Original Article

Physiomimetic Fluidic Culture Platform on Microwell-Patterned Porous Collagen Scaffold for Human Pancreatic Islets

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Abstract

Pancreatic islet transplantation is one of the clinical options for certain types of diabetes. However, difficulty in maintaining islets prior to transplantation limits the clinical expansion of islet transplantations. Our study introduces a dynamic culture platform developed specifically for primary human islets by mimicking the physiological microenvironment, including tissue fluidics and extracellular matrix support. We engineered the dynamic culture system by incorporating our distinctive microwell-patterned porous collagen scaffolds for loading isolated human islets, enabling vertical medium flow through the scaffolds. The dynamic culture system featured four 12 mm diameter islet culture chambers, each capable of accommodating 500 islet equivalents (IEQ) per chamber. This configuration calculates > five-fold higher seeding density than the conventional islet culture in flasks prior to the clinical transplantations (442 vs 86 IEQ/cm^2). We tested our culture platform with three separate batches of human islets isolated from deceased donors for an extended period of 2 weeks, exceeding the limits of conventional culture methods for preserving islet quality. Static cultures served as controls. The computational simulation revealed that the dynamic culture reduced the islet volume exposed to the lethal hypoxia (< 10 mmHg) to ~1/3 of the static culture. Dynamic culture ameliorated the morphological islet degradation in long-term culture and maintained islet viability, with reduced expressions of hypoxia markers. Furthermore, dynamic culture maintained the islet metabolism and insulin-secreting function over static culture in a long-term culture. Collectively, the physiological microenvironment-mimetic culture platform supported the viability and quality of isolated human islets at high-seeding density. Such a platform has a high potential for broad applications in cell therapies and tissue engineering, including extended islet culture prior to clinical islet transplantations and extended culture of stem cell-derived islets for maturation.

Keywords

pancreatic islets, collagen scaffold, dynamic culture, hypoxia, physiomimetic culture

Introduction

Islet transplantation is one of the clinical options for patients with type 1 diabetes for improved glycemic control[1,2]. However, islet transplantation has some challenges, such as shortage of islet source, gradual deterioration of graft function, and lifelong immunosuppression medications for allogeneic islets. Specifically, the cause of the shortage of islet sources is primarily due to a limited number of donors and also contributed by the difficulty of maintaining the number and functions of isolated islets in culture before transplantation.

Islets in a native pancreas are highly vascularized; however, the enzymatic digestion during the islet isolation procedure removes vascular connections between the islets and

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surrounding tissues. Because no vessels transport the required molecules to the isolated islets, all transportation of molecules is diffusion-dependent for their survival[3]. In addition, extracellular matrices supporting islets in the native pancreas tissue are lost in the microenvironment of isolated islets. These critical issues hinder the quality and viability of isolated human islets in conventional *in vivo* culture, and typically, islet mass and viability quickly decrease within a week[4,5].

Alleviating the hypoxic culture environment is one of the keys to improving the survival of isolated islets. However, this would conflict with the practical requirement; high-density cultures of islets are ideal in clinical practice, especially for minimizing the number of culture apparatuses needed. In the high-seeding-density culture flasks, the physical proximity and aggregation of the islets worsens the molecular gradient, resulting in the hypoxia environment[6]. Culture flasks with micropatterned bottoms, such as microwells, have been introduced to separate individual islets and significantly improve islet survival[6,7]. However, even with the micropatterned bottoms, the solid flask bottom limits the diffusion of molecules. In addition, static culture has limitations in regard to the molecular gradient within the culture media.

In this study, we introduce a dynamic culture platform of islets on the microporous collagen microwells. This platform provides a physiological-mimicking microenvironment to extend islet survival in the high-seeding-density culture condition through eliminating the molecular gradient, therefore holds high potential for the extended future applications, such as stem cell-derived islets.

Materials and Methods

Human Islets

Three human islet batches were tested in this study. All human islet isolations were performed at the Southern California Islet Cell Resource Center at City of Hope and obtained through the Integrated Islet Distribution Program[8]. Standardized characteristics, consistent with the recommendations of *Diabetes*[9], are summarized in Supplemental file, Table S1.

Dynamic Culture System on Microwell-Patterned Porous Collagen Scaffolds for the Culture of Human Islets

Microwell-patterned porous collagen scaffolds were fabricated based on the procedures previously published (Fig. 1A)[10,11]. First, hemispheric water droplets ~300 μm in diameter were formed onto a water-repellent polytetrafluoroethylene film (Nitto Denko, Tokyo, Japan) placed on a flat glass plate. The water droplets were then frozen at -80°C to form an ice particulates template. Separately, 2.5 ml of collagen type I aqueous solution (KOKEN, Tokyo, Japan, 1.0 wt% in 0.01 M hydrochloride) was poured onto a sheet of 5 × 5 cm poly (D, L-lactide-*co*-glycolide) (PLGA) mesh (Vicryl® Knitted mesh; ETHICON, Raritan, NJ, USA) and moved in a low-temperature chamber (–2°C). Then, the ice particulates template was pushed over the collagen solution layer, and the constructs were frozen at −80ºC. The frozen constructs were freeze-dried for 24 h (VirTis-Advantage Benchtop Freeze Dryer; SP Industries, Warminster), and cross-linked in a series of ethanol–water mixture solutions, 95/5, 90/10, 85/15, v/v)) containing 50 mM water-soluble carbodiimide hydrochloride (Wako, Tokyo, Japan), 20 mM N-hydroxysuccinimide (Wako), and 0.1 M 2-(N-morpholino) ethanesulfonic acid (Sigma-Aldrich). The microwell-patterned collagen porous scaffolds were then transferred into 0.1 M glycine solution overnight, followed by the washing and freeze-drying. Fig. 1B demonstrated the distribution of islet-mimicking spherical dextran beads for visualization after seeding at a density of 500 beads per cell insert (Cytodex 1, Sigma-Aldrich St Louis, MO, USA) that was colored in a Methylene Blue solution (Sigma-Aldrich)[12].

