Compliant 3D Microenvironment Improves β -Cell Cluster Insulin Expression Through Mechanosensing and β -Catenin Signaling

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Type 1 diabetes is chronic disease with numerous complications and currently no cure. Tissue engineering strategies have shown promise in providing a therapeutic solution, but maintenance of islet function and survival within these therapies represents a formidable challenge. The islet microenvironment may hold the key for proper islet maintenance. To elucidate the microenvironmental conditions necessary for improved islet function and survival, three-dimensional (3D) polyacrylamide cell scaffolds were fabricated with stiffnesses of 0.1 and 10 kPa to regulate the spatial and mechanical control of biosignals. Specifically, we show a significant increase in insulin mRNA expression of 3D primary mouse islet-derived and Min6-derived β -cell clusters grown on compliant 0.1 kPa scaffolds. Moreover, these compliant 0.1 kPa scaffolds also increase glucose sensitivity in Min6-derived β -cell clusters as demonstrated by the increased glucose stimulation index. Our data suggest that stiffness-specific insulin processing is regulated through the myosin light chain kinase (MLCK) and Rho-associated protein kinase (ROCK) mechanosensing pathways. Additionally, β -catenin is required for regulation of stiffness-dependent insulin expression. Through activation or inhibition of β -catenin signaling, reversible control of insulin expression is achieved on the compliant 0.1 kPa and overly stiff 10 kPa substrates. Understanding the role of the microenvironment on islet function can enhance the therapeutic approaches necessary to treat diabetes for improving insulin sensitivity and response.

Introduction

TYPE 1 DIABETES is a disease characterized by the selective destruction of β -cells in the islets of Langerhans, responsible for maintaining glucose and insulin homeostasis. This results in a deregulation of insulin and glucose that requires constant monitoring. The most common treatment for type 1 diabetes is insulin therapy, by insulin injection, implantation of a subcutaneous insulin pump, or wearable infusion pump. The clinical cell-based approaches for treating diabetes, whole pancreas transplantation and islet transplantation, have great potential for future diabetes treatments.^{1,2} However, these treatments have not achieved long-term success. Multiple donors are necessary for each transplant, due to high loss of islet function and necrosis post-transplantation. There have been a series of studies that utilize alternative cell sources, such as stem cells, to overcome the challenge of limited cell source.³ However, much of this loss of function is due

to disruption of the native cellular architecture and microenvironment and still occurs with a surplus of cells. Understanding the role of the microenvironment might help overcome the challenges of limited cell survival.

In the pancreas, islets experience intricate cell–cell interactions that facilitate insulin response and viability.^{4,5} In native islets, cell–cell communication is essential to provide low-insulin release in periods of starvation and sufficient amounts of insulin after food intake. To produce large concentrations of insulin, β -cells rely on multicellular processes to synergistically increase insulin production beyond what can be produced by an individual cell. Even paired β -cells secrete more than twice the amount of insulin than a single cell.⁶ Previous work has demonstrated that insulin production per cell increases with β -cell architecture and that β -cell survival is improved in large clusters.^{7–9}

Microenvironment stiffness is known to play a critical role in cellular response and differentiation, in a variety of

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systems.^{10–13} Specifically, changes in microenvironment stiffness affect intercellular tension and accordingly regulate cellular and nuclear morphology through many different mechanotransduction mechanisms.^{14,15} Matrix interactions that closely mimic the native islet microenvironment in architecture and stiffness could improve insulin output or islet viability.

