# In situ type I oligomeric collagen macroencapsulation promotes islet longevity and function in vitro and in vivo

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## Abstract

Widespread use of pancreatic islet transplantation for treatment of type 1 diabetes (T1D) is currently limited by requirements for long-term immunosuppression, limited donor supply, and poor long-term engraftment and function. Upon isolation from their native microenvironment, islets undergo rapid apoptosis, which is further exacerbated by poor oxygen and nutrient supply following infusion into the portal vein. Identifying alternative strategies to restore critical microenvironmental cues, while maximizing islet health and function, is needed to advance this cellular therapy. We hypothesized that biophysical properties provided through type I oligomeric collagen macroencapsulation are important considerations when designing strategies to improve islet survival, phenotype, and function. Mouse islets were encapsulated at various Oligomer concentrations (0.5–3.0 mg/ml) or suspended in media and cultured for [...] -encapsulated islets showed a density-dependent improvement in in vitro viability, cytoarchitecture, and insulin secretion, with 3 mg/ml yielding values comparable to freshly isolated islets. For transplantation into streptozotocin-induced diabetic mice, 500 islets were mixed in Oligomer and injected subcutaneously, where rapid in situ macroencapsulation occurred, or injected with saline. Mice treated with Oligomer-encapsulated islets exhibited rapid (within 24 h) diabetes reversal and maintenance of normoglycemia for 14 (immunocompromised), 90 (syngeneic), and 40 days (allogeneic). Histological analysis showed Oligomer-islet engraftment with maintenance of islet cytoarchitecture, revascularization, and no foreign body response. Oligomer-islet macroencapsulation may provide a useful strategy for prolonging the health and function of cultured islets and has potential as a subcutaneous injectable islet transplantation strategy for treatment of T1D.

**Keywords:** islet encapsulation, subcutaneous, type I collagen oligomers, type 1 diabetes

## INTRODUCTION

Type 1 diabetes (T1D) is a debilitating and burdensome public health problem affecting an estimated 1.25 million men, women, and children in the U.S. alone (10). The current standard of care is exogenous insulin with whole pancreas transplantation exercised only in the most difficult-to-treat cases in which there exists severe hypoglycemia unawareness. Although insulin therapy is life-saving, it provides inferior control relative to functional islet cells and does not eliminate chronic complications (i.e., cardiovascular disease, kidney failure, retinopathy, and neuropathy) that contribute to a large portion of medical costs and loss of quality of life (41). Even with a strict insulin regimen, T1D patients still experience dangerously high or low blood glucose levels that can potentially be life threatening. In fact, less than one-third of people with T1D in the U.S. consistently achieve target blood glucose levels (25).

Although pancreatic islet transplantation is an attractive therapeutic alternative for T1D patients, this approach remains classified as experimental in the U.S. since long-term safety and efficacy have yet to be achieved (7). At present, clinical islet transplantation involves image-guided infusion of a large number of islets [on average 800,000 islet equivalents (IE) over 2 infusions derived from 4–6 patients] into the portal vein where they become lodged (42). Early results associated with the Edmonton procedure highlighted the potential of this cellular therapy (58). More recently, a multicenter Phase III clinical allogeneic islet transplantation trial was completed, providing favorable data for use in a Biologics License Agreement submission to the U.S Food and Drug Administration (20). Despite these successes, a number of persistent obstacles preclude it from gaining more widespread use. Specifically, there exists a limited supply of quality-controlled donor islets for transplant, and the requirement for long-term immunosuppression has significant side effects (59). Most importantly, the longevity and function of transplanted islets must be improved for patients to achieve long-term insulin independence. Poor islet engraftment and performance have been attributed to loss of critical microenvironmental cues from surrounding extracellular matrix (ECM) and vasculature as a result of islet isolation as well as the rapid blood-mediated inflammatory reaction following islet transplantation (17, 52).

Numerous macro- and microencapsulation strategies, involving natural and synthetic biomaterials, have been developed in an attempt to create a protective microenvironment and physical barrier that prevents detection and accessibility of transplanted islets by the immune system. Unfortunately, these physical barriers also hinder islet engraftment and associated revascularization and reinnervation, which are known to promote islet health and function. Fibrotic capsule formation, largely owing to poor material biocompatibility, further hinders essential mass transport. Incremental improvement has been achieved by *1*) transplanting encapsulated islets into highly vascularized alternative sites (e.g., omentum) (5), *2*) providing supplemental oxygenation strategies (37), and *3*) codelivering angiogenic factors, anti-inflammatory factors, and accessory cells (69, 71); however, reliable and reproducible long-term survival and function of encapsulated islets have yet to be achieved.

Although less understood mechanistically, the interface between islet cells and their extracellular microenvironment provides essential biochemical and biophysical cues that guide not only islet morphogenesis during fetal development but also postnatal homeostatic islet function (26, 27, 65). In vivo, these islet microorgans interact with various ECM components, including fibrillar type I collagen, which is present within and around islets as well as within perivascular capillaries, although species- and age-dependent differences exist (14, 64). Engagement of specific collagen motifs has been shown to promote islet cell survival, differentiation, and β cell function by triggering downstream signaling pathways, in part through integrin and discoidin receptors (53, 56). Additional reports indicate that embedment within three-dimensional (3D) collagen matrixes, in contrast to 2D surface contact, helps to sustain the necessary

cell polarity and organization for prolonged islet function ([12](#), [36](#), [43](#)). Clearly, collagen biophysical properties, including matrix architecture, stiffness, and resistance to proteolytic degradation, are important for guiding proliferation, differentiation, and morphogenesis of various embedded cell types; however, little is known regarding how these parameters influence multicellular functional units, such as islets.

In the present study, we hypothesized that biophysical properties of a rheologically tunable type I oligomeric collagen (Oligomer) were important determinants of islet viability, cytoarchitecture, and function following macroencapsulation in vitro and in vivo. Unlike monomeric (atelocollagen or telocollagen) collagen formulations, which have been previously employed for islet encapsulation, Oligomers retain natural intermolecular crosslinks, which support rapid and robust fibril self-assembly upon neutralization, yielding highly interconnected, D-banded, fibrillar scaffolds ([4](#), [6](#), [32](#), [73](#)). When compared with monomeric collagens prepared at the same concentration, Oligomer matrices exhibit improved mechanical stability (stiffness and strength) and resistance to proteolytic degradation ([6](#), [32](#)). Initial in vitro studies applied confocal microscopy, immunostaining, and glucose-stimulated insulin secretion (GSIS) testing to monitor the dependence of islet viability, cytoarchitecture, collagen remodeling, and function following macroencapsulation at various Oligomer concentrations (fibril densities). Oligomer solutions were then utilized for subcutaneous injection and in situ macroencapsulation of islets within streptozotocin-induced diabetic mice. The subcutaneous space was selected as an alternative transplant site to the portal vein since it offers easy accessibility via injection, potential for monitoring and imaging, as well as retrievability, if necessary, of the transplanted islets. Specifically, short-term (14-day) studies in immunocompromised NOD/SCID mice were used to characterize biocompatibility of Oligomer material and islet engraftment. Follow-up studies evaluated long-term (90-day) survival and function of Oligomer-encapsulated syngeneic islets following subcutaneous delivery in immune competent C57BL/6J mice. Finally, a pilot allogeneic study (60-day) was performed to determine the immunoprotective capacity of Oligomer-encapsulated islets.

