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METHODS ARTICLE

Fibronectin and Collagen IV Microcontact Printing Improves Insulin Secretion by INS1E Cells

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Extracellular matrix (ECM) molecules play significant roles in regulating β-cell function and viability within pancreatic islets by providing mechanical and biological support, stimulating cell survival, proliferation, and their endocrine function. During clinical islet transplantation, the β-cell's ECM environment is degraded by enzymatic digestion. Literature suggests that interactions between islet cells and ECM molecules, such as fibronectin (FN), collagen type IV (Col4), and laminin (LN), are essential for maintaining, or stimulation of islet function and survival, and can effect differentiation and proliferation of the endocrine cells. It is also thought that three-dimensional (3D) culture of β-cells can improve glucose responsiveness by providing a specific niche, in which cells can interact with each other in a more natural manner. Conventional suspension cultures with β-cells results generally in a heterogeneous population with small and large aggregates, in which cells experience different nutrient diffusion limitations, negatively affecting their physiology and function. In this study, we have explored the effect of FN, Col4, and LN111 on INS1E insulinoma cells by using microcontact printing (μCP) to investigate whether a controlled environment and aggregate dimensions would improve their endocrine function. Using this method, we produced a pattern of well-defined circular spots of FN, Col4, and LN111 on polydimethylsiloxane with high spatial resolution. Cell seeding of the INS1E cells on these ECM protein spots resulted in the formation of 3D β-cell aggregates. We show that these INS1E aggregates have very reproducible dimensions, and that the cell culture method can be easily adjusted, leading to a highly accurate way of forming 3D β-cell aggregates on an ECM-functionalized substrate. In addition, we show that ECM molecules can act as anchoring points for β-cells on an otherwise non-cell-adherent material, and this can improve both the endocrine function and viability. We found a significant increase in the secretion of insulin by INS1E cells cultured on μCP FN and Col4 substrates, in comparison to cells that were growing in monolayers on substrates without ECM molecules. Moreover, INS1E cells growing on circular ECM spots in a 3D manner showed improved endocrine function in comparison to their two-dimensional counterparts.

Keywords: β-cells, type 1 diabetes, islet transplantation, β-cell replacement therapies, insulin secretion

Impact Statement

This research deals with finding a proper bioengineering strategy for the creation of improved β-cell replacement therapy in type 1 diabetes. It specifically deals with the microenvironment of β-cells and its relationship to their endocrine function.

Introduction

Clinical islet transplantation requires the isolation of islets from their pancreatic native environment. Isolation of islets is based on the enzymatic digestion of the pancreatic extracellular matrix (ECM), by using collagenase to separate the islets from the surrounding exocrine tissue, a process that also degrades the intraislet ECM.[1,2] ECM molecules are key microenvironmental factors that regulate numerous cellular processes in islets like, islet morphology,[3] cell differentiation,[4,5] intracellular signaling,[6,7] gene expression,[8,9] cell adhesion,[10] cell migration,[10,11] cell proliferation,[12]

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insulin secretion,[10,13] and cell survival.[12] It has been reported that enzymatic disruption of the β-cell-ECM interaction is partially responsible for the rapid loss of islets post-transplantation.[1] In particular, fibronectin (FN), collagen (Col), and laminin (LN) are of high importance to β-cell function and survival.[2,14–18]

The enzymatic degradation of ECM molecules during islet isolation can lead to a loss of integrin binding, which can induce changes in phenotypic characteristics of β-cells. Consequently, this affects the endocrine cell function, morphology, and survival of pancreatic islet cells.[19] The interaction of integrins, such as $\alpha_3\beta_1$ and $\alpha_6\beta_1$, with ECM was proven to affect insulin release; $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, for example, can regulate differentiation, adhesion, and migration of β-cells.[20,21] In addition, β-cells express collagen type IV (Col4) binding $\alpha_v\beta_1$ integrin, which is associated with insulin release, cell motility, and cell adhesion.[14,22] The pancreatic β-cells are also known to bind arginine-glycine-aspartic acid (RGD) sequences on FN by integrins $\alpha_3\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$, which promote cell survival.[23,24] The density of these integrins decreases after enzymatic degradation of the islet basement membrane during isolation, which negatively affects the islet cell's endocrine function.[19] Reintroducing ECM molecules to β-cells has been reported to improve their survival and function. For example, culturing β-cells on ECM secreted by bovine corneal endothelial cells resulted in improved insulin release,[25] islet survival, and proliferation.[26] Furthermore, purified individual ECM molecules, such as collagen type I (Col1), Col4, LN, and FN, can increase the adhesion and insulin release of β-cells.[14,22,27,28]

Although previous studies have provided valuable insights, they were limited by conventional two-dimensional (2D) cultures of β-cells, which can negatively affect their metabolism and functionality.[29,30] Microcontact printing (μCP) can be used to create arrays of specific patterns of proteins on a non-cell-adherent material surface such as polydimethylsiloxane (PDMS), with high spatial resolution in a highly reproducible manner.[31] By carefully designing a pattern consisting of one type of ECM molecule, or a mixture of different ECM molecules, one is able to create a well-defined cell culture substrate that allows one to study the influence of the β-cell microenvironment on their behavior and functionality.