Although the architecture and size of islets has been shown to be critically important, little is known about the effect of microenvironmental cues, such as stiffness, on islet function and survival. Native mouse and human islets share common architectural features; however, they slightly differ in geometry, where mouse islets are more spherical and human islets are more oblong. This difference in structure reflects the body's adjustment to the increased demand rather than a species difference.¹⁶ In mature intact islets, interactions with the natural extracellular matrix (ECM) or synthetic matrix regulate survival, insulin secretion, and proliferation, and aid in the preservation and restoration of islet morphology.^{17,18} β -Cells *in vivo* are surrounded by a rich network of soft tissue (0.1–1 kPa) and vasculature (8–17 kPa), the two main physical interactions the islets experience.^{19,20} However, little is known about the biochemical signaling mechanisms connecting these biophysical cues to viability and insulin processing. Extracellular-signaling-related kinase (ERK) signaling through the Ras-Raf-MEK-ERK signaling pathway is a well-established mechanosensing pathway. Stiff ECM microenvironments increase the formation of complexes between focal adhesion kinase (FAK) and Src and Shc and the mitogen activated protein kinase (MAPK) pathway member Grb2.^{21,22} This complex then enhances FAK-dependent activation of ERK1/2.^{23,24} The cell-matrix interface, which is established by the contractile response to ECM stiffness, directly regulates classical pathways of proliferation for Ras-Raf-MEK-ERK pathway. Another mechanosensing pathway that regulates cell behavior is myosin light chain kinase (MLCK) and Rho/Rho-associated protein kinase (ROCK) kinase.²⁵ Myosin II is believed to be involved in the generation of the contractile force for cell migration.²⁶ The activity of myosin II is mainly controlled by its light chain (MLC) phosphorylation, which is regulated by two classes of enzymes, MLCK and myosin phosphatase (Rho/ROCK).^{27–29} MLCK and Rho/ROCK kinase appear to be two major kinases that phosphorylate MLC.³⁰

The main cell-cell adhesion protein E-cadherin maintains β -cell cluster formation. The adapter protein connecting E-cadherin to the cellular cytoskeleton is β -catenin, a transcription factor for Wnt signaling. Recently, cell-cell adhesion molecules have been recognized as mechanosensors.³¹ Interestingly, β -catenin signaling also is a well-known regulator of insulin sensitivity, possibly linking mechanotransduction to insulin processing.^{32,33}

This work utilizes standard photolithographic techniques to develop a series of polyacrylamide (PA) microwell scaffolds at different stiffnesses to explore the role of stiffness on insulin processing. By understanding the optimal microenvironmental cues necessary for survival and insulin response, we strive to improve long-term islet viability, which could provide a recipient with a dynamic glucose-responsive source of insulin.

Materials and Methods

PA microwell scaffold fabrication

PA microwells were fabricated using a two-step process. First, a micropatterned silicon wafer was created using standard photolithography techniques. A layer of SU-8 2035-negative photoresist (Microchem, Newton, MA) was spun at 1400 rpm for 30 s onto a 3" silicon wafer using a PMW32 spin coater (Headway Research, Garland, TX) and prebaked at 65°C for 5 min and then at 95°C for 15 min. An array of 100- μ m microwells at 100- μ m spacing were patterned into the photoresist using a photomask and exposing the photoresist to UV light for 45 s at an intensity of 9 mW·cm⁻² using a Karl Suss MJB 3 mask aligner (SUSS MicroTech, Inc., Waterbury Center, VT). The SU-8 posts were then postbaked at 65°C for 5 min and then at 95°C for 15 min and developed with SU-8 developer (Microchem) for 10 min under agitation. The wafers were then hard baked at 200°C for 15 min. Second, a PA solution of 5 mL for the 0.1 kPa substrate or 8.75 mL PA for the 10 kPa substrate (Biorad, Hercules, CA) with 4.9 or 1.24 mL water, respectively, was made. A 10% w/v solution of APS (Sigma-Aldrich, St Louis, MO) was created in 100 μ L of water and added to the PA solution followed by 10 μ L of TEMED (Biorad). The solution was gently inverted and poured over the SU-8-templated wafer. The PA was allowed to polymerize for 1 h and then removed from the wafer and allowed to soak in phosphate-buffered saline (PBS) for 5 days.

Hydrogel stiffness measurements

Stiffness measurements of the hydrogel were taken by microindentation. Hydrated PA was attached to a glass slide and mounted onto the stage of an Asylum MFP-3D atomic force microscope (Asylum Research, Goleta, CA) coupled to a Nikon TE200U microscope. Force measurements were obtained using an Olympus silicon nitride cantilevers with a spring constant of 2.0 N/m. Displacement versus position data were converted to force versus indentation based on the contact position, which was fitted to a Hertzian model to obtain Young's moduli. Force loading was applied to at least three samples using a 10 $\times$ 10 indentation matrix with a 10- μ m borosilicate tip at a 0.6 N/m spring force.