## MATERIALS AND METHODS

**Mouse islets.** Mouse pancreatic islets were isolated from 8- to 14-wk old C57BL/6J or CD1 mice (Jackson Laboratory, Bar Harbor, ME) as described ([66](#)). Islet isolations were approved by the Indiana University Institutional Animal Care and Use Committee using AAALAC guidelines. Isolated islets were incubated in RPMI 1640 medium supplemented with 10% fetal bovine serum (HyClone, ThermoFisher Scientific, Waltham, MA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma Aldrich, St. Louis, MO) in a humidified environment of 5% $CO_2$ in air at 37°C before experimental use the next day.

**Oligomeric collagen encapsulation of mouse islets for in vitro culture.** Type I oligomeric collagen (Oligomer) was acid-solubilized from the dermis of market-weight pigs and lyophilized for storage as described previously ([32](#)). The Oligomer formulation was standardized on the basis of molecular composition as well as polymerization capacity according to the voluntary consensus standard ASTM F3089–14 ([2a](#)). Here the polymerization capacity is defined by the matrix shear storage modulus, G′ (in Pa), as a function of oligomer concentration in the polymerization reaction.

Oligomer polymerization kinetics were measured using an AR2000 rheometer (TA Instruments, New Castle, DE) equipped with a stainless steel, 40-mm-diameter parallel plate geometry. Upon lowering the geometry, the Peltier plate was maintained at 4°C for 2 min and then increased to 37°C for 10 min to induce collagen polymerization. Time-dependent changes in shear storage modulus (G′) were measured at 1% controlled oscillatory strain. Polymerization half-time was defined as the time required for G′ to reach half-maximum value ($n = 4$–5 for each formulation).

Oligomer was diluted with 0.01 N HCl and neutralized to final concentrations of 0.5, 1.5, or 3.0 mg/ml, corresponding to polymerized matrices with G′ values of 40, 200, and 1,000 Pa, respectively. Mouse islets

were suspended in the neutralized Oligomer solutions, aliquoted into 96-well plates (30 islets/100 µl; Cellvis, Sunnyvale, CA), and allowed to polymerize at 37°C. Oligomer solutions (in the presence or absence of cells) were maintained on ice (4°C) before warming to 37°C to induce rapid polymerization. Immediately following polymerization, culture medium was added, and the Oligomer-islet constructs were cultured for up to 14 days with medium changes made daily. For comparison purposes, mouse islets were also cultured in a conventional suspension format.

**Assessment of mouse islet viability, cytoarchitecture, and function following in vitro culture.** Islets cultured in suspension or embedded in Oligomer were treated with calcein AM and propidium iodide (Molecular Probes, Eugene, OR) for live-dead determinations. Images were collected using laser scanning confocal microscopy on an Olympus IX81 inverted microscope adapted with Olympus Fluoview FV1000 (Olympus, Tokyo, Japan). Image stacks of 40- to 100-µm thickness with a 3-µm step size were obtained using a 20× air objective, and z-projections were created using Imaris software (Bitplane, Concord, MA).

Immunofluorescence was used to qualitatively assess islet cytoarchitecture and protein expression. Islets cultured in suspension or embedded in Oligomer were fixed in 3% paraformaldehyde (Mallinckrodt, Derbyshire, UK), permeabilized with 0.1% Triton X-100 (Sigma Aldrich), and blocked with 1% bovine serum albumin (Jackson ImmunoResearch, West Grove, PA). Samples then were treated overnight at 4°C with primary guinea pig anti-insulin (PA1–26938, Invitrogen, Carlsbad, CA) and rabbit anti-glucagon antibodies (ab10988, Abcam, Cambridge, MA). Samples were rinsed and then treated with secondary antibodies (A11073, goat anti-guinea pig Alexa Fluor 488 conjugate and A11035, goat anti-rabbit Alexa Fluor 546 conjugate, Life Technologies, Carlsbad, CA) overnight at 4°C. After rinsing, samples were treated with DRAQ5 (Cell Signaling Technologies, Danvers, MA) to stain nuclei.

Quantitative assessment of islet function was performed via GSIS testing. Islets cultured in suspension or encapsulated in Oligomer were prepared within 24-well Transwell culture inserts (Corning, Kennebunk, ME) for up to 14 days. Basal insulin secretion was stimulated by incubating the samples for 1 h with 2.8 mM glucose in Krebs Ringer buffer (0.1% BSA, 25 mM HEPES, 115 mM NaCl, 24 mM NaHCO$_3$, 5 mM KCl, 1 mM MgCl$_2$·6H$_2$O, 2.5 mM CaCl$_2$·2H$_2$O). Following basal secretion, samples underwent static incubation for 1 h each, with low (2.8 mM) followed by high (28 mM) glucose concentrations in Krebs Ringer buffer. Insulin secretion was measured using a STELLUX insulin enzyme-linked immunosorbent assay (ELISA) kit (Alpco, Salem, NH). Stimulation indexes (SI), which represent the ratio of insulin secreted with high glucose over insulin secreted with low glucose, were calculated and normalized to values obtained for freshly isolated (*day 0*) islets.

**Subcutaneous islet transplantation in diabetic mice.** Mouse islet transplantation procedures were approved by the Indiana University Institutional Animal Care and Use Committee using AALAC guidelines. Male 8-wk to 14-wk old NOD.CB17-Prkdc[scid]/J (immunocompromised model; Jackson Laboratories, Bar Harbor, ME) or C57BL/6J (syngeneic and allogeneic models) recipient mice were injected with low-dose streptozotocin (55 mg/kg) for 5 days to chemically induce diabetes before islet transplantation (39). C57BL/6J (immunocompromised and syngeneic models) or CD1 (allogeneic model) islets were mixed with neutralized Oligomer solutions. Oligomer-islet suspensions were then injected subcutaneously through small-bore needles (26 gauge) into diabetic mice. Each mouse received 2 injections, one positioned laterally on each side of the back, with 250 islets/500 µl Oligomer per site for a total of 500 islets/mouse. Additional mice received 2 injections of islets suspended in saline (islets only control; 250 islets/500 µl saline per site) or neutralized Oligomer solution (Oligomer only control; 500 µl Oligomer/site) for comparison. For the immunocompromised model, experimental groups included 1.5 mg/ml Oligomer (*n* = 3), 2.2 mg/ml Oligomer (*n* = 3), 3.0 mg/ml Oligomer (*n* = 3), Oligomer only control (*n* = 3), and islets-only control (*n* = 4). For the syngeneic model, 3.0 mg/ml Oligomer (*n* = 3) and islets only (*n* = 3) were used. For the allogeneic model, 3.0 mg/ml Oligomer (*n* = 3) and 4.2 mg/ml Oligomer (*n*

= 5) were used. Nonfasting blood glucose was measured 3 times per week after transplantation. Diabetes was classified as two consecutive blood glucose levels above 300 mg/dl. Based on preferences and standards established in the literature, a blood glucose level of 250 mg/dl was selected as the "diabetic threshold" ([31], [34]). At the end of the study, the pancreas was removed and histopathological analysis performed to confirm destruction of endogenous islets.