In this study, we set out to optimize μCP to study the influence of a pattern of circular ECM spots on the function and behavior of β-cells. We hypothesize that INS1E β-cell function and viability can be improved by growing them on an ECM protein functionalized substrate, while growing β-cells into three-dimensional (3D) aggregates using the same ECM proteins guided by μCP could further help improve their endocrine function. To study the effect of biofunctionalized surfaces, we explored the effect of FN, Col4, and LN111 on the glucose responsiveness of rat INS1E insulinoma cells on uniformly coated tissue culture polystyrene in comparison to PDMS. We showed that uniform coatings of these ECM proteins on standard tissue culture plastic, and μCP spots of FN and Col4 on a soft PDMS substrate significantly increase the insulin secretion of INS1E cells compared to cells grown on noncoated tissue culture plastic controls. Interestingly, LN111 seems to adversely affect insulin secretion in comparison to cells grown on the noncoated surfaces. INS1E cells grown into 3D aggregates guided by the μCP ECM spots on PDMS outperformed the same cells grown in a monolayer on uniformly coated tissue culture plastic in terms of relative insulin secretion per DNA. μCP revealed to be an effective manner to engineer uniform cell clusters by limiting cell adhesion to only the ECM-coated spots and guiding the formation of uniformly shaped and sized β-cell aggregates on and otherwise non-cell-adherent PDMS substrate. This study provides a method in which interactions of β-cells with ECM components can be studied in a very controlled manner. The μCP technique described in this report can be used to study the effect of ECM proteins on the endocrine function and behavior of β-cells, and guide the formation of equally sized and shaped 3D β-cell aggregates grown on demarcated functionalized micrometer-sized circular ECM spots.

Materials and Methods

Fabrication of μCP substrates

To fabricate μCP patterns of ECM molecules on a non-cell adhesive PDMS substrate, we modified the method published by Mendelsohn *et al.*[32] We used a silicon-based master wafer mold prepared by microphotolithography to create a pattern of ultra violet (UV) crosslinked SU8 to create PDMS-based stamps for printing. In brief, liquid SU8 is spin coated onto a silicon wafer, followed by selective UV polymerization using a photomask with a predefined pattern. The pattern design consists of 2500 circular spots/cm^2, each spot having a diameter of 100 μm. After photopolymerization, one is left with a negative replica of the intended pattern in SU8, which can be used as a mold for PDMS film casting. Subsequently, PDMS stamps with a thickness of 1–1.5 mm were prepared by negative replica molding using a 1:10 w/w mixture of Sylgard 184 elastomer (Dow Corning, USA). The stamps were treated by plasma oxidization (Plasma-Prep II plasma ether; SPI supplies, USA) at an oxygen pressure of 1.0 bar and at 40 mA for 20 s to increase the hydrophilicity of PDMS, and to clean the surface from any remaining organic contaminants. Subsequently, the stamps were inked with 50 μL of 100 μg/mL solubilized FN (rat derived), Col4 (human derived), or LN111 (mouse derived) (Merck-Millipore, The Netherlands; cat. no.: 341668, cat. no.: CC076, and cat. no.: CC095) in phosphate-buffered saline (PBS). We verified homogenous transfer of the proteins by labeling with the fluorophore DyLight 488 or 549 NHS Ester (Thermo Fisher Scientific) and fluorescence microscopy. Afterward, the excess inking solution of FN, Col4, or LN111 was removed from the stamp by washing with Milli-Q water and then dried under a stream of N_2. PDMS cell culture substrates with a thickness of 3–5 mm were prepared by film casting on flat silicon wafers. The PDMS films were treated by plasma oxidization at an oxygen pressure of 1.0 bar and at 40 mA for 90 s. Subsequently, the micropatterned PDMS stamps were placed on the flat plasma-treated PDMS films for 20 min. During transfer, a 25 g/cm^2 weight was placed on top of each stamp to ensure homogeneous transfer of the molecules on the underlying PDMS film, Successively, the nonprinted areas of the substrate were blocked by incubation of 10% w/v Pluronic F108 in PBS for 3 h at room temperature. After μCP, the PDMS films were gently washed with PBS, disinfected with 70% ethanol for 2 min to ensure sterility, and washed with sterile PBS. Verification of homogenous and accurate printing was

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done by fluorescence microscopy. By using the abovementioned stamp configuration, we printed around 2420 spots per sample; 20% ($\sim 1.9 \times 10^7$ μm^2) of the total surface area of 1.5 cm diameter PDMS films is therefore covered with circular ECM protein containing spots. We compared the biofunctionalized ECM μCP PDMS samples with uniformly coated and noncoated tissue culture plastic (TCP) as controls.

Cell culture

INS1E rat insulinoma cells were cultured in RPMI 1640 medium with 2.05 mM L-glutamine (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum, 100 U/mL penicillin (GIBCO, Bleiswijk, The Netherlands), 100 U/mL streptomycin (Lonza, Verviers, Belgium), 10 mM HEPES, 1 mM sodium pyruvate, and 50 μM freshly added β-mercaptoethanol. Cells were incubated in humidified air (5% CO_2) at 37°C.