An overview of the fabricated dynamic culture system for human islets is shown in Fig. 1C, D This culture system was fabricated in a well of 6 six-well plate integrated with cell culture cassette including four cell culture inserts (Fig. 1E; Supplemental Fig. S1). The cell culture inserts' legs 1 mm in height made the gaps between the silicon-cell insert membrane and the bottom of the six-well plate. Stainless tubing (0.25 mm in inner diameter and 0.51 mm in outer diameter) was vertically inserted in the center of the cell culture cassette, which functioned as an inlet of the culture media. Next, the suction tubing (stainless tubing, same as the inlet tubing) was placed on the lid of the six-well plate. These inlet and outlet tubes were connected to the flow rate-controlled peristaltic pumps (BPU and KS-1, Tokai Hit, Fujinomiya, Japan) set at 40 μl/min (Fig. 1F).

The resultant dynamic culture system had the vertical media flow from the bottom to the top of the cell culture insert. Prior to start the cell culture, the vertical flow of the media within the culture chamber was visually confirmed by injecting the colored water solution (Methylene Blue solution diluted with water at 1:50, Sigma-Aldrich) at 40 μl/min into the water-prefilled chamber (Supplemental Fig. S2A–C). Under the conditions where (1) the pump flow is equally divided among four cell culture inserts, providing 10 μl/min per insert, (2) the bottom area of each insert is 60.8 mm², and (3) assuming a uniform vertical media flow is achieved, the simply calculated media flow rate is 0.164 mm/min (equivalent to 2.7 μm/s). This rate is within the physiological range of interstitial fluid flow that has been reported previously[13]. In the initial preparation, 75 ml of culture media was prepared to start circulation. Static culture with four cell inserts placed in 75 ml of media in a 150 mm dish was prepared for controls. The culture media was changed weekly for both the dynamic and static culture conditions. For the dynamic culture, this entailed replacing the old culture media reservoir with a new reservoir containing 75 ml of fresh culture media. The modified CMRL