**Intraperitoneal glucose tolerance test.** Intraperitoneal glucose tolerance tests (GTT) were performed at specified times following subcutaneous transplantation to assess islet responsiveness to glucose challenges. Mice were fasted overnight, and then injected intraperitoneally with 2 g/kg of 20% glucose. Blood glucose levels were measured at baseline before injection (*time 0*) and 15, 30, 60, 90, and 120 min following glucose injection.

**In vivo histology and immunofluorescence.** Injection sites and surrounding tissues were removed at specified times and placed in 10% formalin before paraffin embedding and sectioning. Sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MTC). For immunofluorescence, sections were deparaffinized, rehydrated, and stained with primary guinea pig anti-insulin (A0564, Dako, Santa Clara, CA) and rabbit anti-glucagon (sc-13091, Santa Cruz Biotechnology, Dallas, TX) at 1:500 dilutions. Sections were then treated with secondary Alexa Fluor 488 goat anti-guinea pig ([A11073], Life Technologies) and Alexa Fluor 568 goat anti-rabbit ([A11036], Life Technologies) at 1:50 and 1:200 dilutions, respectively. For identification of vascular endothelium, sections were stained with primary mouse anti-CD31/PECAM-1 (BBA7, R&D Systems, Minneapolis, MN) then treated with secondary Alexa Fluor 546 donkey anti-mouse ([A10036], Life Technologies). Nuclei were counterstained with DAPI.

## RESULTS

**Oligomer fibril architecture affects viability, cytoarchitecture, and function of cultured islets.** It has been suggested that loss of critical microenvironmental cues upon islet isolation and culture decreases component cell viability and function, thereby compromising islet health and transplant outcomes ([59], [65]). To better define how biophysical properties of fibrillar type I collagen affects overall islet health and function, isolated C57BL6/J mouse islets were macroencapsulated in Oligomer ranging from 0.5 to 3.0 mg/ml and cultured for 14 days. Previous reports show that increasing Oligomer concentration yields matrices of increased stiffness (G′) as a result of increased fibril density ([32]). Freshly isolated islets and islets maintained in conventional suspension culture served as positive and negative controls, respectively.

As expected, suspension islets showed a progressive loss of viability over the 14-day culture period ( [Fig. 1A]), contributing to altered morphology ([Fig. 2A]) and significant loss of function ($68.2 \pm 2.8\%$), as measured by GSIS ([Fig. 2B], $P < 0.05$). By contrast, Oligomer-encapsulated islets displayed improved viability ([Fig. 1, B–E]) and morphology ([Fig. 2A]), which was dependent upon fibril density. Islet cells actively adhered and exerted contractile forces on the surrounding collagen-fibril matrix, which was evident both macroscopically (construct contraction) and microscopically (islet-collagen interactions). Although the majority (≥66%) of Oligomer-0.5 constructs contracted, hindering further analysis, all Oligomer-1.5 and Oligomer-3.0 constructs retained their volume, displaying minimal to no islet cell death ( [Fig. 1, B and C]) and progressively less local fibril reorganization with increased concentration ( [Fig. 1, D and E]). Immunostaining confirmed that encapsulated islets stained positively for insulin and glucagon, indicating maintenance of cytoarchitecture and phenotype ([Fig. 2A]). Additionally, islets maintained in Oligomer-1.5 and Oligomer-3.0 over the 14-day culture period retained glucose-sensitive insulin release, with normalized SI values of $73.2 \pm 3.0\%$ and $98.9 \pm 5.6\%$, respectively, when compared with freshly isolated islets ([Fig. 2B]).

**Subcutaneous injection and in situ oligomer macroencapsulation of islets supports rapid engraftment and reversal of diabetes in immunocompromised mice.**

Since Oligomer-islet macroencapsulation prolonged islet viability and function in vitro, Oligomer formulations were evaluated for their ability to support subcutaneous delivery and engraftment of islets in chemically induced diabetic mice. This minimally invasive transplantation strategy involved mixing islets within Oligomer solutions followed by subcutaneous injection into streptozotocin-induced diabetic mice as shown in Fig. 3A. Each mouse received 2 injections, one on each side of its back. All mice receiving transplanted islets were injected with a total of 500 islets (250 islets/500 μl per site). Immediately following injection, the Oligomer-islet suspension self-assembled in situ, forming a stable and continuous collagen-fibril matrix that encapsulated and protected resident islets between the panniculus carnosus muscle and skeletal muscle fascial layer (Fig. 4). The polymerization half-time for Oligomer at body temperature (37°C) was $20.26 \pm 0.12$ s, $16.28 \pm 0.03$ s, $16.35 \pm 0.11$ s, as measured rheometrically for Oligomer −1.5, −2.2, and −3.0 mg/ml, respectively.

Initial studies involved transplantation of C57BL/6J mouse islets into NOD.CB17-Prkdc[scid]/J mice, which exhibit acute inflammation but no T- and B cell mediated immune reactions. The goal was to assess short-term (14-day) morphology and function of transplanted islets as well as Oligomer biocompatibility and tissue response. For these studies, Oligomer solutions at concentrations of 1.5, 2.2, and 3.0 mg/ml ($n = 3$ for each group), which correspond roughly to Oligomer stiffness values 200, 500, and 1,000 Pa based upon standardized polymerization capacity, were employed. Based on in vitro results, these groups were selected to define how Oligomer concentration (collagen fibril density) modulated islet survival, phenotype, and transport properties associated with their glucose-sensitive insulin release. Prior to islet transplant, mean blood glucose values were $533 \pm 72$ mg/dl, which was the highest among the mouse strains used for this work.

As shown in Fig. 3B, islets + Oligomer-3.0 provided the most reproducible reversal of diabetes in all animals (consistent maintenance of blood glucose values below the diabetic threshold) and highly regulated blood glucose levels (small SD bars). Additionally, nonfasting blood glucose decreased below the diabetic level within 24 h and remained below the diabetic level for the entire 14-day study period (Fig. 3B). In contrast, both islet only and Oligomer only control groups remained hyperglycemic, above the diabetic level, for the entire 14-day study (Fig. 3B). As shown in Fig. 4, the most uniform encapsulation of individual islets was achieved with Oligomer-3.0, with islets maintaining their normal morphology with evidence of functional revascularization. Islet aggregates were noted within Oligomer-1.5 and -2.2, suggestive of insufficient fibril density for encapsulation and/or inadequate mixing. Immunostaining of Oligomer-islet constructs confirmed a multicellular islet cytoarchitecture with both insulin- and glucagon-producing cells (Fig. 4, D, H, L, and O). Surrounding the islets, the self-assembled Oligomer appeared as normal collagenous connective tissue, which increased in density with concentration. A density-dependent fibroblast infiltration was observed with no evidence of inflammation or foreign body response, which is consistent with previous in vivo studies (8, 76). The fibrillar Oligomer was highly stable and well-integrated within the subcutaneous space for all islet + Oligomer and Oligomer only ($n = 3$) groups (data not shown). In contrast, for the islet only group ($n = 4$), large granulomatous regions were observed with no identifiable islets (Fig. 4, M–O). Such findings are consistent with previous reports that suggest that the subcutaneous space is an inhospitable microenvironment for transplantation of islets alone (30, 46).