Cell seeding density

To have an equal cell density on the micropatterned substrates and tissue culture plastic samples, 4×10^5 INS1E cells/cm^2 were seeded on μCP FN-, Col4-, and LN111-patterned PDMS, while 1×10^5 INS1E cells/cm^2 were seeded on uniformly coated and noncoated tissue culture plates (polystyrene). After 6 h, the samples were supplemented with fresh medium. The cell seeding density was determined by quantifying the DNA content per sample in triplicate using a Quant-iT Pico-Green dsDNA assay kit (Invitrogen) according to the manufacturer's protocol.

Cell morphology

To study the effect of the μCP ECM proteins on INS1E cell morphology, samples were fixed in 4% (w/v) paraformaldehyde in PBS for 1 h at room temperature after 1, 3, and 7 days of culture. Subsequently, samples were dehydrated using an increasing concentration series of ethanol from 70% to 100% for 30 min in each step. Afterwards, samples were prepared for scanning electron microscopy using a Balzer's CPD 030 and BAL-TEC critical point dryer, and gold coated by a sputter coater (Cressington, UK). Electron micrographs were made using an XL30 ESEM-FEG environmental scanning electron microscope (Philips/FEI, The Netherlands). Furthermore, to verify cell growth, samples from each experimental condition were fixed in 4% (w/v) paraformaldehyde in PBS for 1 h at room temperature during cell culture at 3 and 7 days and imaged using standard bright-field phase contrast microscopy (Nikon TE300, Japan).

Cell viability

A two-color fluorescent LIVE/DEAD viability/cytotoxicity Kit (Invitrogen) was used to evaluate the viability of INS1E cells at each time point. The assay was performed on cells cultured on noncoated and uniformly coated tissue culture plastic plates, and μCP PDMS films functionalized with ECM proteins after 3 and 7 days. Green fluorescent Calcein AM indicates live cells by labeling intracellular esterase, and red fluorescent Ethidium homodimer-1 indicates dead cells by complexation to DNA, which can only occur when plasma membranes are damaged. Samples ($n = 3$) were imaged using confocal fluorescence microscopy (Olympus 1×71, Japan). Cell viability was determined by fluorescence as the percentage of Calcein AM-positive cells versus Ethidium homodimer-1-positive cells of the total amount of cells observed (Fig. 2).

Glucose-stimulated insulin secretion test

Glucose-stimulated insulin secretion tests were performed on INS1E cells after 3 and 7 days of culture ($n = 3$/condition). Each sample was preincubated for 1 h in a glucose-free incubation buffer consisting of a modified Krebs-Ringer bicarbonate buffer with HEPES and 10 mM theophylline (KRBH) containing 115 mmol/L NaCl, 5 mmol/L KCL, 24 mmol/L $NaHCO_3$, 2.2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 20 mmol/L HEPES, and 2 g/L human serum albumin at pH 7.4. Subsequently, samples were incubated during three consecutive steps, exposing them to low glucose (1.6 mmol/L), high glucose (16.7 mmol/L), and another low glucose (1.6 mmol/L) containing KRBH buffer for 90 min at 37°C each time. Tissue culture media samples were collected after each incubation step and analyzed for insulin content using an ELISA assay (Mercodia, Uppsala, Sweden) according to the manufacturer's protocol. Finally, the total amount of cells was determined by DNA quantification as described above. Subsequently, the DNA measurements were used to compare the relative insulin secretion in each experimental condition to each other by normalizing the insulin secretion to DNA, allowing one to determine the glucose responsiveness of β-cells between the different samples irrespective of total cell number/condition.

Statistical analysis

We presented glucose responsiveness of INS1E cells as mean insulin secretion (pMol/μg DNA) ± standard deviation. Statistical analysis was performed using a Student's *t*-test, one-way analysis of variance, and a least-significant difference multiple comparison test by application of SPSS statistic software (Chicago, IL). Statistical significance was considered at $p < 0.05$.

Results

μCP of ECM molecules

To evaluate the effect of ECM molecules on function, viability, and morphology of the INS1E cells, three different ECM molecules were applied as a uniform coating or μCP pattern (Fig. 1A). FN, Col4, and LN111 were selected due to their abundance in islets and their previously reported roles on β-cell behavior. Cell cultures on noncoated tissue culture plastic plates served as negative controls. Fluorescence microscopy indicated that homogenous 100 μm diameter circular spots were μCP with FN, Col4, or LN111 (Fig. 1B, C). Evaluation of the fluorescent intensity indicated that the covalently immobilized FN, Col4, and LN111 remained stable for at least 7 days after μCP (Fig. 1D). After seeding INS1E cells on μCP substrates, the cells only adhered to the ECM containing spots and after proliferation formed equally shaped and sized rounded aggregates (Fig. 1E). Since cell–cell interactions can play an important role in β-cell function, we ensured that identical cell amounts in each condition were used at the start of each culture. The initial cell seeding

