mu$ m thick, were acquired at 5  $\mu$ m intervals and then projected into single plane images. Stained cells were manually counted in images ( $n \geq 3$ ) of each sample. To quantify the viability staining results, the percentages of live cells relative to the total number of stained live and dead cells were calculated, and to quantify the apoptosis staining results, the percentages of apoptotic cells relative to stained apoptotic and necrotic cells were calculated.

### 2.4. Encapsulated $\beta$ -cell glucose-stimulated insulin secretion

At specified time points, encapsulation samples were removed from culture medium for glucose challenge. Samples were first placed in a low glucose concentration solution (1.1 mM) for 45 min, followed by incubation in a high glucose concentration buffer (16.7 mM) for 1 h [23]. The high glucose buffer solutions were collected for insulin measurement by ELISA. The insulin sandwich ELISA utilized two monoclonal insulin antibodies (Bioscience International, Saco, ME), streptavidin horse radish peroxidase (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and TMB ultra as the HRP substrate (Pierce, Rockford, IL).

The ATP content of each encapsulation sample was measured using the CellTiter-Glo<sup>®</sup> Luminescent Cell Viability Assay (Promega, Madison, WI). Encapsulated cell samples were incubated in 0.5 ml RPMI without phenol red combined with 0.5 ml CellTiter-Glo reagent for 30 min on an orbital shaker (~200 rpm). Sample solutions were transferred in 200  $\mu$ l aliquots to opaque 96-well plates, and the luminescence of each sample measured by a microplate reader (Perkin-Elmer Wallac Victor<sup>2</sup>, 1420 Multilabel Counter). Measured insulin release from each sample was normalized by the ATP content of the respective sample. ATP was selected instead of DNA or total protein, because the smaller molecular size of ATP affords simple extraction from hydrogel samples, eliminating the error introduced by the physical destruction of the gels required for DNA or protein measurement. Control experiments confirmed that sample ATP content corresponds with sample cell number and DNA content (data not shown).

### 2.5. Statistical analysis

All results are presented as average values  $\pm$  standard deviations. Statistical significance between experimental conditions in each study was determined using a two-tailed, unpaired Student's *t*-test. Differences between conditions were considered statistically significant when *P* was less than 0.05.

## 3. Results

MIN6  $\beta$ -cells encapsulated in unmodified PEG hydrogels in the absence of cell–cell and cell–matrix interactions exhibit reduced cell viability within the 10 days of culture ( $17 \pm 15\%$ , Fig. 1). We hypothesize that  $\beta$ -cell viability will be preserved in a hydrogel encapsulation environment functionalized with critical matrix contacts, and further, that  $\beta$ -cell function will depend on the presence of specific ligand interactions. As two-dimensional substrates for  $\beta$ -cell adhesion [2,3,5,6] and as a three-dimensional culture environment [3], collagen type I and laminin have been identified as matrix components that may provide critical interactions for improving  $\beta$ -cell survival and function. The adhesive peptide sequences listed in Table 1 are found within these ECM proteins and were selected for encapsulation with MIN6  $\beta$ -cells. For each integrin binding target listed in Table 1, at least one subunit has been found to be expressed by pancreatic  $\beta$ -cells [24].

### 3.1. Encapsulated $\beta$ -cell survival as a function of peptide sequences

MIN6  $\beta$ -cells were encapsulated in functionalized PEG hydrogels containing individual peptides, each at a concentration of 5 mM. The viability of  $\beta$ -cells within peptide-containing hydrogel samples was observed after 1, 5, and 10 days with the LIVE/DEAD<sup>®</sup> cytotoxicity assay. The percentage of live cells in each sample with time is presented in Fig. 1. In control samples, containing no peptide functionality, encapsulated  $\beta$ -cells exhibit significantly decreased survival after five and ten days,  $60 \pm 7\%$  and  $17 \pm 15\%$ , respectively. Encapsulated MIN6  $\beta$ -cell survivability is maintained in hydrogels containing the laminin-derived sequences, IKLLI and IKVAV (Fig. 1A). In the presence of the adhesive sequences LRE, PDSGR, and YIGSR, also found within laminin,  $\beta$ -cell survival is improved relative to control samples (Fig. 1B), but the percentage of observed live cells is slightly less than that in samples containing IKLLI and IKVAV. The survival of  $\beta$ -cells encapsulated with the RGD peptide found in most matrix proteins, including laminin, is similar to that in control samples after 5 days in culture, but remains at approximately 60% after 10 days. The collagen-derived sequence DGEA does not improve encapsulated  $\beta$ -cell survival beyond that of control samples over the course of the experiment (Fig. 1C).