Cell culture

Whole islets were isolated from Black 6 lab mice by the Islet Core at UCSF using standard islet isolating techniques. Islets were dissociated with 0.05% trypsin in PBS (Cell Culture Facility, San Francisco, CA) to a single-cell suspension. The Min6, a mouse β -cell line, was cultured using standard cell culture techniques in DMEM high-glucose media (Cell Culture Facility) with 10% fetal bovine serum and 1% penicillin/streptomycin (Cell Culture Facility). Cell cultures were maintained in a humidity-controlled 5% CO₂ incubator at 37°C. The Min6 cell line was chosen because it exhibits glucose metabolism and glucose-stimulated insulin secretion similar to normal islets. Cells were treated with the MEK1 inhibitor PD98059 at 10 μ M (Cell Signaling, Danvers, MA) or deoxycholic acid (DCA), a β -catenin activator (Sigma-Aldrich), at 10 μ M for 1 h prior to sample collection. Cells were treated with the β -catenin inhibitor IRW-1 (Sigma-Aldrich) at 10 μ M, ROCK inhibitor Y27632

(Calbiochem, Darmstadt, Germany) at 10 μ M, or the MLCK inhibitor ML-7 (Calbiochem) at 10 μ M during seeding and samples were collected after 24 h of culture.

Glucose stimulation insulin secretion assays

Before seeding, cells were trypsinized and resuspended in a complete medium. Cells were then seeded into the PA scaffold at 10⁶ cells per mL and cultured for 24, 48, or 72 h. After which cells underwent a glucose stimulation insulin secretion (GSIS) time course, cells were cultured in 5 mM of glucose-balanced HEPES buffer for 60 min and then stimulated at 15 mM glucose-balanced HEPES buffer for 15 and 60 min. The media were prepared as previously described.³⁴ The conditioned media from the Min6 and primary islets cultured on the 0.1 kPa microwells and the 10 kPa microwells were harvested after each GSIS stimulation step. Insulin production was measured with an ELISA (Mercodia, Winston Salem, NC) according to the manufacturer's instructions. Analysis of variance (ANOVA) followed by a Tukey test was used to evaluate statistical significance.

Fluorescent microscopy

Cells were fixed using 4% paraformaldehyde (Fisher Scientific, Waltham, MA) for 30 min at room temperature and permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) for 30 min. After three washes in PBS, cells were blocked with 5% goat serum for 1 h at room temperature. Primary antibodies ERK2 (D-2; Santa Cruz) and pERK 1/2 (12D4; Santa Cruz) were added at a 1:100 dilution. Samples were incubated in primary antibody at 4°C overnight. Samples

were incubated with secondary antibodies for 1 h at room temperature. F-actin and nuclei were stained with Fluor 488 phalloidin (Life Technologies, Eugene, OR) and DAPI (Life Technologies), respectively. Cells were imaged using a spinning-disk confocal (Nikon Eclipse Ti-E motorized inverted microscope with Yokogawa CS22 Spinning Disk Confocal from Solamere Technology Group, Acquisition with Micro-Manager 1.4) and analyzed using ImageJ (National Institutes of Health, Bethesda, MD). Nuclear circularity was measured in four clusters where 25 cells from each cluster were measured; this measurement was done in technical triplicate. The equation used for circularity was $C = (4\pi A)/(p^2)$, where A is the area of the nucleus and p is the perimeter. An ANOVA followed by a Tukey test was used to evaluate statistical significance.

Quantitative real-time polymerase chain reaction

mRNA was isolated using RNeasy column purification (Qiagen, Valencia, CA). The concentration and purity of RNA were determined using a Nano Drop ND-1000 Spectrophotometer (Thermo Scientific, Waltham, MA). cDNA was synthesized with iScript cDNA synthesis kit (Biorad). mRNA expression was evaluated using an Applied Biosystems Viia7 real-time polymerase chain reaction system. Forward and reverse primers and SYBR green fast mix (Life Technologies) were used to amplify each cDNA of interest. A minimum of three biological triplicates and one technical triplicate were run for each treatment and normalized to the housekeeping gene L19 or a geometric mean of GAPDH and TBP. An ANOVA followed by a Tukey test was used to evaluate statistical significance.

FIG. 1. Polyacrylamide (PA) can be used to construct primary islet-derived and Min6-derived three-dimensional (3D) β -cell clusters. (A) Schematic describes PA fabrication using standard photolithography to create. (B) Atomic force microscopy was used to measure substrate stiffness. (C) PA microwells after seeding with β -cells and (D, E) culturing for 24 h, 3D β -cell clusters are formed. ($p < 0.001$). Color images available online at www.liebertpub.com/tea

FIG. 2. β -Cell insulin expression is regulated by microwell stiffness. **(A)** Min6-derived and **(B)** primary islet-derived clusters have increased insulin expression in 0.1 kPa microwells (* $p < 0.05$).