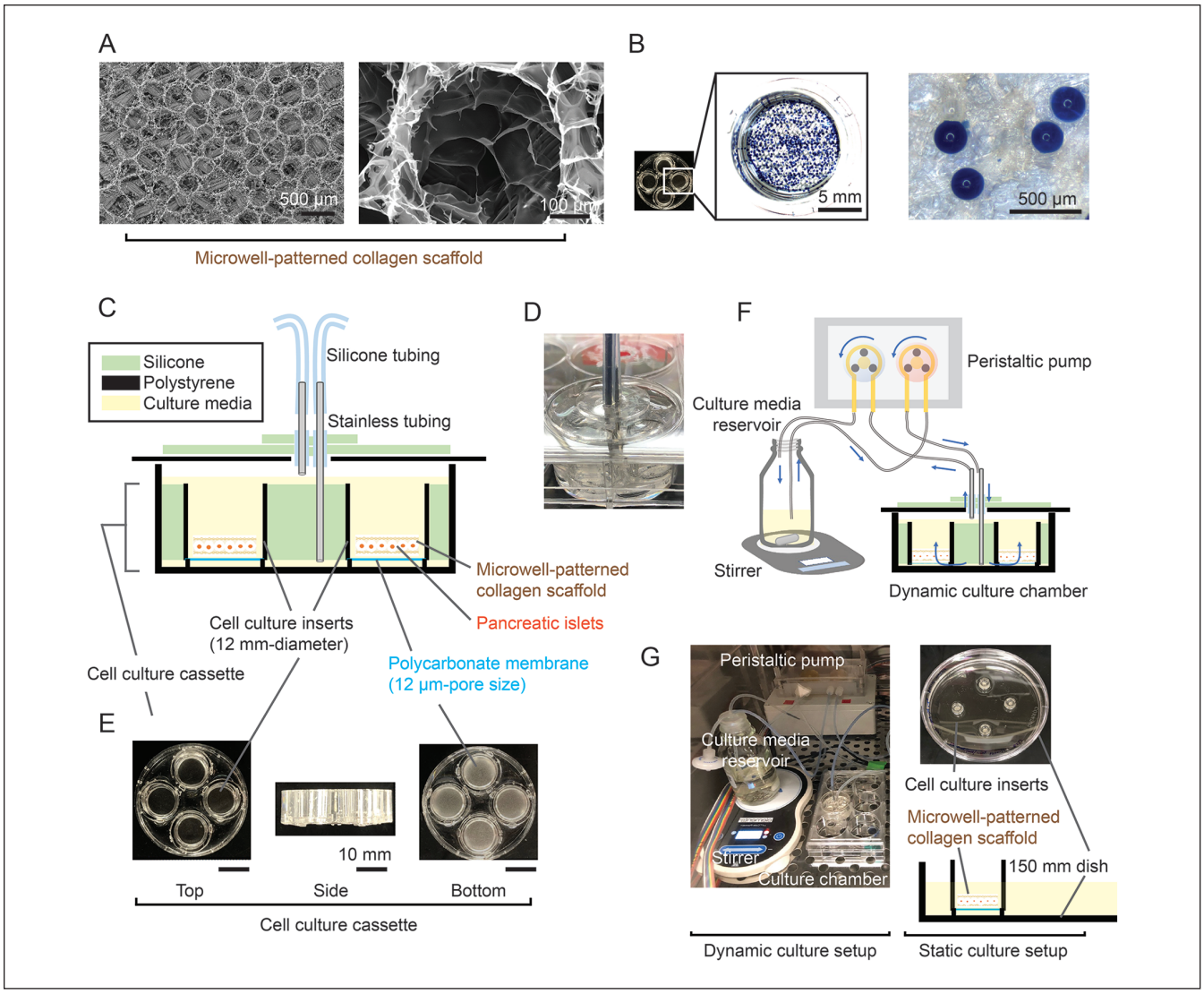

Figure 1. Fabrication of the dynamic culture system for the long-term culture of human islets at high-seeding density (A) Left panel: A scanning electron microscopy (SEM) image of the microwell-patterned microporous collagen scaffold. Scale bar: 500 μm. Right panel: An SEM image in high magnification. Scale bar: 100 μm. (B) Left panel: The distribution of islet-mimicking dextran beads for visualization (500 beads per cell insert). Scale bar: 5 mm. Right panel: An enlarged photo. Scale bar: 500 μm. (C) A schema of the fabricated dynamic culture system in a cross-sectional view. Islets held on the porous collagen scaffold were placed in small cell culture insert chambers. The culture system provides the vertical culture media flow to the islets through the porous collagen scaffold. (D) An image of the actual dynamic culture system prepared in a six-well platform. (E) A fabricated dynamic culture chamber within a silicon body. Top view, side view and bottom view. (F) A schema of the dynamic culture circuit system, including the dynamic culture chambers, flow rate-controlled peristaltic pumps, and the culture media reservoir on the stirrer. (G) A photograph of dynamic culture setup (left panel) and static culture in a 150 mm dish and cell inserts. Static and dynamic cultures have the same culture media volume of 75 ml.

1066-based media (Corning Life Sciences, Corning, NY, USA) supplemented with 0.5% human serum albumin, 0.1 μg/ml insulin-like growth factor-1 (Cell Sciences, Newburyport MA), 10 U/ml heparin sodium (Sagent Pharmaceuticals, Schaumburg, IL), and 10 mM glutamine (Gibco, Waltham, MA, USA) penicillin–streptomycin (Gibco)[6]. The entire setup was placed in a 5% CO_2 incubator at 27°C (Fig. 1G), based on our previous study demonstrating the better maintenance of islets in lower temperature conditions compared with 37°C[5,12].

Human islets (500 islet equivalent [IEQ][14]) were sandwiched between two layers of 300 μm microwell-patterned microporous collagen scaffolds 12 mm in diameter and placed onto the cell culture insert. The seeding density was calculated at 442 IEQ/cm^2, which is > 5-fold higher seeding density compared with the conventional islet culture used in preparation for the clinical islet transplantations (86 IEQ/cm^2 [15,000 IEQ/T175 flask]). Note that islets were prepared on the microwell-patterned microporous collagen scaffolds in both conditions, static and dynamic cultures.

Computational Model Oxygen Environment of Cultured Islets

The computational model of oxygen (O_2) tension was constructed using a finite element method (COMSOL Multiphysics 5.3, COMSOL, Stockholm, Sweden). The parameters used for the simulation are listed in Table S2. The O_2 transport properties are simulated based on the diffusion–convection–reaction equation:

$$\frac{\partial}{\partial t}c = D\nabla^2 c - \nabla \cdot (\mathrm{uc}) - R$$

where c is the O_2 concentration, D is the diffusion coefficient of O_2, u is the velocity field of medium flow, R is the O_2 consumption rate of human islets governed by the Michaelis–Menten type kinetics:

$$R = OCR_{max}\left(\frac{c}{c + K_m}\right)$$

where OCR_{max} denotes the maximum O_2 consumption rate of human islets and K_m represents the Michaelis constant. Henry's law is applied to track the continuity of O_2 partial pressure across boundaries of medium and islets:

$$c = S \cdot pO_2$$

where S is the O_2 solubility and pO_2 is the partial pressure of O_2.

The simulation geometry is based on experimental setups described in the previous section. Human islets are assumed to be evenly distributed across the culture surface. Therefore, we construct a unit cell comprising a single islet to reduce computational demand. The geometry, boundary conditions, and exemplary computational results of the unit cell are illustrated in Fig. S3A–B. Within this unit cell, the islet is positioned at the center of a hexagonal prism. The bottom plate is subject to a pO_2 boundary condition equivalent to the pO_2 found in the medium of the culture chamber (146 mmHg). The side walls are designated as symmetric planes to ensure that the distribution of islets reflects the actual islet seeding density. In the static culture condition, the velocity field is assumed to be zero, indicating O_2 can only be supplied by diffusion. In the dynamic culture conditions, the laminar velocity field is correlated to the medium flow rate (20 or 40 µl/min).