If our hypothesis that incorporated peptides can improve encapsulated cell survival by providing critical matrix interactions is true, we would expect cell death in the absence of matrix contacts to be the result of apoptosis, or more specifically anoikis. To test this, the occurrence of early stage apoptosis among cells encapsulated with each peptide was explored using fluorescently-labeled annexin V staining. Apoptotic and necrotic cells were identified, and the percentage of cells stained positive for apoptosis in each sample was calculated. As displayed in Fig. 2, samples

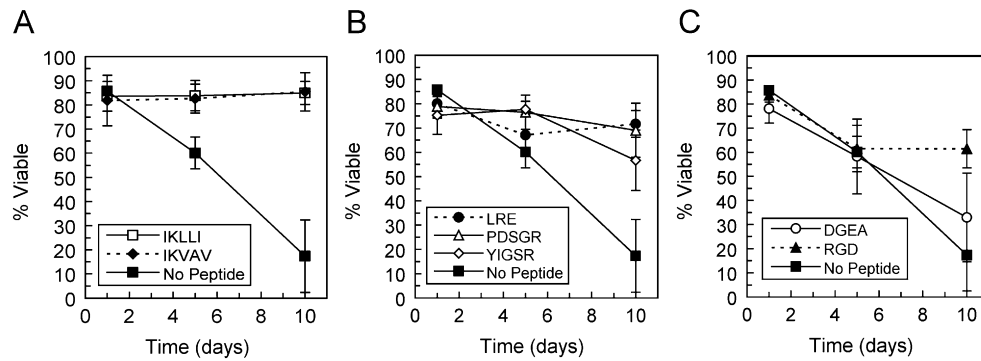


Fig. 1. Percentage of live  $\beta$ -cells encapsulated in peptide functionalized PEG hydrogels as a function of culture time. Fluorescently stained live and dead cells were imaged within hydrogel samples containing the adhesive sequences, (A) IKLLI and IKVAV, (B) LRE, PDSGR, and YIGSR, and (C) RGD and DGEA. The percentage of viable cells observed with each condition was calculated and compared to that in control gels with no cell–matrix interactions.

Table 1

Adhesive peptide sequences incorporated into  $\beta$ -cell-laden hydrogels and investigated for their effects on encapsulated MIN6  $\beta$ -cell survival and function [7,10,24,25]

Peptide sequence	ECM protein/localization	Known receptor (s)
IKLLI	Laminin $\alpha$ 1 chain	$\alpha$ <sub>3</sub> $\beta$ <sub>1</sub> and cell surface heparin
IKVAV	Laminin $\alpha$ 1 chain	110 kDa laminin receptor protein
LRE	Laminin $\gamma$ 1 chain	Unknown
PDSGR	Laminin $\beta$ 1 chain	Unknown
RGD	Laminin $\alpha$ 1 chain	$\alpha$ <sub>v</sub> $\beta$ <sub>3</sub> , $\alpha$ <sub>5</sub> $\beta$ <sub>1</sub>
YIGSR	Laminin $\beta$ 1 chain	67 kDa laminin receptor protein
DGEA	Type I collagen $\alpha$ 1(I)-CB3 fragment	$\alpha$ <sub>2</sub> $\beta$ <sub>1</sub>