**Long-term reversal of diabetes following delivery of oligomer-encapsulated syngeneic islets.** To assess long-term survival and function of Oligomer-encapsulated islets following subcutaneous delivery, C57BL/6J mouse islets were injected into C57BL/6J mice (syngeneic transplant). For this study, only Oligomer-3.0 was used. Mean blood glucose levels on the day of transplantation were $344 \pm 27$ mg/dl. Once transplanted, Oligomer-encapsulated islets showed rapid engraftment, again reversing diabetes within 24 h of transplantation in all recipients (Fig. 5A). Additionally, all mice remained below the diabetic level throughout the 90-day study period with small standard deviations, indicative of tight regulation of blood glucose. In contrast, mice transplanted with islets only remained diabetic with blood glucose values over

400 mg/dl (Fig. 5A). Intraperitoneal glucose tolerance testing (GTT) was performed on *day 15* (
Fig. 5, *B* and *C*) and *90* (Fig. 5, *D* and *E*) to further assess glucose responsiveness of transplanted islets.
Interestingly, mice transplanted with Oligomer-encapsulated islets achieved normoglycemia 120 min post-
glucose injection while blood glucose in islet only mice remained elevated above basal levels (
Fig. 5, *B* and *D*). Analysis of area under the curve (AUC) indicated that values for Oligomer-islet mice
were significantly lower than those for islet only mice at both 15- and 90-day time points ($P < 0.05$;
Fig. 5, *C* and *E*). Histopathologic and immunostaining analyses showed Oligomer persistence at 90 days
with integration into the surrounding subcutaneous tissue compartment and no evidence of chronic
inflammatory or foreign body response (Fig. 6A). Within Oligomer, islets maintained their rounded,
multicellular architecture insulin- and glucagon-positive cells (Fig. 6, *A–C*) and nearby patent vasculature (
Fig. 6B) and CD-31 positive cells (Fig. 6, *D* and *E*). As expected, islet-only explants showed a robust
foreign-body response indicative of graft failure (Fig. 6, *F* and *G*).

### Pilot studies show reversal of diabetes with immune modulation following in situ oligomer encapsulation of allogeneic islets.

To assess function and immunoprotection of Oligomer-encapsulated islets following subcutaneous delivery,
pilot allogeneic transplantation studies were performed, where CD1 mouse islets were injected into diabetic
C57BL/6J mice. Prior to islet transplant, mean blood glucose values were $378 \pm 74$ mg/dl. When
Oligomer-3.0 was applied, blood glucose values declined within the first 24 h; however, animals remained
hyperglycemic (Fig. 7A). Hypothesizing that the level of immune protection may be related to the amount
of Oligomer (or fibril density), follow-up animals were performed using an Oligomer concentration of 4.2
mg/ml, which corresponds roughly to an Oligomer stiffness value of 2,000 Pa and polymerization half-time
of $16.30 \pm 0.02$ s. In this case, diabetes was reversed within 24 h with blood glucose values gradually
increasing above the diabetic threshold after ~40 days (Fig. 7A). Histopathologic and immunostaining
analyses of 60-day Oligomer-islet explants showed insulin- and glucagon-positive islets surrounded by
fibrillar Oligomer (Fig. 7, *B–D*). Interestingly, a subset of islets showed no evidence of a foreign body
response, while others showed very mild inflammatory infiltrate. In some instances, nearby vasculature was
evident (Fig. 7, *E* and *F*); however, vascularization and inflammation events did not appear to be correlated.

## DISCUSSION

Previous attempts to restore islet-ECM interactions via micro- or macroencapsulation strategies have
yielded variable improvement in islet survival and function, with few studies focusing on the role of
material biophysical properties. In this study, we show Oligomer fibril architecture, and its capacity to be
precision tuned, is important when developing a macroencapsulation strategy for sustaining functional islet
masses in vitro and in vivo. Oligomer rapidly transitions from liquid to a solid scaffold, facilitating in situ
macroencapsulation not only within cultureware but also subcutaneously following simple injection
through small-bore needles (26 gauge). The higher-order suprafibrillar assembly and improved proteolytic
resistance imparted by natural intermolecular crosslinks present within Oligomer molecules (6) addressed a
number of shortcomings associated with previous collagen-encapsulation and subcutaneous transplantation
strategies.

Upon islet isolation and conventional culture in suspension, insulin-producing β cells undergo multiple cell
death processes including apoptosis, anoikis, and necrosis, which has been attributed to the loss of critical
cell-ECM interactions, biophysical cues, and vascularization (23, 44, 54, 70). While there is ample
evidence that entrapment of islets in conventional monomeric type I collagen enhances islet survival and
insulin secretory function in vitro (11, 24, 36, 43), these constructs are reported to be prone to contraction,
poor mechanical stability, and rapid degradation (21, 24). This has prompted the use of collagen in
combination with chemical crosslinking agents (21), nature-derived and synthetic copolymers (19, 21, 33,
40), and microfabricated scaffolds (13) to improve mechanical properties and resistance to proteolytic

degradation. For example, Ghahary's group ([21]) reported that treatment of fibroblast populated collagen matrices with glutaraldehye crosslinking and polyvinyl alcohol (PVA)-borate networks yielded improved in vitro islet viability and function compared with untreated controls. Alternatively, islets have been cultured on the surface of collagen-containing decellularized tissues, such as intestinal submucosa ([74]), liver ([75]), lung ([1]), and pancreas ([28]), which, for the most part, are processed to maintain their native architecture and mechanical properties.

In this report, we show that isolated islets, like a variety of stromal cell populations, actively engage Oligomer fibrils, in an attempt to establish tensional homeostasis and a favorable mechanical environment for cell function ([22]). As fibril density and associated matrix stiffness increase, islet cells are less able to induce traction-mediated matrix deformation and reorganization. It is noteworthy that Oligomer matrices possess a higher stiffness-to-fibril density ratio and broader range of tunability compared with their monomeric collagen counterparts ([32]), offering greater resistance to cellular traction forces. Decreasing islet-induced matrix remodeling by fine tuning Oligomer stiffness improved islet survival and cytoarchitecture over the 14-day culture period as well as sustained glucose-responsive β-cell function comparable to freshly isolated islets. The fact that no disintegration or degradation of Oligomer-islet constructs occurred in vitro can be attributed to native intermolecular crosslinks, which are known to decrease collagen turnover in vivo ([3]). While the observed density-dependent phenotypic modulation was likely mediated, in part by, integrin-mediated mechanotransduction pathways, more detailed studies are necessary to elucidate relevant molecular players.