Morphological Assessment of the Human Islets

Constructs of cultured islets with collagen scaffolds were quickly fixed in 10% formalin (Thermo Fisher Scientific) for 30 min and embedded in 3% agar (Sigma-Aldrich) to maintain the spatial relations between the islets and scaffold before the conventional paraffin embedding process. Hematoxylin and eosin (H&E) stain and dual-color immunohistochemistry (IHC) were performed for morphological assessments. IHC was performed on Ventana Discovery Ultra IHC autostainer (Ventana Medical Systems, Roche Diagnostics, Indianapolis, IN, USA) and images were captured using an IX50 microscope (Olympus) and cellSens software (Olympus)[6,12]. Primary antibody incubation was performed using Insulin-DAB (for brown color, rabbit monoclonal antibody, dilution at 1/2,000, Cell Signaling Technology, Danvers, MA) and Glucagon-Purple (rabbit polyclonal antibody, no dilution [ready-to-use reagent], Ventana).

Metabolic Assessments of the Human Islets

Consumption of glutamine was measured using the culture media samples and the Glutamine/Glutamate-Glo™ Assay (Promega, Madison, WI, USA) for freshly prepared culture media samples and the media sampled from 7-day-cultured islets. Glutamine consumption was calculated as the difference between the glutamine amount in the culture media of day 0 (fresh) and day 7. Similarly, the consumption of glucose was measured using the culture media samples (LifeScan, Malvern, PA, USA). Lactate released into the culture media from the cultured islets was measured using the L-Lactate Assay Kit (Abcam, Waltham, MA). All data were normalized by the IEQ applied to the culture.

Viability and Function of the Human Islets

Islet viability was analyzed using live/dead staining by a semi-automated method previously developed[15,16]. For the cultured islets in the dynamic culture, islets were retrieved from the scaffold by flushing the culture media. The overall viability of 50 IEQ of islets was calculated using the cellSens software (Olympus)[16].

Glucose-stimulated insulin secretion (GSIS) was assessed using 50 IEQ and Krebs–Ringer buffer (KRB) solution containing 2.8 mM glucose (low glucose) for 1 h followed by the 28 mM glucose (high glucose) for 1 h. Data of secreted insulin in low-glucose and high-glucose KRB solutions were normalized using total insulin content in islets[12]. The insulin concentration of the samples was measured using a human insulin ELISA (enzyme-linked immunosorbent assay) kit (Mercodia, Uppsala, Sweden). The insulin secretion ratio between high glucose over low glucose was used to calculate the stimulation index (SI).

Gene Expression Assessments of the Human Islets

RNA was isolated from 250 IEQ of islets at pre-culture and post-culture in two conditions (static and dynamic culture conditions) to synthesize cDNAs according to the

manufacturer's instructions (cDNA Synthesis Kit for RT-qPCR, Thermo Fisher Scientific). Microfluidic quantitative real-time PCR (qRT-PCR) was performed using the BioMark 48.48 Dynamic Array system (Standard Biotools, South San Francisco, CA, USA). The expressions of the genes of interest were normalized to the internal control gene (*ACTB*). TaqMan probes (Life Technologies, Carlsbad, CA, USA) used in the qRT-PCR are listed in Table S3. Relative quantities of each transcript were expressed as a fold increase to the average of pre-cultured islets. Gene expression data were obtained from three islet donor batches.

Statistical Analysis

Data were reported as the mean ± standard error of the mean (SEM). Statistical analyses for individual comparisons were performed using Wilcoxon/Kruskal–Wallis test (one-way test with chi-square approximation) or Student's *t* tests, depending on the data distribution (JMP 16 program [SAS Institute, Cary, NC, USA). Data are presented graphically with results from $n = 3$ individual donor batches. All biological assays were performed in technical triplicates within each islet batch.

Results

Computational Simulation Demonstrates the Improved Oxygen Environment in Dynamic Culture Over Static Culture

We first assessed the O_2 level of the culture media using a computational model, using three conditions (static, dynamic at 20 µl/min and dynamic at 40 µl/min). The static condition induced a severe hypoxic media environment surrounding islets while the dynamic cultures improved the media O_2 at the level of islet surface (27.3, 110.4 and 110.8 mmHg in static, dynamic at 20 µl/min and dynamic at 40 µl/min, respectively; Fig. 2A). Fig. 2B demonstrates the intra-islet O_2 environment by the convection of culture media containing O_2; both dynamic culture conditions at slow and moderate flow rates reduced the islet volume exposed to the hypoxia compared with the static condition. The dynamic culture significantly reduced the islet volume exposed to lethal hypoxia compared with the static condition (Fig. 2C). For example, the estimated volume exposed at $pO_2 < 1$ mmHg was 9.1%, 1.2%, and 1.1% in static, dynamic at 20 µl/min and dynamic at 40 µl/min conditions, respectively. Two dynamic culture conditions did not demonstrate a significant difference in mitigating hypoxia; hence, we conducted the subsequent *in vitro* studies comparing the dynamic at 40 µl/min to the static condition.