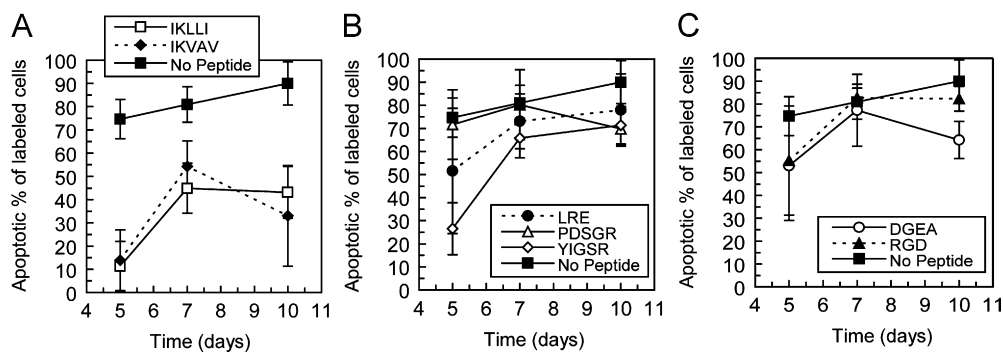


Fig. 2. Percentage of encapsulated  $\beta$ -cells positively stained for apoptosis in peptide derivatized hydrogels with culture time. Apoptotic cells and necrotic cells were identified by staining with annexin V and propidium iodide, respectively, and the relative percentages of apoptotic cells within gels incorporating (A) IKLLI and IKVAV, (B) LRE, PDSGR, and YIGSR, and (C) RGD and DGEA were calculated. Viable  $\beta$ -cells are not represented in this data.

encapsulated with the sequences that most preserved  $\beta$ -cell survival, IKLLI and IKVAV, also exhibit the lowest levels of apoptosis (Fig. 2A). Reduced apoptosis was also observed for encapsulated  $\beta$ -cells exposed to the laminin sequence YIGSR. With each of the remaining sequences, apoptosis was not statistically different from controls over the experimental time course. While the viability results in Fig. 1 represent the percentage of live cells relative to the number of live and dead cells visualized with the LIVE/DEAD<sup>®</sup> staining kit, the results in Fig. 2 represent the percentage of apoptotic cells observed when both apoptotic and necrotic cells were visualized with the Vybrant<sup>®</sup>

apoptosis staining kit. Because live, healthy cells are not observed with the apoptosis stain, the live cell population is not represented in the data in Fig. 2.

### 3.2. Glucose-stimulated insulin secretion as a function of peptide sequences

Individual MIN6  $\beta$ -cells encapsulated in the presence of the same adhesive peptide sequences from the previous survival studies were stimulated with glucose (16.7 mM), and the amount of cell-secreted insulin measured by ELISA. To account for differences in insulin secretion

due to differences in the number of cells in each samples, the amount of secreted insulin was normalized by the amount of ATP for each sample. Insulin release data for  $\beta$ -cells encapsulated with peptide sequences is displayed in Fig. 3, organized in the same order as the viability and apoptosis results. MIN6  $\beta$ -cells encapsulated in the presence of IKLLI and IKVAV secrete significantly higher amounts of insulin relative to  $\beta$ -cells encapsulated without matrix contacts and those in gels functionalized with the other tested peptide sequences. Normalization by ATP removes the influence of cell death over the experimental time course, such that insulin release values remain relatively constant even for conditions in which cell death is occurring.

### 3.3. Effects of peptide concentration on $\beta$ -cell survival and function: IKLLI and IKVAV

MIN6  $\beta$ -cells were encapsulated with 0.05, 0.5, and 5 mM concentrations of IKLLI and IKVAV, individually, with two experimental goals: first, to investigate the effects of peptide concentration on the peptide's influence on encapsulated  $\beta$ -cell function, and second, to establish an active range of peptide concentrations that do not affect the influence of the specific cell–matrix interaction. The latter information may be useful in the design of a clinically applicable encapsulation environment. With peptide concentrations between 0.05 and 5.0 mM, no statistically significant differences were observed in encapsulated cell viability or insulin release over the time course of the experiments for either IKLLI or IKVAV (Figs. 4 and 5). Interestingly, with the lowest concentration of IKVAV, 0.05 mM, average values of insulin release were less than control values at each time point, but this trend is not statistically significant at days 1 or 10.