Results

To study the effect of microenvironment stiffness on islet function, standard photolithography was used to create PA square microwells of 100 μm by 100 μm length by width with 60- μm depth (Fig. 1A). The length and width of the microwells were controlled by the mask, while the depth of the microwells was controlled by the spin rate and subsequent thickness of the photoresist. Microwells with the just mentioned dimensions allowed for primary islet-derived and Min6-derived three-dimensional (3D) β -cell clusters <100 μm in diameter to be created (Fig. 1D, E). β -Cell clusters of 100 μm in diameter are small enough that oxygen permeability and nutrient exchange are not be significantly affected.^{35,36} Further, this cluster size maximizes insulin expression and secretion per volume (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/tea).

The mechanical properties of PA hydrogels were altered by varying the PA concentration. The elastic modulus of the PA hydrogels was measured by atomic force microscopy (Fig. 1B). The PA stiffness was chosen for this study to

replicate the two common stiffnesses found in the pancreas ECM: 0.1 kPa (compliant) that models the pancreatic ECM and 10 kPa (stiff) that models the surrounding vasculature.

To examine the effect of substrate stiffness on β -cell function, insulin expression and secretion were measured on both compliant and stiff, flat and microwelled substrates (Fig. 2). Microwelled substrates facilitated spherical cluster formation (Fig. 1C–E) known to improve insulin processing. However, only islet-derived and Min6-derived β -cell clusters cultured on the compliant microwelled substrates increased insulin expression significantly above control levels (Fig. 2A, B). This specific response demonstrates that insulin expression is increased not only by cluster formation, but also in response to stiffness of the surrounding microenvironment.

For long-term insulin secretion studies, a GSIS was performed that replicates glucose levels during starvation and after eating to stimulate the phases of insulin secretion. Min6-derived β -cell clusters were cultured on both compliant and stiff microwelled substrates and a GSIS was performed after 24, 48, and 72 h (Fig. 3). Surprisingly, even

FIG. 3. Min6-derived β -cell clusters in compliant 0.1 kPa microwells have increased glucose sensitivity. **(A)** After 24 h of incubation, there is no significant change in insulin secretion rate. **(B)** After 48 h, there is a slight increase in insulin secretion on compliant microwells at 15 mM glucose. **(C)** And after 72 h, there is an overall decrease in the insulin rate on compliant stiffness microwells. **(D)** The Glucose Stimulation Index shows an increase in the insulin sensitivity on the 0.1 kPa substrate (* $p < 0.05$).

FIG. 4. Min6-derived β -cell clusters maintain a circular nuclear shape in compliant PA microwells. (A) Nuclear staining of cells cultured in compliant 0.1 kPa and stiff 10 kPa microwells. (B) The decrease in circularity of the nuclei on stiff microwells indicates that cells experience more cellular tension. (* $p < 0.05$). Color images available online at www.liebertpub.com/tea

though insulin expression is increased on compliant microwells, after 24 h there is no significant difference in the glucose stimulation profile between the Min6-derived β -cell clusters cultured on the compliant and stiff microwelled substrates (Fig. 3A). Similar results are seen at the 48- and 72-h time points as well (Fig. 3B, C). However, analysis of the glucose stimulation index profile shows significant improvement at the 48-h time point (Fig. 3D). An increased glucose stimulation index demonstrates that the β -cell clusters are more efficient at secreting insulin in response to glucose, a hallmark of healthy insulin-secreting clusters. Both insulin secretion rate and glucose stimulation index are important for maintaining proper insulin response.

Intercellular tension in response to stiff microenvironments can cause a change in nuclear morphology. Tension in cytoskeletal attachments to the nucleus can distort the circular configuration of tensionless nuclei. Thus, nuclear circularity within β -cell clusters can indicate the relative amount of internal cellular tension. As expected the Min6-derived β -cell clusters cultured in stiff microwells, which would be expected to have higher internal cellular tension, have nuclei that are significantly less circular than those on compliant substrates (Fig. 4).