Dynamic Culture Prevents Morphological Degradation of Pancreatic Islets in Long-Term Culture With Improved Oxygen Environment

Histological analyses of 2-week-cultured human islets at high-seeding density demonstrated that the static culture induced degradation of islets, whereas dynamic cultures maintained robust islet morphology (Fig. 3A). Furthermore, dual-color IHC demonstrated that the dynamic cultures maintained the insulin-producing beta cells and glucagon-producing alpha cells for 2 weeks, which are two major endocrine cell types in the pancreatic islets. In live/dead staining, the 2-week-cultured islets in static conditions exhibited the marked cell damage (Fig. 3B). In contrast, those in dynamic culture conditions showed less cell damage. Analysis of the viability revealed that the dynamic culture conditions significantly improved the viability compared with the static condition ($P = 0.020$). The lactate level in culture media, a metabolite of the aerobic glycolysis[17], suggested that the dynamic culture provided aerobic condition (Fig. 3C). We further investigated the mitigation of hypoxia by the dynamic culture in gene expressions of long-term cultured islets in static and dynamic culture conditions. Dynamic culture mitigated the increase in representative HIF1A downstream genes (*GLUT1,GLUT3*, *and VEGFA*)[18–20] compared with static culture (Fig. 3D). It is important to note that hypoxia may not alter HIF1A mRNA expression levels, as it is the protein expression of HIF1A that plays a critical role in transmitting the signaling[21].

Dynamic Culture Improves Islet Metabolism and Function Over Static Culture in a Long-Term Culture

We measured the consumption of glutamine, an essential nutrient for islet metabolism and survival for islets[22]. Dynamic culture condition demonstrated significantly high glutamine consumption compared with the static culture ($P = 0.001$, Fig. 4A). Glucose consumption by islets, demonstrated a similar trend to the glutamine consumption but with no statistical significance ($P = 0.061$, Fig. 4B). We further evaluated the islet endocrine function with GSIS. Raw insulin secretion data in low-glucose and high-glucose conditions demonstrated the improved response in dynamic culture ($P = 0.003$) to static condition ($P = 0.044$) (Fig. 4C), although stimulation index of GSIS showed no statistical difference between the conditions tested (Fig. 4D).

Discussion

In this study, we introduced the unique dynamic culture platform using porous collagen microwells for primary human islets. Notably, our platform mitigated the hypoxia-induced