### 3.4. Synergistic effects of peptide combinations

Single adhesive peptides offer simplistic models of whole matrix proteins. Because cell–matrix signaling may involve an array of binding events even with a single matrix protein, the effects of combinations of recognition

sequences on encapsulated  $\beta$ -cells were investigated. MIN6  $\beta$ -cells were encapsulated in hydrogels including the two peptides identified in this study to improve glucose-stimulated insulin secretion, IKLLI and IKVAV, at a total peptide concentration of 5 mM (2.5 mM each). Additionally,  $\beta$ -cells were encapsulated with two known synergistic laminin-derived sequence pairs found in the literature, IKVAV and YIGSR [26] and PDSGR and YIGSR [27,28], also at a total peptide concentration of 5 mM.

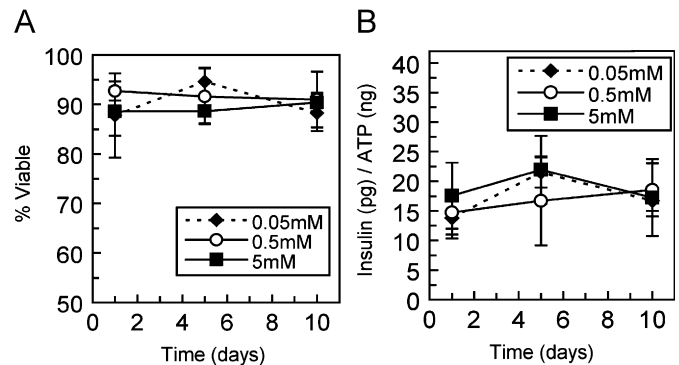


Fig. 4. (A) Viability and (B) glucose-stimulated insulin release of  $\beta$ -cells encapsulated in hydrogels containing varying concentrations (0.05 mM, 0.5 mM, or 5 mM) of the adhesive sequence IKLLI as a function of time.

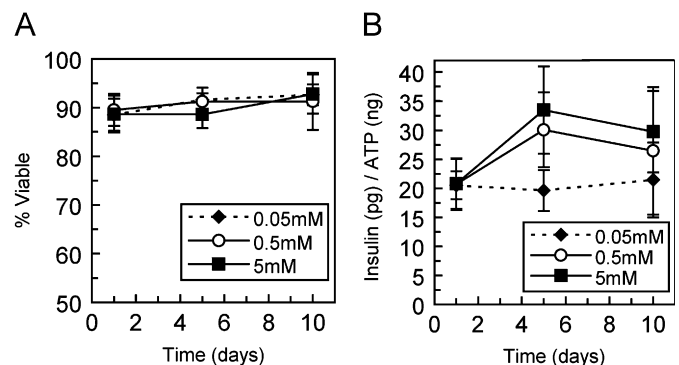


Fig. 5. (A) Viability and (B) glucose-stimulated insulin release of  $\beta$ -cells encapsulated in hydrogels containing varying concentrations (0.05, 0.5, and 5.0 mM) of the adhesive sequence IKVAV as a function of time.