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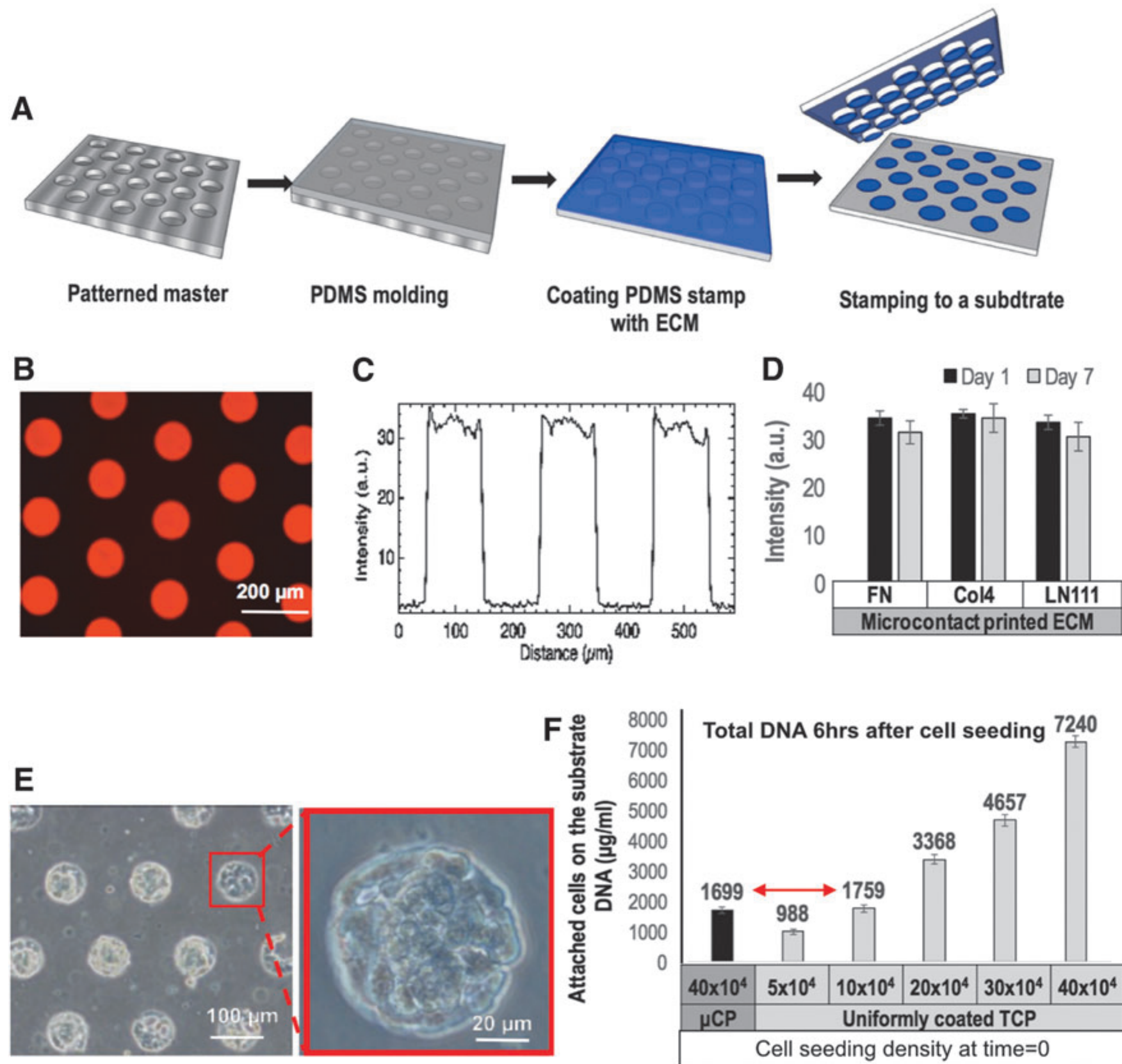

FIG. 1. Fabrication of cell-seeded ECM μCP substrates. **(A)** Schematic outline of the creation of the μCP ECM arrays. **(B)** Homogenous distribution of fluorescently labeled FN validated proper homogenous transfer of ECM proteins. **(C)** Homogeneity of the μCP spots was confirmed using fluorescence intensity distribution quantification. **(D)** Consecutive fluorescent intensity measurements of μCP FN, Col4, and LN111 demonstrated that the patterns were stable in cell culture medium at 37°C for at least 7 days. **(E)** INS1E cells were able to grow on μCP spots, but not on nonprinted areas. **(F)** An equal cell density between patterned and nonpatterned, or uniformly coated substrates was determined using DNA quantification. μCP, microcontact printing; Col4, collagen type IV; ECM, extracellular matrix; FN, fibronectin; LN, laminin; PDMS, polydimethylsiloxane; TCP, tissue culture plastic. Color images available online at www.liebertpub.com/tec

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amount in each condition was verified by a series of cell seeding optimization steps, after which the total amount of DNA present after cell had adhered was determined. On μCP PDMS samples we printed, the entire surface (1.9×10^7 μm^2 of $1\,cm^2$) is covered with circular cell-adherent ECM spots, while in control samples, cells can adhere to the entire surface. Cells unable to adhere were removed 6 h after seeding when the medium was refreshed before DNA analysis. Based on these outcomes, 4×10^5 INS1E cells/cm^2 on μCP FN, Col4, and LN111 substrates, and 1×10^5 INS1E cells/cm^2 on noncoated and uniformly coated samples were used for cell seeding in the follow-up experiments (Fig. 1F).