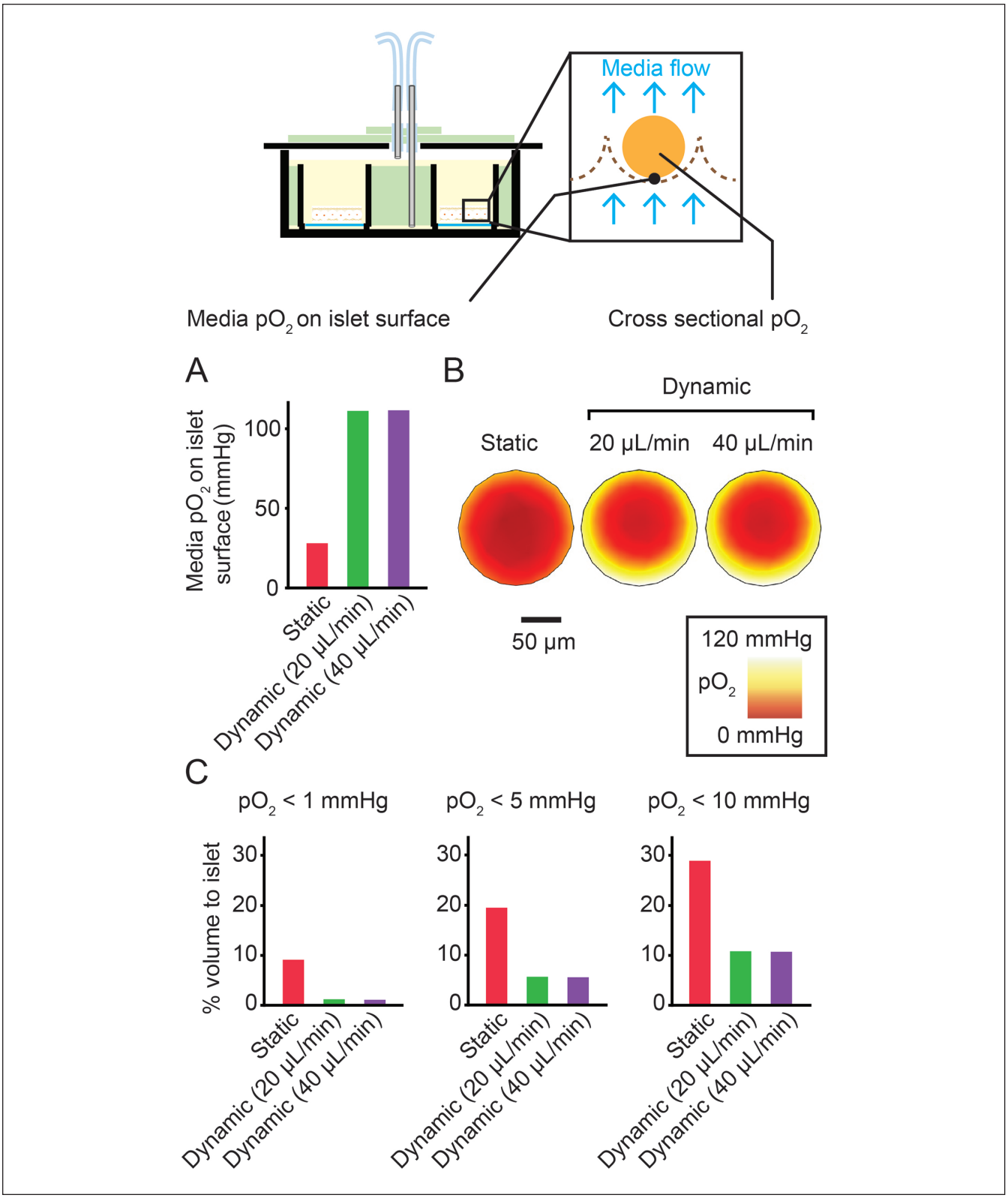

Figure 2. Computational simulation of intra-islet O_2. (A) The O_2 level of culture media at the bottom surface of islets in static, dynamic flow rate at 20 μl/min and dynamic flow rate at 40 μl/min conditions. (B) Vertical cross-sectional view of the intra-islet O_2 distribution. An islet of 150 μm diameter, an average-sized islet, was used for the simulation. Scale bar: 50 μm. (C) Cell volume exposed to the hypoxic condition was calculated. Hypoxic conditions were defined at $pO_2 < 1$ mmHg (left panel), $pO_2 < 5$ mmHg (middle panel), and $pO_2 < 10$ mmHg (right panel).