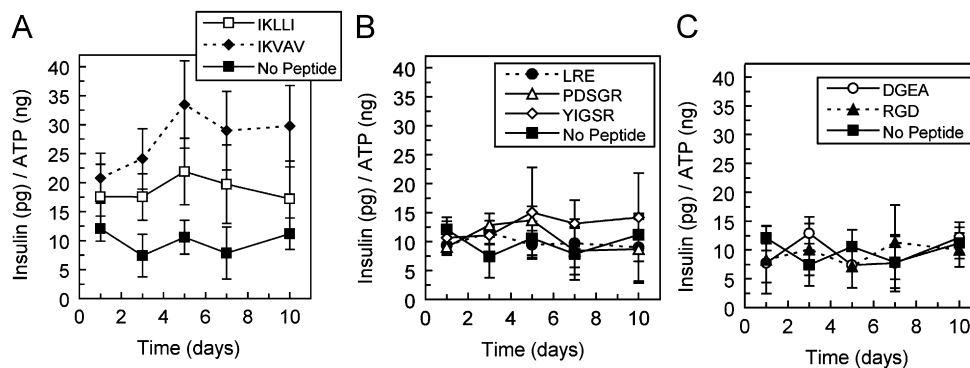


Fig. 3. Glucose-stimulated insulin secretion of  $\beta$ -cells encapsulated in peptide-functionalized hydrogels normalized by sample ATP content as a function of time.  $\beta$ -cells were encapsulated in gels containing 5 mM (A) IKLLI or IKVAV, (B) LRE, PDSGR, YIGSR, and (C) RGD, and DGEA, individually.

In samples with the two-peptide pairs containing IKVAV, glucose-stimulated insulin secretion was followed to test the influence of peptide interactions on  $\beta$ -cell function. Viability was not investigated because the presence of IKVAV alone preserves cell survival. Insulin release from  $\beta$ -cells encapsulated with IKLLI and IKVAV was similar to that from cells encapsulated with only IKVAV at each experimental time point (Fig. 6A). The average insulin release values for samples containing IKVAV and YIGSR were greater than those for IKVAV alone, however, they were not statistically significant at days 5 and 10 (Fig. 6B).

For the peptide pairing of PDSGR with YIGSR, both viability and insulin release were measured. As observed in Fig. 7A, the presence of PDSGR and YIGSR individually improved encapsulated cell survival, but after 10 days encapsulated  $\beta$ -cell viability is greater in samples containing both peptides. Glucose-stimulated insulin release is also greater from samples containing both peptides, relative to each peptide individually (Fig. 7B). The synergistic effect of

the laminin sequences PDSGR and YIGSR is evident in these results.

#### 4. Discussion

Contact with extracellular matrix has previously been shown to promote islet survival and function, specifically laminin and collagen types I and IV in both two-dimensional culture on matrix coated surfaces [2,4–6] and three-dimensional culture within collagen type I gels [3]. In this work, we investigated cell–matrix contacts in three dimensions using short adhesive recognition peptides covalently immobilized within a model hydrogel encapsulation environment. Five laminin-derived sequences, one sequence from collagen type I, and the RGD peptide found in most ECM proteins including laminin were covalently incorporated throughout PEG hydrogels used for  $\beta$ -cell encapsulation.

PEG hydrogels are hydrophilic and resistant to protein adsorption creating an encapsulation environment void of matrix signaling. Dispersed MIN6  $\beta$ -cells encapsulated in unmodified PEG hydrogels with no cell–cell contact and no cell–matrix interactions exhibit diminished viability within 5 days of encapsulated culture ( $17 \pm 15\%$  after 10 days, Fig. 1). When encapsulated in PEG gels functionalized with peptide the binding sequences IKLLI, IKVAV, LRE, PDSGR, RGD, and YIGSR,  $\beta$ -cell viability is greater than in control samples after 10 days, but to varying degrees. Matrix contacts influence many aspects of cell behavior, including survival. Cell–matrix signaling can prevent apoptosis, specifically anoikis. Anoikis, derived from the Greek work for homelessness, is the programmed cell death that occurs when cells do not receive proper signaling from their surrounding matrix [29]. Anoikis is critical in development and tissue homeostasis, and resistance to anoikis has been implicated in the malignancy of several types of cancer. Cell–matrix signaling influences cell survival via protein kinase signaling pathways involving the integrin signaling molecules FAK (focal adhesion kinase), Shc, and ILK (integrin-linked kinase) [30]. In studies with  $\beta$ -cells specifically, culture on a laminin-5 rich cell-secreted matrix led to higher phosphorylation levels of FAK [31]. Cell–matrix interactions directly contribute to changes in cytoskeletal organization, and several signaling molecules and apoptosis regulators are associated with the cytoskeleton and may regulate anoikis by sensing cytoskeletal integrity [30]. In the design of an islet encapsulation barrier, anoikis may be prevented by introducing critical cell–matrix contacts within the encapsulation microenvironment.