The stiffness of the microenvironment is converted to an intercellular response through mechanosensing. Three mechanosensing signaling pathways—ERK, MLC, and ROCK signaling—were explored in clustered Min6-derived β -cell clusters. To confirm the role of each signaling pathway, MEK1 (ERK inhibitor), ML-7 (MLCK inhibitor), and Y27632 (ROCK inhibitor) were used. Interestingly the MEK1 inhibitor has no effect on insulin expression (Fig. 5A).

Further, the absence of ERK nuclear localization and complete absence of pERK, the activated form of ERK, in both substrates indicates that ERK signaling is not involved (Fig. 5B). However, treatment with ML-7 and Y27632 reduces insulin expression on compliant substrates to levels achieved on the stiff substrates (Fig. 6).

To determine the effect of substrate stiffness on β -catenin signaling, gene expression of *GSK3 β* , a competitive inhibitor of β -catenin, and *Lef* and *Tcf*, binding partners of β -catenin, was analyzed. At 24 h, gene expression for *GSK3 β* is decreased while *Lef* and *Tcf* expression is increased on the compliant substrates (Fig. 7A). A decreased expression of *GSK3 β* and increased expression of *Lef* and *Tcf*, the transcriptional binding partners for β -catenin, on compliant microwell substrates suggest that canonical Wnt signaling may be upregulated to confer improved insulin sensitivity and expression. To confirm the role of β -catenin, we used the β -catenin inhibitor IRW-1 and the β -catenin activator DCA. When β -catenin signaling is inhibited, insulin expression is decreased on the compliant substrates to the level observed on the stiff substrate (Fig. 7B). However, when β -catenin signaling is activated, insulin expression is increased on the stiff substrates to the level observed on the compliant substrates (Fig. 7B). This demonstrates reversible control of insulin expression on the compliant and stiff substrates through β -catenin signaling manipulation.

Discussion

Tissue engineering strategies have the power to explore the roles of architecture and microenvironment on β -cell biology.

FIG. 5. Erk signaling is not required for stiffness-sensitive insulin expression. (A) Incubation with an MEK1 inhibitor shows no change in insulin expression. (B) Absence of ERK activation is demonstrated by the lack of nuclear staining overlap between ERK (green) and the nucleus (red), and by the absence of pERK (green) staining. ERK, extracellular-signaling-related kinase. Color images available online at www.liebertpub.com/tea

FIG. 6. MLCK and ROCK signaling is required for stiffness-sensitive insulin expression. Addition of MLCK and ROCK inhibitors significantly decreases insulin expression on compliant 0.1 kPa microwells to the levels in the stiff 10 kPa microwells ($p < 0.001$). MLCK, myosin light chain kinase; ROCK, Rho-associated protein kinase.

Accordingly, research has been focused on showing importance of spherical architecture on β -cell clusters for increased insulin production and viability.⁹ They have also shown that larger β -cell clusters secrete more insulin per cell.⁷ Additionally, the importance of 3D cell cluster formation for optimal β -cell function using a variety of different natural and synthetic materials has been demonstrated.^{18,37–39} These studies have focused on the biochemical cues initiated by surface molecule binding, such as the role collagen and laminin, the two major ECM proteins of islets.⁴⁰ Improved islet function has been demonstrated when the ECM interactions are reconstructed. Although this is useful to understand the role of architecture and ECM binding, they focus on surface chemistry that mediates islet survival and function and ignore the physical and mechanical properties that guide that β -cell response. Precise manipulation of these physical cues promises new insights into cellular behavior and tissue function, which could improve clinical outcomes of islet transplantation. In our study we have created a system in which the biomechanical properties of the material and cell can be controlled to explore their effect, a previously under-acknowledged regulator of β -cell function.

PA was chosen because it is a biocompatible hydrogel, with a tunable stiffness range. By attenuating the polymer

formulation, microwell scaffolds that have ranges of physiological stiffness can be fabricated using standard photolithographic techniques. Additionally, when hydrated and incubated at 37°C, it does not rupture due to swelling; additionally, if interested, the acrylate handles can be exploited to covalently functionalize the surface.