Viability of INS1E cells on micropatterns of ECM

To validate the cytocompatibility of INS1E cells on ECM micropatterned substrates, the viability of INS1E cells cultured on conventional ECM coatings and μCP ECM spots was investigated after 1 and 7 days (Fig. 2A). Uncoated tissue culture plastic was used as a control. Live/dead staining revealed cell viability of around 90% for all μCP samples after 1 and 7 days of culture. The relative percentage of viable cells on μCP ECM spots was somewhat higher than those of INS1E cells cultured on either uniformly coated, or noncoated tissue culture plastic samples after 7 days. No major differences between all groups were observed as can be seen in the comparative histology (Fig. 2B).

μCP ECM spots induce INS1E cells to form 3D aggregates

We assessed the effect of μCP FN, Col4, and LN111 on INS1E cell morphology using SEM after 1, 3, and 7 days of culture. The results indicated that INS1E cells initially

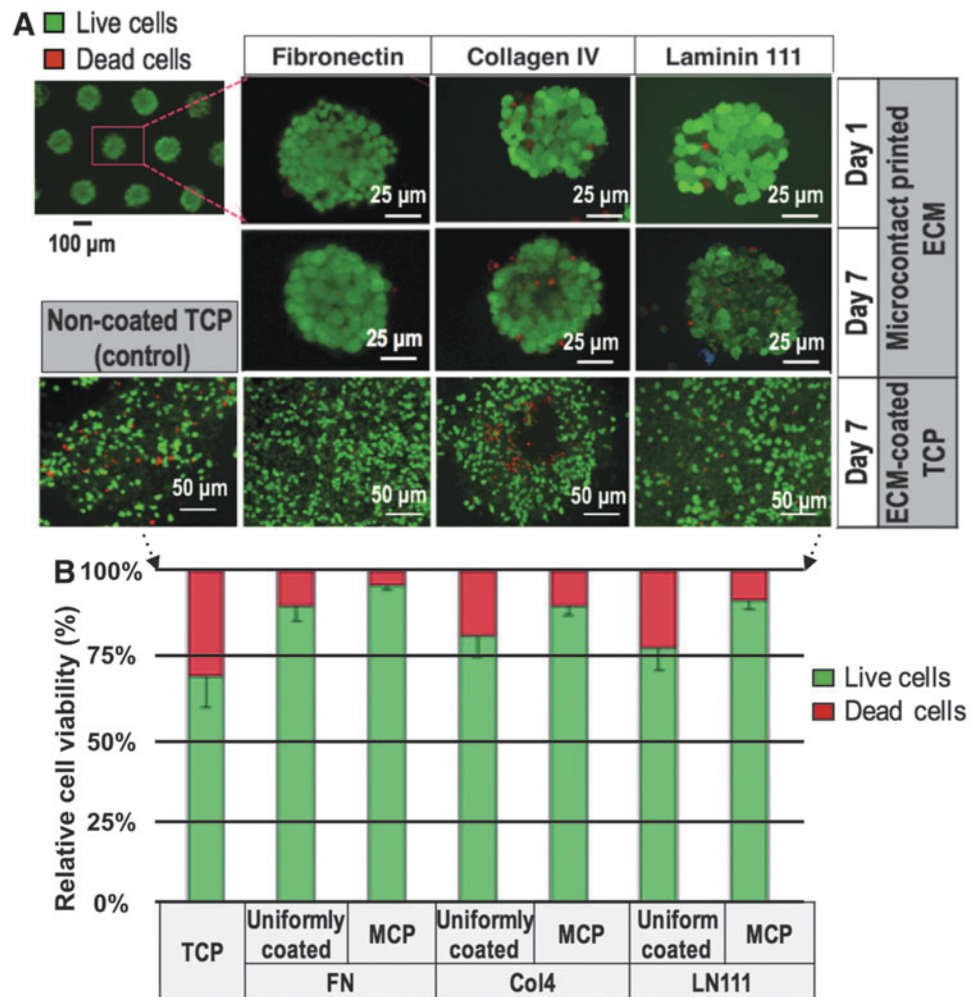

FIG. 2. Evaluation of the cell viability in the presence of FN, Col4, and laminin 111 on a nonpatterned (uniformly coated) TCP plate and µCP PDMS. **(A)** Live/dead staining of INS1E cells on days 1 and 7. **(B)** Image-based semiquantification of the amount of live and dead cells after 7 days. Color images available online at www.liebertpub.com/tec

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attach in a homogeneous manner to the µCP ECM spots. Subsequently, the cells grow into a monolayer on day 1, then form a multilayer on day 3, and ultimately form 3D rounded β-cell aggregates on day 7 (Fig. 3A). On day 7, the aggregate dimensions were as follows: width 72 ± 10, 76 ± 9, and 75 ± 12 µm, height of 23.3 ± 3.6, 38.7 ± 4.8, and 41.5 ± 4.2 µm for FN, Col4, and LN111 µCP spots (Fig. 3B, C). To determine the number of INS1E cells, which had adhered to the nonprinted and µCP samples after 3 and 7 days, we determined the total amount of DNA after each time point. Although all samples had initially been seeded with the same number of cells at the start of the experiment, we found that the DNA content on the LN111 µCP samples was higher than those of the Col4- and FN-printed samples (Fig. 3D). In detail, the DNA content for control samples as well as homogenously coated samples with FN, Col4, and LN was 5,400 ± 3000, 25,100 ± 4500, 35,300 ± 7000, and 43,400 ± 9000 µg/mL on day 7. Moreover, the DNA content on day 7 for µCP FN, Col4, and LN samples was 20,600 ± 3000, 28,200 ± 5000, and 38,200 ± 5700 µg/mL (Fig. 3D).