To test the role of anoikis in the cell viability observed in gels containing peptide sequences and the cell death in gels void of matrix interactions, apoptosis within encapsulation samples was observed by staining with fluorescently-labeled annexin V. Interestingly the conditions that resulted in the highest live cell percentages (IKLLI and IKVAV) also contributed to the lowest apoptotic percentages, indicating

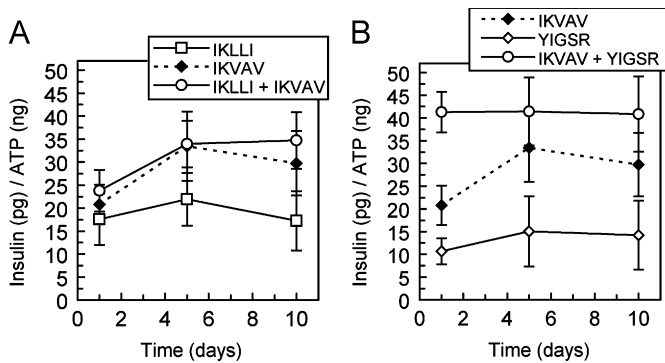


Fig. 6. Glucose-stimulated insulin secretion of  $\beta$ -cells encapsulated in PEG hydrogels containing equimolar concentrations (2.5 mM each, 5.0 mM total) of the peptide pairs (A) IKLLI and IKVAV and (B) IKVAV and YIGSR with culture time. Insulin measurements were normalized by the ATP content of each sample and plotted with released insulin values from  $\beta$ -cells encapsulated with individual peptides.

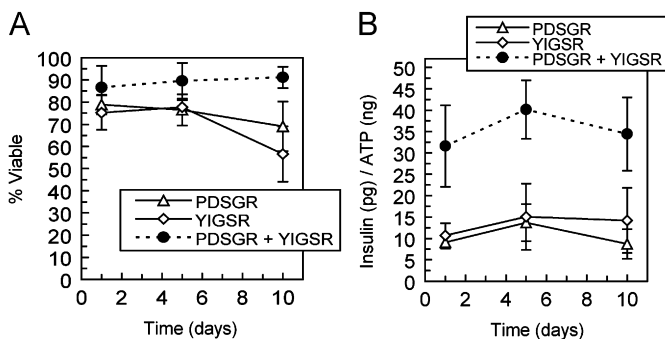


Fig. 7. (A) Viability and (B) glucose-stimulated insulin secretion of  $\beta$ -cells encapsulated in PEG hydrogels containing equimolar concentrations (2.5 mM each, 5.0 mM total) of the peptide pair PDSGR and YIGSR with culture time. Live cell percentages and insulin secretion normalized by ATP content were graphed along with the data from samples containing the individual peptides.

that the matrix interactions provided by covalently incorporated adhesive peptides promote cell survival by preventing apoptosis, possibly anoikis.

Just as cell–matrix contacts lead to signaling events that control cell survival, they can influence other intracellular events such as glucose-stimulated insulin release in the case of the pancreatic  $\beta$ -cell. Increased  $\beta$ -cell insulin release was observed in samples encapsulated with the laminin-derived peptide sequences IKLLI and IKVAV, also shown to promote  $\beta$ -cell survival. IKLLI is known to bind integrin  $\alpha_3\beta_1$  and cell surface heparan sulfate [24], while IKVAV associates with a 110 kDa laminin receptor protein [32]. While the intracellular results of these specific binding events are not fully understood, these results agree with previous work that demonstrated that attachment to laminin in two-dimensional culture increases glucose-stimulated insulin secretion from  $\beta$ -cells [5,6] and suggest that the observed increase is related to  $\beta$ -cell interactions with the recognition sequences IKLLI and IKVAV. Changes in the  $\beta$ -cell cytoskeleton are thought to influence glucose-stimulated insulin secretion in addition to survival. F-actin remodeling has been shown to influence insulin release from MIN6  $\beta$ -cells and primary rat  $\beta$ -cells, specifically through association with the t-SNARE complex responsible for insulin granule plasma membrane docking and fusion [33]. Also, Nuclear Factor- $\kappa$ B (NF- $\kappa$ B) is activated in  $\beta$ -cells attached to matrix substrates, and this activation is proposed to contribute to changes in actin organization in the interior of  $\beta$ -cells, as well as the expression of genes required for proper glucose-stimulated insulin secretion [34]. Interesting future studies would include examining actin organization and intracellular signaling of primary  $\beta$ -cells in these highly controlled three-dimensional microenvironments with varying peptide functionalities.