Present-day clinical islet transplantation involves infusion of isolated islets through the portal vein into the liver where they become lodged. Although this procedure is minimally invasive, a number of alternative sites have been evaluated recently ([18], [50]). Our in situ macroencapsulation strategy targeted the subcutaneous space, an advocated site for clinical islet delivery since it provides adequate space for implant accommodation, ease of access for transplant administration and removal (if necessary), and amenability for posttransplantation monitoring ([77]). Although the subcutaneous space facilitates administration, major challenges for islet transplantation include poor oxygen tension and inadequate vascularization ([60]). In fact, islet transplantation, whether alone or encapsulated, into an unmodified subcutaneous site has never reversed diabetes in animals or humans as the microenvironment has been deemed inhospitable to cell survival ([46]). It has been suggested that stimulation of angiogenesis is critical to successful subcutaneous islet transplantation ([47], [51], [55], [68]), prompting investigation of a number of vascularization strategies. Specifically, vascularization using empty devices or other synthetic materials pretransplantation of islets ([16], [35], [45], [46], [61], [62]), oxygen generators ([37], [38]), and cotransplantation of soluble factors (e.g., fibroblast growth factor, hepatocyte growth factor, and vascular endothelial growth factor) ([29], [71]) or cells (e.g., fibroblasts, mesenchymal stem cells, and endothelial cells) ([48], [49], [71]) have all been explored with variable success. For most of these strategies, there is a 1- to 4-wk posttransplantation delay for animals to achieve normoglycemia as islet health and site vascularization and mass transport properties presumably become sufficient for normal glucose homeostasis.

Our approach, which involved subcutaneous injection and in situ Oligomer-islet macroencapsulation, resulted in rapid lowering, within 24 h, of blood glucose values below the diabetic threshold with histological evidence of functional vascularization within 14 days. This uncommon rapid recovery of normoglycemia was observed in immunocompromised, syngeneic, and allogeneic models, suggesting that the natural and stable fibrillar scaffold formed Oligomer acted to sustain the glucose-sensitive insulin secreting function of islets as well as support the necessary molecular diffusion and transport. The mass transport properties of Oligomer, and their dependence on fibril density and molecular size, have been previously defined experimentally and computationally ([72]). Following injection and throughout the study duration, Oligomer-islet constructs remained identifiable, facilitating retrievability from their subcutaneous locations. Oligomer material persisted, appearing as normal collagenous connective tissue with density-

dependent fibroblast infiltration and no evidence of inflammatory or foreign body response, which is consistent with previous reports where Oligomer has been implanted in other anatomical locations ([8], [76]). Clearly, the stability and persistence of Oligomer was critical to prolonged islet survival and function, with multiple groups previously attributing failure of collagen-encapsulated islet transplantation to rapid in vivo degradation ([15], [21], [69]). Also, the volume ratio of fibrillar Oligomer to islets was critical to uniform encapsulation and consistent maintenance of a functional β cell mass, with the highest fibril densities required for allogeneic islet transplants. In the present study, Oligomer contributed to an engraftment response marked by "tissue integration" rather than a fibrotic, foreign-body reaction, which is known to be detrimental to islet health and glucose-insulin transport ([2], [46]). These results were encouraging since alginate, as well as other nature-derived and synthetic materials, have a long history of biocompatibility issues leading to early graft failure due to fibrosis ([9], [57], [67]). To the best of our knowledge, this is the first report of successful subcutaneous islet transplantation with rapid lowering of blood glucose using an injectable in situ Oligomer-islet macroencapsulation strategy.

In conclusion, Oligomer fibril architecture was an important determinant of viability, morphology, and function of transplanted islets as well as islets cultured in vitro. While the exact mechanisms underlying the observed improvement in islet function have yet to be determined, we suppose that the ability of oligomers to recreate fibrillar collagen in its natural and stable format contributes in a multifaceted fashion. More specifically, its high in vivo biocompatibility, with no inflammatory reaction, likely contributes to improved islet health and β cell mass engraftment. The fact that increased survival and function of macroencapsulated islets was observed both in vitro and in vivo suggests that physical support provided by the scaffold, together with receptor-mediated (e.g., integrin) engagement, assists in maintaining essential survival and differentiation signaling pathways. The integration of the oligomer scaffold within the subcutaneous space and associated vascularization supports essential oxygen diffusion and molecular transport while reducing inflammatory cell infiltrate and activation, even in the presence of allograft islets. Additional studies are currently under way to further validate and optimize islet subcutaneous delivery as well as define islet dose-response. The ability of Oligomer to provide important microenvironmental cues that positively impact islet survival while overcoming major shortcomings of conventional encapsulation strategies positions it well as a tool for *1*) in vitro maintenance of islet viability and function following isolation as well as *2*) the design of functional subcutaneous islet transplant solutions.

## GRANTS

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

C.H.S., S.A.T., R.G.M., R.V.C., and S.L.V.-H. conceived and designed research; C.H.S., K.S.O., and A.J.A. performed experiments; C.H.S. and S.L.V.-H. analyzed data; C.H.S. and S.L.V.-H. interpreted results of experiments; C.H.S. and S.L.V.-H. prepared figures; C.H.S. and S.L.V.-H. drafted manuscript; C.H.S., K.S.O., A.J.A., S.A.T., R.G.M., R.V.C., and S.L.V.-H. edited and revised manuscript; C.H.S., K.S.O., A.J.A., S.A.T., R.G.M., R.V.C., and S.L.V.-H. approved final version of manuscript.

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## Figures and Tables

**Fig. 1.**

Islets macroencapsulated in Oligomer show sustained viability and fibril density-dependent traction forces following 14 days of in vitro culture. Representative images of calcein AM (green)- and PI (red)-stained islets after 14 days of culture in suspension (*A*), macroencapsulated in Oligomer-1.5 (*B*), or macroencapsulated in Oligomer-3.0 (*C*). Confocal reflection images show islet-induced collagen-fibril deformation (arrows) within Oligomer-1.5 (*D*) but not Oligomer-3.0 (*E*). Scale bar = 50 µm (*A–C*) and 100 µm (*D* and *E*).