Glucose responsiveness of INS1E cell clusters

Glucose-stimulated insulin secretion of INS1E cells was assessed in INS1E cells cultured on µCP PDMS substrates, nonpatterned TCP plates, and noncoated TCP plates after 7 days. Insulin secretion was significantly higher in cells cultured on both Col4- and FN-coated (13.9 ± 3.5 and 17.9 ± 4.8 picomol/µg DNA) and µCP (11.6 ± 1.5 and 15.5 ± 2 picomol/µg DNA) samples compared to tissue culture plastic control samples (6.2 ± 1.0 picomol/µg DNA). In contrast, cells cultured on both coated and µCP LN111 samples showed reduced insulin secretion (1.2 ± 0.1 and 3.8 ± 0.9 picomol/µg DNA) compared to the control group. Regardless of which ECM protein was used, the insulin secretion was significantly higher when cells were grown on µCP PMDS surfaces compared to uniformly coated tissue culture plastic surfaces.

Discussion

ECM molecules play a prominent role in the cellular microenvironment where they can influence function, survival, morphology, proliferation, and differentiation of cells. Isolation of islets from their native microenvironment using enzymatic digestion disrupts the interaction between the islet cells and important ECM molecules such as FN, Col4, and Lm.[1,2] Improving the interaction of insulin-producing β-cells with inert biomaterial surfaces by ECM proteins can be an interesting strategy to help restore the lost ECM and mimic the pancreatic islet microenvironment.

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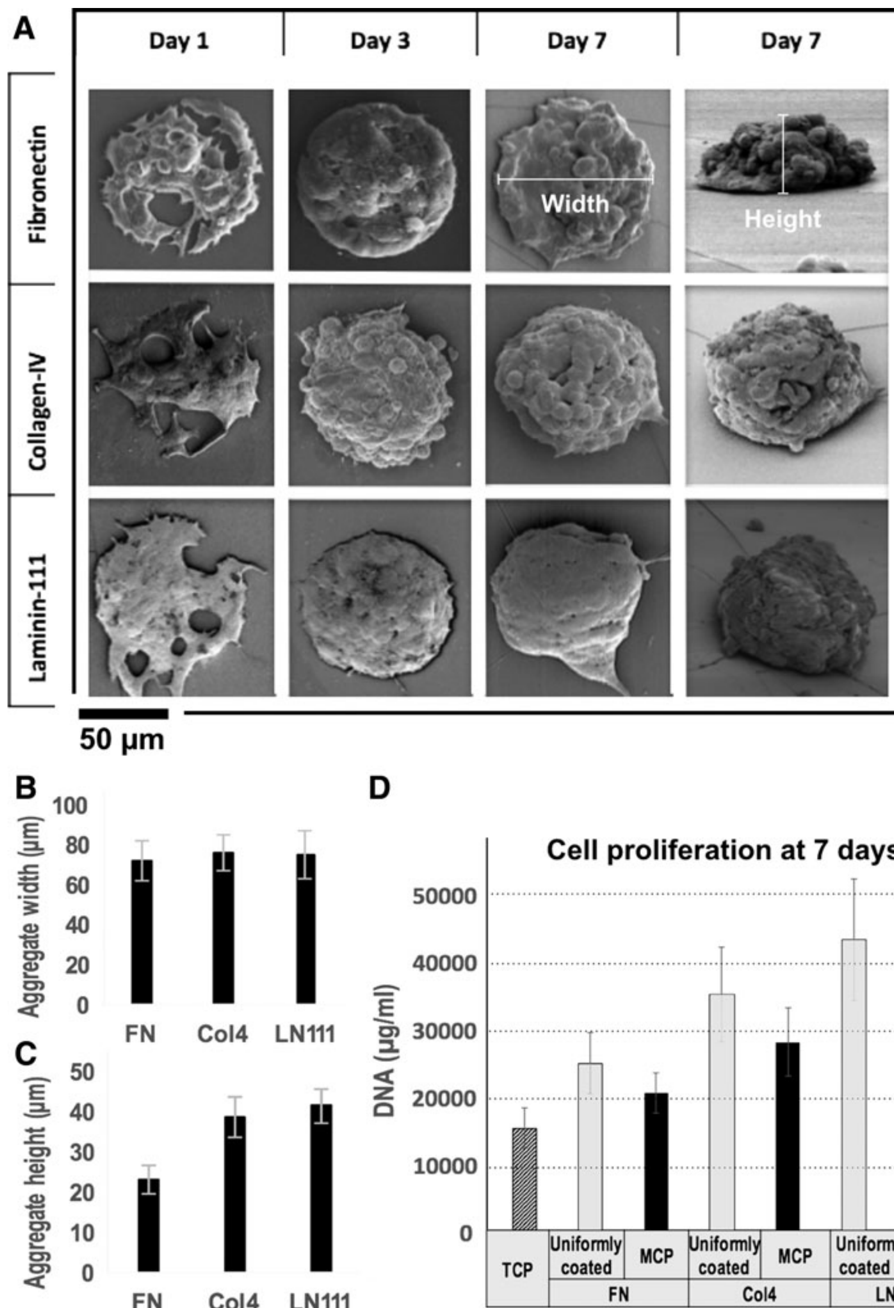