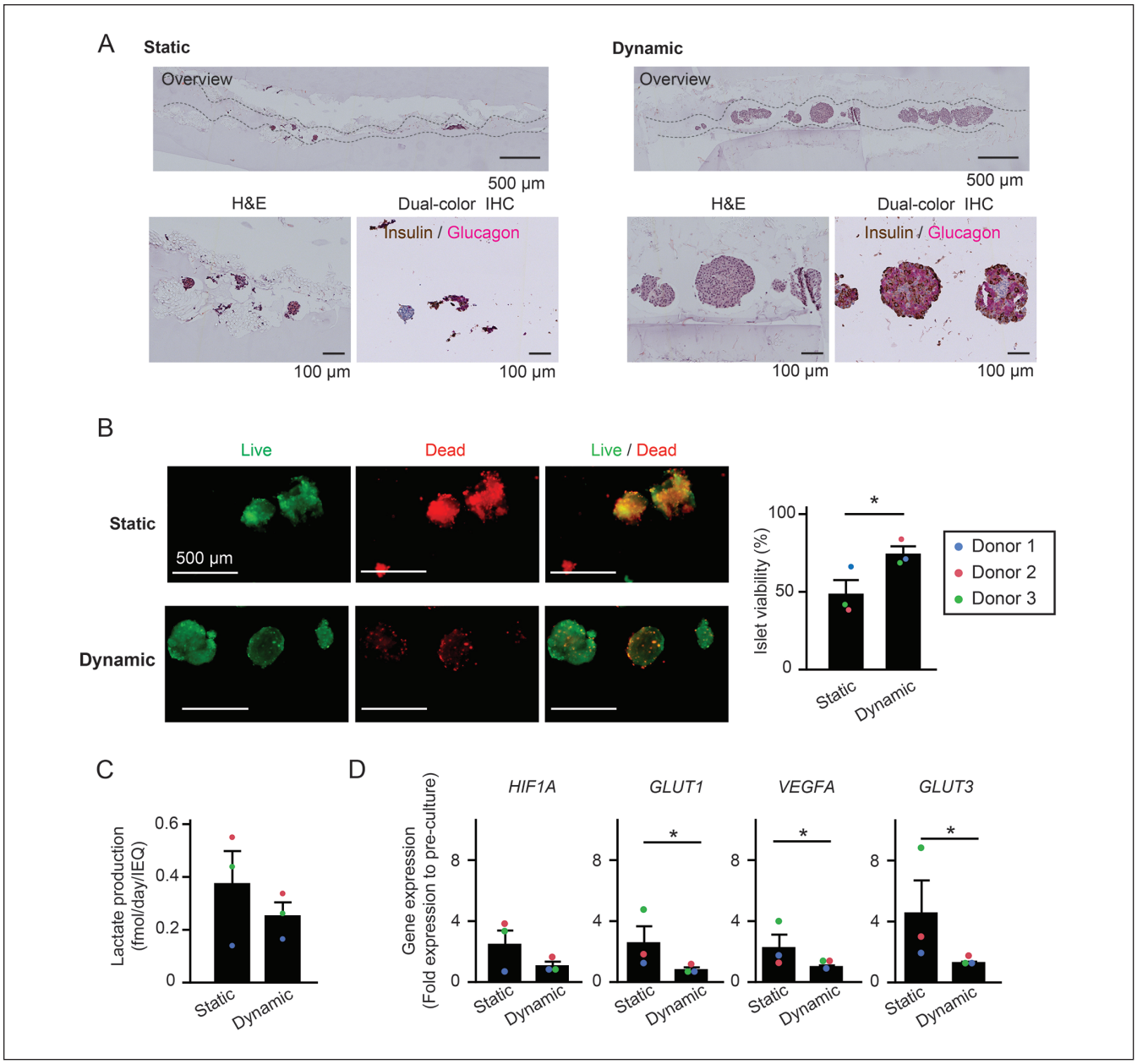

Figure 3. Mitigation of hypoxia-induced islet damage by the dynamic culture over 2 weeks. (A) Histological assessment of pancreatic islets in a long-term culture on collagen scaffolds in static and dynamic culture conditions. Histology sections demonstrate the vertical cross-sections of the samples; the upper and bottom layers in the Overview pictures are the scaffolds, and the middle layer is the islet layer. Borderlines in Overviews between the scaffold and islet layers are indicated by the dotted lines. Overview in H&E stain. Scale bar: 500 μm. Enlarged, detailed appearance of the cultured islets in H&E stain (left) and dual-color IHC (right; brown for insulin-positive cells and pink for glucagon-positive cells). Scale bar: 100 μm. (B) Representative live/dead stain images of the 2-week-cultured islets. Islets were retrieved from the scaffold for staining. The top row for static and the bottom row for dynamic conditions. Scale bar: 500 μm. Right panel: analyzed data of viability based on the live/dead stain images. $n = 3$ human islet batches. (C) Lactate released by the cultured islets into the media. (D) Gene expressions of long-term cultured islets on collagen scaffolds were quantified by qPCR and expressed as fold increase to pre-culture. *$P < 0.05$ in Wilcoxon/Kruskal–Wallis test.

islet damage in the long-term culture at quite a high-seeding density compared with the conventional culture. This has a high potential for clinical applications to improve the current islet transplantation strategies in multiple ways.

Several studies, including ours, demonstrated the importance of O_2 and nutrients to maintain isolated islets in culture by providing exogenous O_2 and nutrients to mitigate islet cell death[3,5,15,22]. Although the extra supply of critical molecules

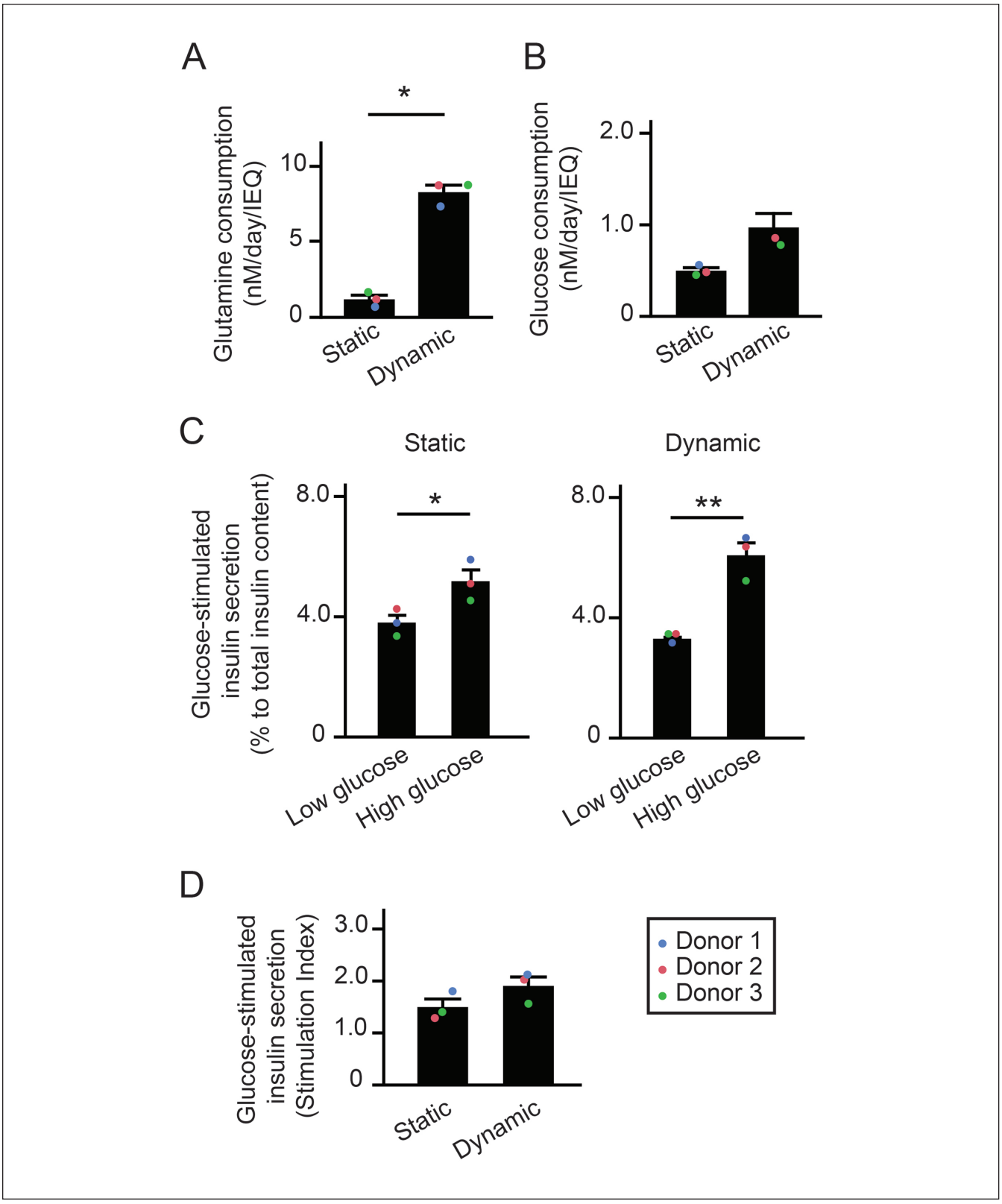

Figure 4. Metabolism and function of pancreatic islets in a long-term culture on collagen scaffolds (A) Glutamine consumption by islets during the culture. $n = 3$ human islet batches. Pairwise t test. (B) Glucose consumption by islets during the culture. $n = 3$ human islet batches. Pairwise t test. (C) Glucose-stimulated insulin secretions of 2-week-cultured islets in static condition (left) and dynamic condition (right). (D) Islet function measured by stimulation index of glucose-stimulated insulin secretion. In all graphs, individual donor data were plotted. Student's t tests $^{*}P < 0.05$ and $^{**}P < 0.01$.