**Fig. 2.**
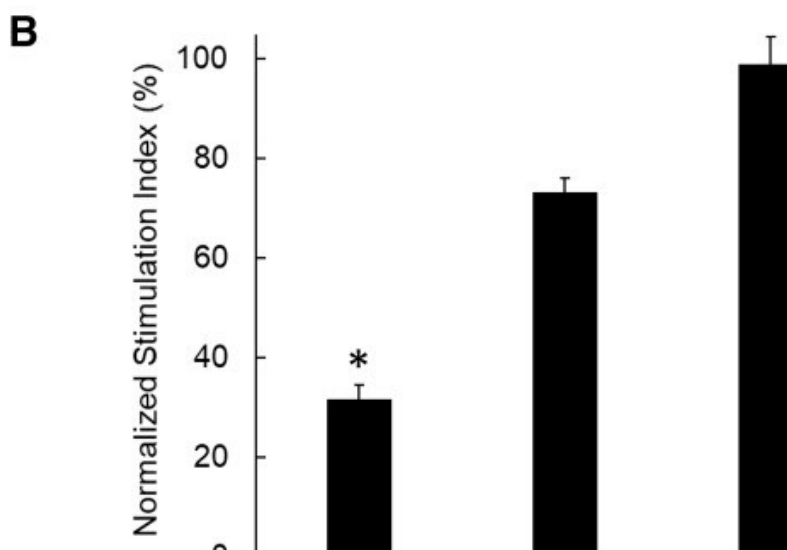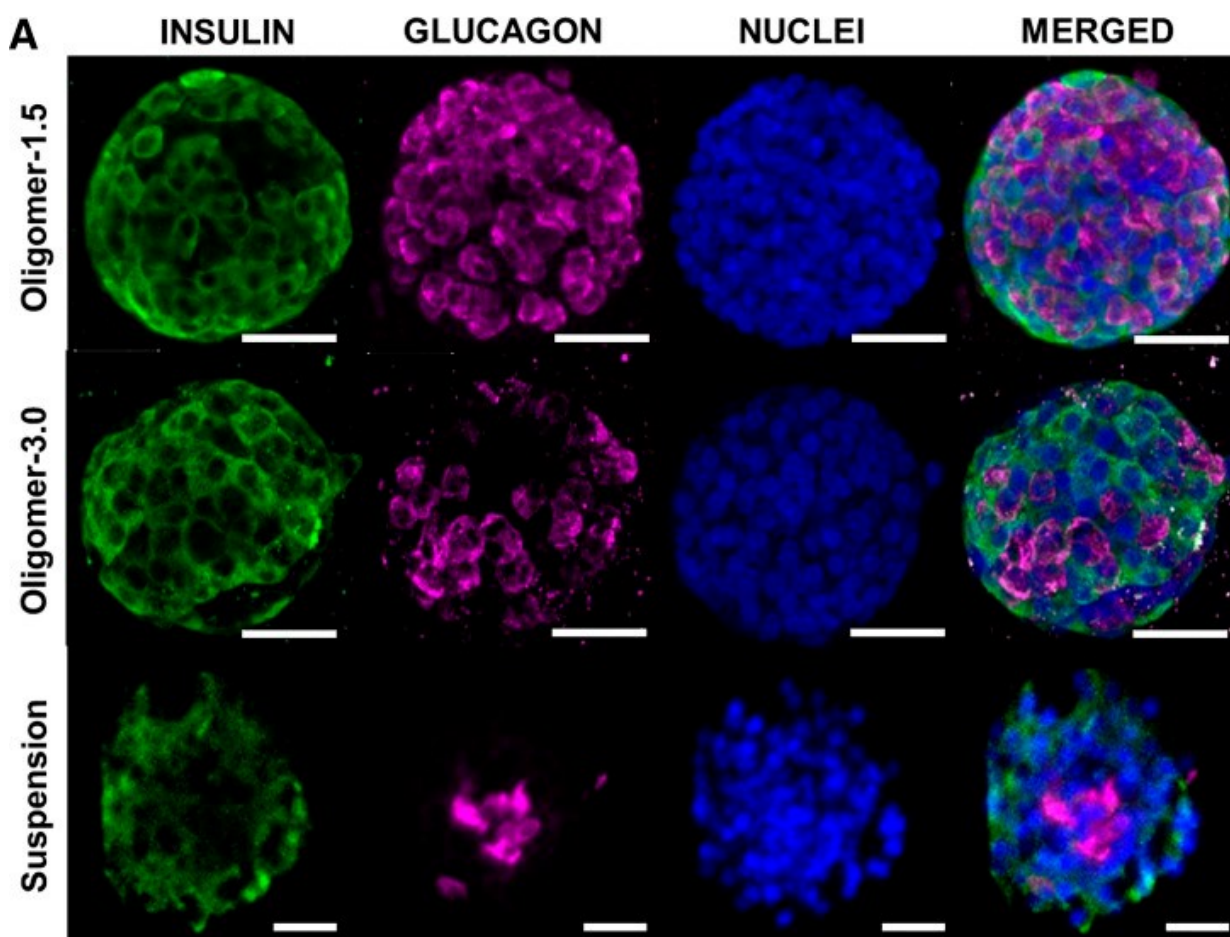


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Islets macroencapsulated in Oligomer maintain morphology and function following 14 days of in vitro culture. *A*: immunostaining shows islet cytoarchitecture with insulin- (green) and glucagon-positive (purple) cells within Oligomer-1.5 and Oligomer-3.0. Islet cell nuclei (blue) were visualized with DRAQ5. Scale bar = 30 μm. *B*: 14-day normalized stimulation indexes (means ± SD; $n$ = 8–12) as measured by glucose-stimulated insulin release. Values were normalized to stimulation indexes for freshly isolated (*day 0*) islets. *Mean SI value for 14-day suspension islets was significantly less than the value for freshly isolated islets ($P < 0.05$).