FIG. 3. **(A)** Electron micrographs of INS1E cells cultured on μCP FN, Col4, and LN111 substrates after 1, 3, and 7 days of culture. **(B)** Height of aggregates on FN, Col4, and LN111 μCP substrates after 7 days. **(C)** Width of the aggregates on FN, Col4, and LN111 μCP substrates. **(D)** The total cell number of INS1E cells on μCP and coated FN, Col4, and LN111 after 7 days of culture.

In this study, we report on the effect of μCP FN, Col4, and LN111 on INS1E cells cultured on PDMS and tissue culture polystyrene. μCP provides an easy method to study the interaction between cells and ECM proteins on a micropatterned functionalized PDMS substrate to which cells normally do not adhere due to its hydrophobic properties. Recently, many studies have focused on minimizing the drawbacks of μCP on soft pliable surfaces, such as ink-transfer issues and stamp deformation. We compared β-cell behavior on uniformly coated and μCP surfaces comprising either a uniform coating of FN, Col4, or LN111 or circular printed spots with a diameter of 100 μm of the same ECM molecules on PDMS. Consecutive fluorescent intensity measurements of μCP FN, Col4, and Lm patterns demonstrated that the ECM proteins were homogeneously transferred and remained stable in medium at 37°C for at least 7 days. After cell seeding and prolonged culture, the micropatterned ECM spots enabled aggregation of INS1E cells into similarly shaped rounded aggregates with an average size of around 75 μm (diameter) × 35 μm (height). We observed that in these multilayered cell aggregates, only the cells on the bottom were in direct contact with the underlying μCP biofunctionalized substrate. Previous studies indicated that cell–cell contact and islet size are important factors influencing the function and survival of β-cells. Some studies indicate that relatively large aggregates (>150 μm) quickly lead to apoptosis of β-cells, ultimately resulting in loss of endocrine function.[33,34] Moreover, while

β-cell clusters of ∼100 μm diameter do not demonstrate a significant amount of cell loss by apoptosis, they were reported to secrete less insulin compared to smaller (<100 μm) β-cell clusters, suggesting that diffusion limitation might not be the cause, but cell–cell interactions.[35]

Our results revealed that the FN- and Col4-coated spots prepared by μCP on PDMS can improve the function of INS1E cells compared to cells grown on noncoated tissue culture plastic surfaces. We observed that the amount of DNA isolated from INS1E cell cultures on Col4 and LN111 uniformly coated tissue culture plastic or μCP PDMS samples was considerably higher after 7 days then on noncoated tissue culture plastic control samples, while in all conditions, an equal amount of cells was seeded, suggesting a lower proliferation rate in the latter conditions. These observations suggest a positive effect on INS1E proliferation when they are in contact with Col4 or LN111. In contrast, the glucose responsiveness of INS1E cells in the presence of LN111 was significantly less than when in contact with FN and Col4, as can be seen in Figure 4. This suggests that LN111 plays an important role in cell proliferation rather than enhancing the glucose responsiveness of INS1E cells. In all conditions where biofunctionalized ECM surfaces were used as a cell culture substrate, the insulin secretion was significantly higher in cells cultured on μCP PDMS surfaces compared to uniformly coated tissue culture plastic controls. Although there is an obvious difference in mechanical properties between PDMS and tissue culture plastic (polysterene), which might influence cell behavior, most cells in 3D aggregates are not directly exposed to the underlying substrate. The intensified cell–cell contacts due to the formation of 3D aggregates by INS1E cells guided by the ECM μCP spots could possibly explain the improved insulin secretion, since cells grown into 2D monolayers have less 3D cell–cell interactions. Our findings regarding aggregate formation are in line with a study performed by Mendelsohn *et al.*,[32] who showed that with the same cell-type aggregate formation occurs on μCP surfaces with square spots. Although they only performed a 24-h cell culture with varying spot dimensions, they observed the formation of multilayered aggregates on 60×60 μm square spots and suggested that with sufficient cell seeding numbers, a multilayered aggregate can be formed on larger square-shaped spots of LN in 24 h.[32] A possible reason for the enhanced insulin secretion could be that cells directly in contact with the underlying ECM proteins are performing better due to this interaction, while the cells above perform better due to more intense cellular interactions. There are a number of studies that support our observations that FN and Col4 can improve the glucose responsiveness of β-cells.[36,37] It has been reported that FN increases insulin secretion at a similar level as RGD-functionalized substrates,[38] which suggests the importance of the RGD-integrin binding. Some studies have shown a positive role of LN111 on survival, differentiation, and insulin gene expression of islets.[39,40] However, information on the effect of different laminins remains scarce and underlying mechanism of the effect of LN111 on insulin release has not been unraveled yet, and warrants a more elaborate study in the future. A more dedicated study using biofunctionalized surfaces, using various combinations of ECM molecules on primary islet cells could elucidate which integrins and signal transduction pathways are involved to stimulate their endocrine function and what exact role-specific combinations of ECM proteins play in the β-cell niche. In this study, we observed a positive effect of Col4 and LN111 on cell proliferation, an outcome which is in line with observation done by Weber *et al.*,[13] who showed a positive effect on the survival rate of β-cells when ECM molecules were used in 3D cultures. These observations seem to be dependent on different culture systems with inherent higher cell survival rates. For example, encapsulation of the MIN6-B1 cells in hydrogels without ECM molecules has resulted in high survival rates, suggesting that an entirely 3D culture perhaps better mimics