Toward the goal of identifying an active concentration range for adhesive peptide sequences immobilized within a hydrogel encapsulation environment, MIN6  $\beta$ -cells were encapsulated with the individual peptides IKLLI and IKVAV over a concentration range spanning two orders of magnitude (0.05–5 mM). With either peptide, no differences in  $\beta$ -cell viability or insulin release were observed at any concentration. These results suggest that even at the lowest peptide concentration, sufficient signaling is achieved and additional peptide is excess. Little is known concerning the concentrations of individual integrins and laminin-specific receptors on the  $\beta$ -cell surface, limiting the design of an encapsulation environment with a complementary adhesive peptide concentration. These results provide a concentration range over which the peptides IKLLI and IKVAV can be incorporated into an encapsulation environment without altering the influence of matrix signaling on encapsulated cell behavior.

MIN6  $\beta$ -cells were encapsulated in PEG hydrogels that incorporated adhesive peptide pairs: the two peptides identified in this work to increase  $\beta$ -cell insulin release, IKLLI and IKVAV, as well as two laminin-derived pairs

shown to be synergistic in the literature, IKVAV and YIGSR [26], and PDSGR and YIGSR [27,28]. In the presence of either IKVAV-containing pair, glucose-stimulated insulin secretion was not statistically greater than that from samples presented with IKVAV alone. These results suggest that the signaling events that occur upon interaction between the 110 kDa laminin receptor and IKVAV are not further augmented by either the known binding of integrin  $\alpha_3\beta_1$  to IKLLI or the established interaction between the 67 kDa laminin receptor and YIGSR. However, cells encapsulated with a combination of PDSGR and YIGSR exhibited greater viability and increased insulin release relative to samples containing the individual peptides. This peptide combination was previously shown to promote attachment of corneal epithelial cells to poly(dimethyl siloxane) surfaces [27], as well as the attachment and migration of HT-1080 cells from a human fibrosarcoma [28]. PDSGR and YIGSR are in close proximity in the  $\beta_1$  chain of laminin [24], and while the target receptors for PDSGR are unknown, the proximity of these peptides may suggest a synergistic role in the conformation of native laminin that provides a higher affinity cell–matrix interaction [28].

## 5. Conclusions

In summary, hydrogels functionalized with short adhesive peptides provided cell–matrix interactions to encapsulated  $\beta$ -cells. Specific recognition sequences from laminin, IKLLI and IKVAV, promoted  $\beta$ -cell viability and insulin secretion when immobilized at concentrations ranging from 0.05 to 5.0 mM.  $\beta$ -cells encapsulated in gels that incorporated both PDSGR and YIGSR were more viable and secreted more insulin than cells encapsulated with PDSGR or YIGSR individually. These results identify candidate recognition sequences for consideration in the design of future islet encapsulation barrier materials, as well as suggest a highly controlled three-dimensional culture environment for fundamental studies of the role of extracellular signals on  $\beta$ -cell function. Additionally, the  $\beta$ -cell-peptide interactions investigated herein could be applied to improve encapsulated cell survival and function in both micro- and macro-encapsulation devices fabricated from any immunisolating polymer barrier.

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## Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biomaterials.2007.03.005

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