is effective, it may not be sufficient to create the ideal microenvironment for cultured islets. In the native physiological tissue microenvironment, many molecules necessary for sustaining the cell function are continuously supplied to the islet cells; at the same time, secreted molecules, including the hormones as well as biological byproducts, are transported into the systemic circulation. This exchange of molecules is not achieved with conventional static culture system, in which the formation of molecular gradient around the islet cells is inevitable. The dynamic culture system addresses such issues to minimize this detrimental molecular gradient. Our dynamic culturing platform mitigated hypoxia, as demonstrated by the mitigation of hypoxia markers, consistent with the improved oxygenation in the O_2 simulations.

Our culture platform, the combination of a vertical media flow system and porous microwell scaffold, has several advantages over the previously reported perfusion systems. In fact, dynamic cultures of islets and stem cell-derived islet cells have been reported to sustain their function effectively[13,23,24]. In developing the dynamic culture circuit, determining the optimal flow rate of the culture media is critical for balancing molecule supply and stress alleviation; therefore, we set the flow rates in our tests based on the previously reported fluidic flow rate[25,26]. A precisely controlled flow is critical for determining the optimal flow rate for islet culture. Previously introduced dynamic culture setup generally utilizes the horizontal media flow, in which the media runs over the cultured islets sitting on the bottom of the culture dishes. In such cases, the actual flow hitting the islets may not be uniform, thus, limiting the availability of essential molecules to some islets. We addressed the concern by developing the dynamic culture chambers with a vertical media flow, which was ideal for providing a uniform flow for entire islets sitting on the porous scaffold. A porous collagen scaffold placed on the culture cell insert enables the media to flow through the scaffold, where the islets settle.

Our dynamic culture system developed for isolated islets has potential for multiple applications. First, the most straightforward application would be the use of this platform for the long-term human islet culture, which allows us to combine low-yield islets from multiple donors to transplant a sufficient number of islets for the recipient, though the potential increase of immunogenicity in the use of multiple donors should be carefully considered in allo-islet transplantations.

Second, our platform holds potential for the maturation of stem cell-derived islets. Since the stem cell-derived islets mature with extended culture to acquire the appropriate glucose responsiveness[27], our long-term culture platform provides ideal *in vivo*-mimicking environment for the cells. Quite importantly, the collagen scaffold accommodating the stem cell-derived islets can be used as a transplantation platform for extrahepatic islet transplantations. The retrieval of cultured islets upon the completion of the culturing period is unnecessary. Collagen scaffold enhances the post-transplant revascularization and improves the engraftment in the extrahepatic islet transplantations[28].

Although dynamic culture demonstrated several advantages over static culture in this study, there are some study-specific limitations. First, although using human pancreatic islets from deceased donors in our study has an advantage in developing a clinically relevant strategy, obtaining the human islets in uniform quality is difficult compared with the islets from animals. It is well-known that the characteristics of the isolated human islets are quite diverse[29]. Characteristics of human islets, such as morphology, function, and metabolism are significantly affected by the donor factors[30,31]. This biological variability makes data analysis challenging, especially when demonstrating statistical significance. Thus, islet batch-to-batch differences should be carefully considered in data interpretations.

Second, our study demonstrated the proof of concept of dynamic culture on the microwell-collagen scaffold in a small-scale setting at 500 IEQ per a 12 mm chamber within a 35 mm well platform. For clinical application, we could fabricate the up-scaled 10 × 10 cm platform at a similar islet seeding density, which accommodates ~45,000 IEQ (450 IEQ/cm^2). As a potential example for the subcutaneous site Islet transplantation, we could layer four of the 10 × 10 cm scaffolds with islets after the culture period and transplant the layered-islet scaffolds into three different sites (ie, 12 scaffolds in total), which accommodates a total of 540,000 IEQ, the typical islet number for clinical transplantations. In fact, the simplicity and cost-effectiveness of our method, which can be readily scaled up and potentially automated, represent significant advantages of our platform. The process of depositing water droplets in a moisture-controlled environment to create collagen microwell scaffolds is easily adaptable to larger sizes as required. Furthermore, integrating the dynamic culture circuit with commercially available large cell inserts to accommodate the scaffolds is technically feasible and straightforward. We are progressing with the upscaling of our dynamic culture systems as described above.

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Authorship

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Data Availability Statement

All data required to evaluate the conclusions in the study are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be available to researchers upon request.

Ethical Approval

Human pancreata of deceased donors processed in this study were approved for research by the Institutional Research Board of City of Hope (IRB # 01046), and informed consent was obtained from family or relatives of the donors. No animal experiments were involved in this study.

Statement of Human and Animal rights

Experimental animal procedures are not involved in this study.

Statement of Informed Consent

Human islets were isolated from the human pancreata of deceased donors with informed research consent in place.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: This study was performed as a collaborative study between Tokai Hit and Arthur Riggs Diabetes & Metabolism Research Institute of City of Hope. KI, NK, and TT are the employees at Tokai Hit. The remaining authors declare no competing interests.

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Supplemental Material

Supplemental material for this article is available online.

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