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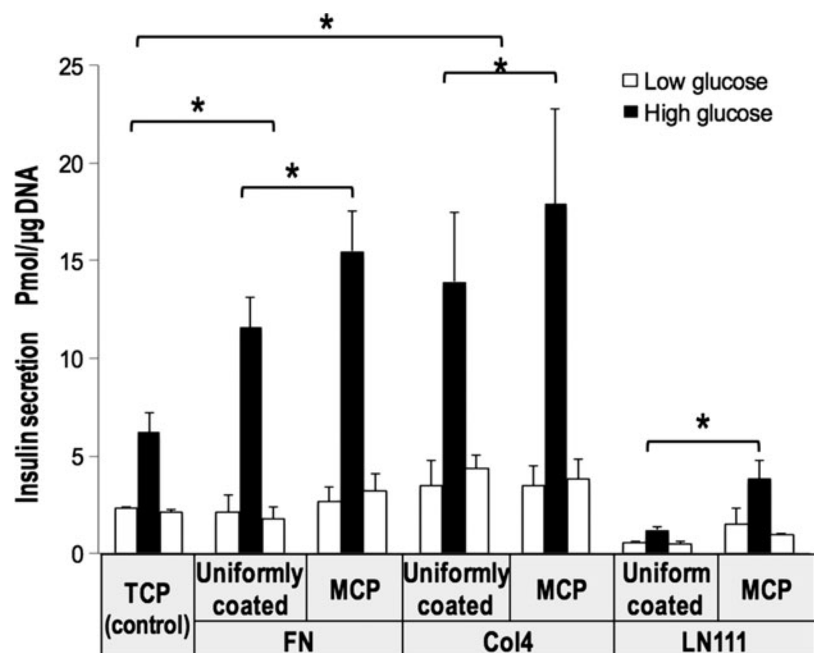

FIG. 4. Glucose-stimulated insulin secretion test of INS1E cells on nonpatterned (uniformly coated), and μCP FN, Col4, and LN111 after 7-day cell culture. *Indicates significant differences between groups ($p < 0.05$).

the native rounded islet morphology leading to improved cell behavior.[13,41]

Comparative morphology of the different aggregates cultured on the different ECM molecules showed that cells cultured on μCP spots of LN111 assemble into more compact aggregates than cells cultured on FN and Col4, a behavior that could potentially negatively affect their endocrine function. There is currently no study available regarding the effect of laminins on compaction of β-cells in literature. However, some studies suggest that extracellular stimuli, as well as cell–cell adhesion molecules, can regulate specific cell signaling involved in morphological changes of epithelial cells.[42]

Conclusions

We demonstrated that FN, Col4, and LN111 are homogenously printable by μCP on a flat PDMS surface. Our results indicated that μCP provides an effective method to reproducibly enable the formation of well-defined β-cell aggregates. Moreover, we revealed that μCP-guided 3D cell aggregate formation can improve the function of INS1E cells compared to conventional 2D monolayer cultures. β-Cells cultured on conventionally ECM-coated surfaces do not present a natural 3D rounded morphology, and display lesser endocrine function, compared to cells cultured in 3D aggregates on ECM μCP surfaces. In this study, we reported that μCP can be used to enable formation of β-cells into 3D aggregates to study the effect of cell–cell and ECM-cell interactions and their endocrine function. This approach holds great potential for applications in tissue regeneration and drug discovery, stem cell research, and many other cell-based analyses and devices. We showed that μCP can provide an interesting technology platform to study the effect of ECM molecules on β-cells for the development of improved functionalized biomaterials and scaffolds for treatment of type 1 diabetes.

In this study, we reported on a method to generate well-defined β-cell aggregates of ~75 μm diameter in a highly reproducible and controlled manner using μCP circular ECM spots on non-cell-adherent PDMS. We showed that FN and Col4 can have a positive effect on glucose responsiveness of INS1E cells, but that LN111 does not. Col4 and LN111 seem to positively affect the proliferation of INS1E cells, while the formation of 3D aggregates guided by μCP ECM on PDMS significantly improves insulin secretion in comparison to 2D cell cultures on tissue culture plastic surfaces. One has to keep in mind that ECM molecules in tissues form an intricate network and different molecules can act simultaneously on cells residing in this matrix. *In vitro* systems cannot entirely mimic this complex microenvironment, and this and other studies are limited by the source of ECM proteins used and their availability. Based on the outcomes of this study, we think that further studies on the effect of different combinations of ECM proteins on primary islet cells could lead to more insight in how the pancreatic islet niche can affect β-cell behavior.

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Disclosure Statement

No competing financial interests exist.

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