As expected from previous research, microwell scaffold alone improves insulin expression. However, compliant microwell scaffolds, which combine 3D clusters with physiologically relevant stiffness, greatly improve the glucose stimulation index and insulin expression. Although there is striking increase in insulin expression on compliant substrates, there is only a moderate change in the insulin secretion. This could be due to the necessity of both: physical interactions with the substrate, and biochemical signaling interactions initiated by ECM-adhesion binding proteins to further promote insulin secretion and sensitivity.^{40,41}

Interestingly, increased insulin expression in β -cells cultured on compliant substrates was downregulated with ROCK and MLCK inhibitors, but it was unaffected by ERK inhibition. This result was surprising as ERK signaling has been linked to focal adhesion formation and mechanosensing.⁴² The ablation of the increased insulin expression on the compliant substrates with the MLCK and ROCK inhibitors suggests that a mechanosensing mechanism regulates changes in insulin, and that the MLCK inhibitor appears to have a stronger inhibitory affect. Although both the MLCK and ROCK inhibitors decrease insulin expression on the compliant substrate, the expression is never below the expression level of the stiff substrate. This suggests that these inhibitors decrease insulin expression by ablating cellular recognition of stiffness rather than the change in cellular tension of the cells regulating the changes in insulin expression.

Additionally, the nucleus has been proposed to act as a cellular mechanosensor, with changes in nuclear shape causing conformational changes that directly affect transcriptional regulation.^{15,43–45} The decreased circularity in the stiff microwell substrates was consistent with the increased cellular tension experienced in the more rigid scaffolds. If cellular tension regulates insulin expression, then inhibition of mechanosensing would be expected to increase insulin expression. However, there is no increase in insulin expression with the inhibition of mechanosensing, suggesting that mechanosensing, not intercellular tension, is necessary for stiffness-

FIG. 7. Stiffness-sensitive insulin expression is mediated by β -catenin signaling. (A) Decreased expression of GSK3 β and increased expression of β -catenin adaptor proteins Lef and Tcf suggest increased insulin sensitivity by β -catenin signaling. (B) Incubation with 1RW-1, a β -catenin inhibitor, reduces insulin expression on the compliant 0.1 kPa substrates to the level observed on the stiff 10 kPa substrates where addition of DCA, a β -catenin activator, increases the insulin expression on the stiff 10 kPa substrate (** $p < 0.005$).

controlled insulin expression. Additionally, cells cultured with a β -catenin inhibitor decreased insulin expression to the expression level of the stiff substrate whereas cells cultured with a β -catenin activator increase the insulin expression on the stiff substrate to the levels of the compliant substrate. This demonstrates that β -catenin can reversibly control insulin expression between the stiff and compliant substrate. Interestingly β -catenin is an established regulator of insulin sensitivity through Wnt signaling; however, it had not previously been linked to mechanosensing for insulin sensitivity. These findings are consistent with the decreased expression of *GSK3 β* that when inactive, activates β -catenin and results in its nuclear localization. The increased expression of *Tcf* and *Lef*, the binding partners of β -catenin, is also consistent with the β -catenin signaling. This indicates that β -catenin signaling is required to mediate stiffness-dependent insulin expression. This suggests that MLCK and ROCK mechanosensing and β -catenin signaling through the canonical Wnt pathway modulate stiffness-dependent insulin expression and secretion.

This study demonstrates the ability of discrete biophysical cues to affect β -cell insulin expression and sensitivity in a 3D system. To our knowledge, this is the first study that investigates stiffness-mediated 3D β -cell cluster's insulin processing. The results of this study have promising implications for not only on tissue engineering but also for diabetes treatment in transplantation and immune isolating systems. These findings can direct future islet studies, specifically improving strategies for increased glucose sensitivity. By understanding the microenvironment responsible for improving the islet's insulin response, we can build on the current cell-encapsulating technologies and improve the potential to cure diabetes.

Conclusions

To measure the role of stiffness alone on insulin processing, a 3D microwelled system that is mechanically tunable, biocompatible, and fabricated using standard photolithography was designed. Three-dimensional Min6-derived and primary islet-derived β -cell clusters were created and a stiffness-dependent change in insulin expression and sensitivity was demonstrated. The importance of ROCK and MLCK signaling through mechanosensing was shown in stiffness-dependent insulin processing. Additionally, the decrease in nuclear circularity suggests increased intercellular tension. However, this did not contribute to changes in insulin processing. This work demonstrated that stiffness-sensitive insulin processing requires β -catenin signaling. Further, this suggests that β -catenin signaling through the Wnt pathway regulated by mechanosensing tunes insulin processing. Understanding the microenvironment can play a key role in future diabetes studies. This information can be directly translated to current islet transplantation methods and immune isolation devices for the long-term treatment of diabetes.

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Disclosure Statement

No competing financial interests exist.

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