**Fig. 3.**
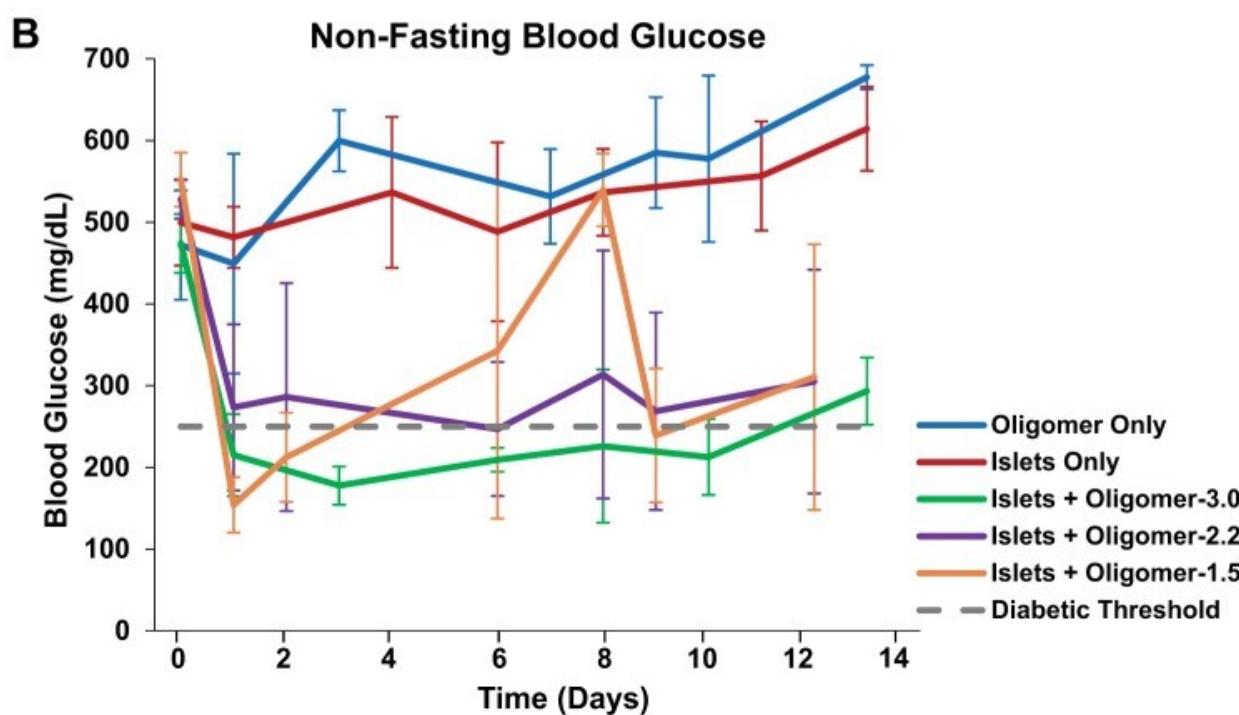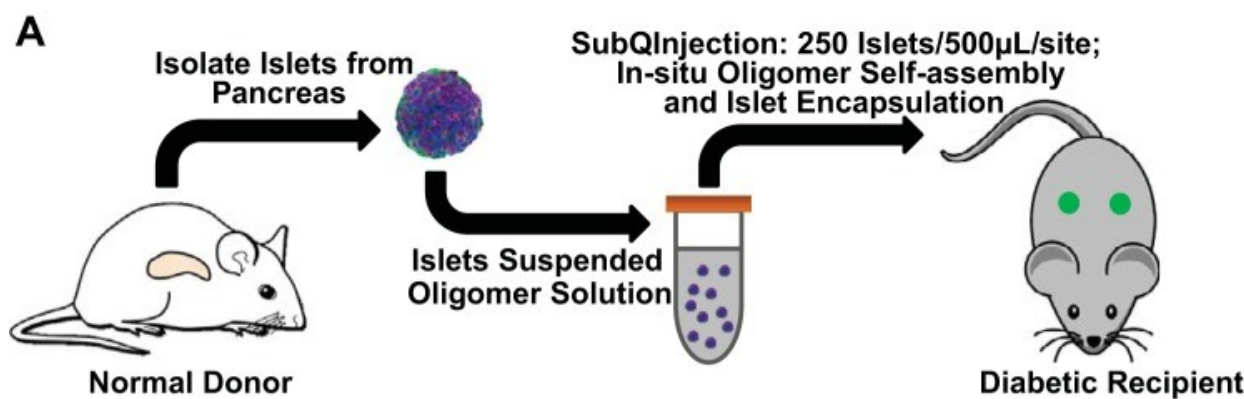


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*A*: schematic summarizing in situ subcutaneous Oligomer-islet macroencapsulation within diabetic recipients. Islets were isolated from normal donor mice, suspended within neutralized Oligomer solutions, and injected subcutaneously into two dorsal sites within streptozotocin-induced diabetic mice. Immediately upon injection, the Oligomer-islet suspension self-assembled, transitioning from a liquid to a solid collagen-fibril scaffold and entrapping islets. *B*: nonfasting blood glucose levels (means ± SD) following subcutaneous macroencapsulation of C57BL/6J islets in various Oligomer formulations ($n$ = 3 for each formulation) into diabetic NOD.SCID mice compared with subcutaneous injection of islets only ($n$ = 4) and Oligomer only ($n$ = 3) control groups.

**Fig. 4.**
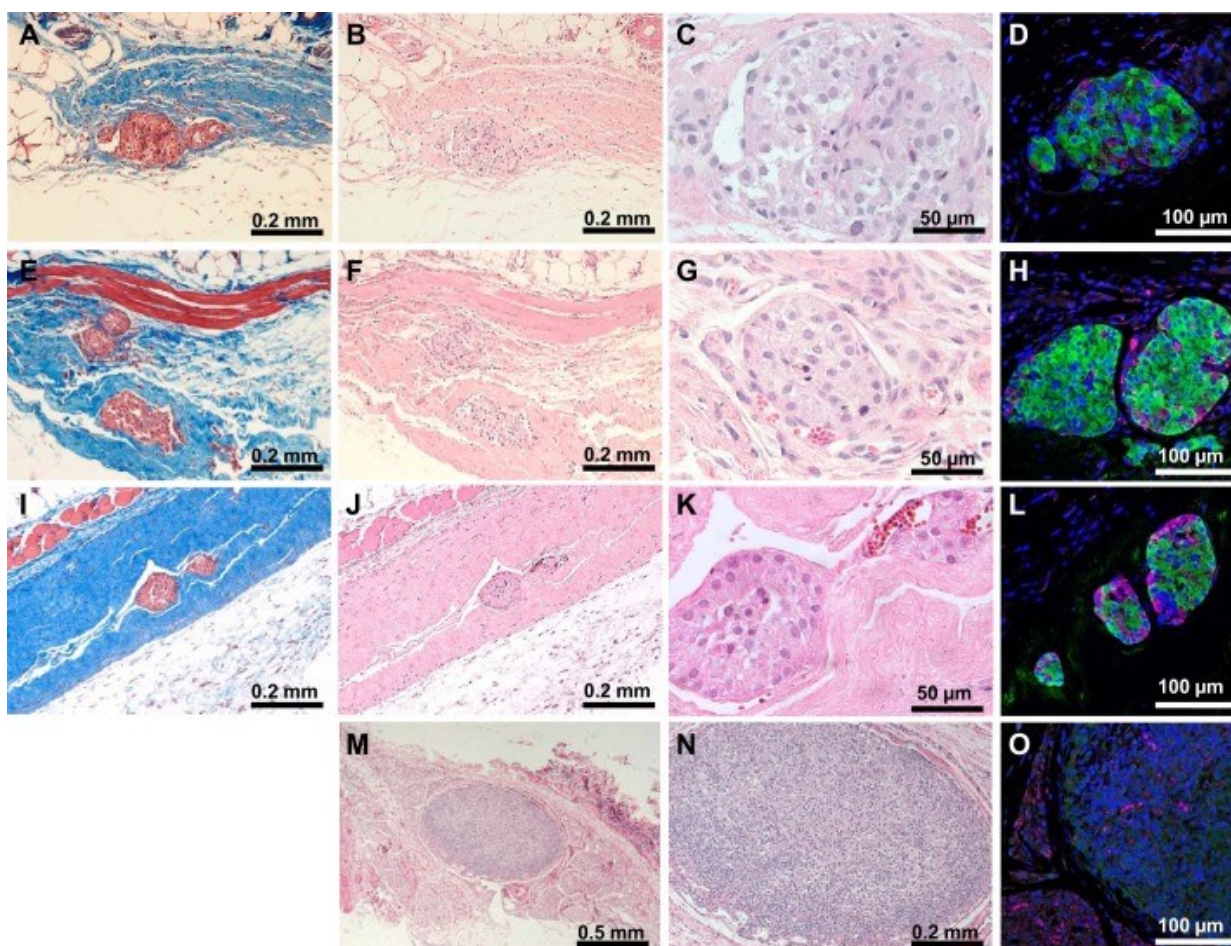


Histopathological and immunohistochemical analysis of C57BL/6J islet explants in 1.5 mg/ml (*A–D*), 2.2 mg/ml (*E–H*), 3 mg/ml (*I–L*), and saline (*M–O*) 14 days following subcutaneous transplantation in diabetic immunocompromised NOD.SCID mice. Oligomer-islet constructs were readily identifiable between the panniculus carnosus muscle (PCM) and the skeletal muscle facial layers (SMF). Masson's trichrome-stained sections (*A, E, I*) showed an increase in the amount of fibrillar collagen and more uniform islet encapsulation as Oligomer concentration was increased. Islets maintained multicellular cytoarchitecture with cells staining positively for insulin (green) and glucagon (purple) (*D*, *H*, *L*, *O*). Cell nuclei were stained with DAPI. H&E-stained sections indicated functional vascularization of islets and no evidence of Oligomer degradation, inflammation, or foreign body response. In contrast, injection of islets only resulted in formation of a large granuloma with loss of normal multicellular morphology and protein expression (*M–O*).
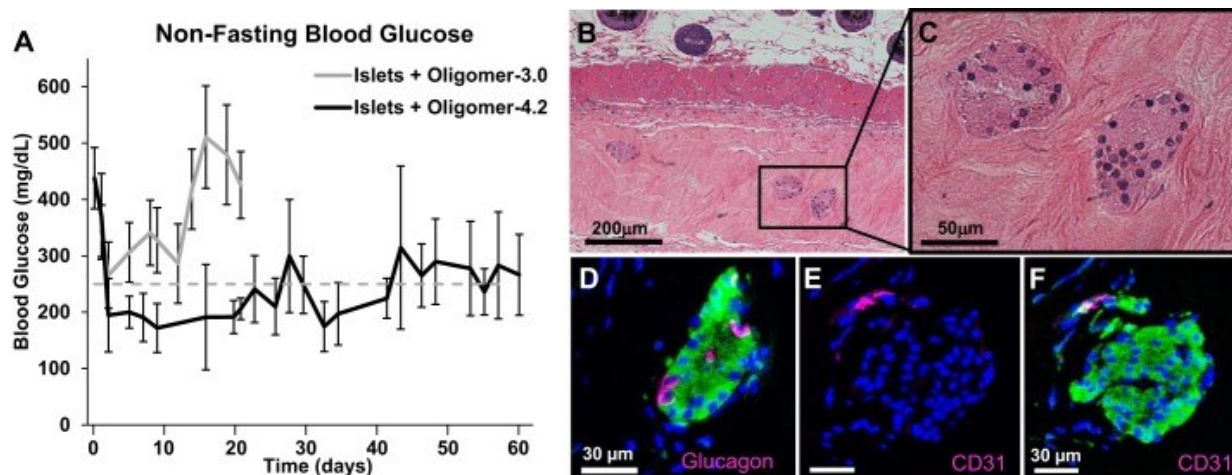
**Fig. 5.**
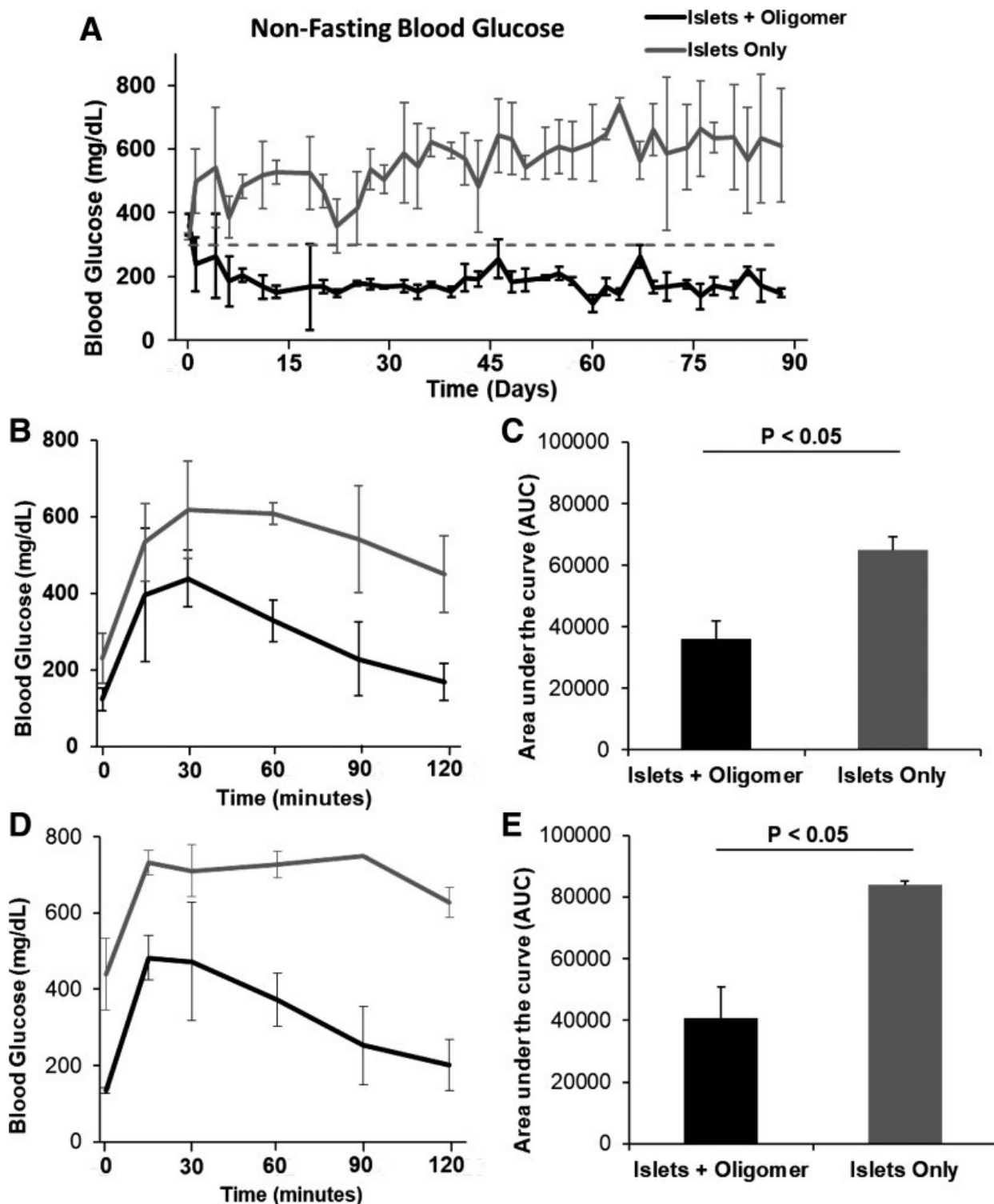
*A*: nonfasting blood glucose levels (means ± SD) following subcutaneous in situ macroencapsulation of syngeneic islets in Oligomer (3 mg/ml; *n* = 3) within diabetic C57BL/6J mice compared with islet only group (*n* = 3). Mice receiving macroencapsulated islets achieved normoglycemia within 24 h following transplantation. Blood glucose remained below the diabetic threshold (<250 mg/dl) throughout the 90-day study period. The control group remained diabetic throughout the study with widely varying blood glucose values. *B–E*: GTT (mean ± SD) and associated AUC (mean ± SD) analysis 15 (*B, C*) and 90 (*D, E*) days following transplantation demonstrated the capacity of islets + Oligomer group but not islets-only group to rapidly regulate blood glucose levels following glucose injection. AUC values for islets + Oligomer group

were significantly ($P < 0.05$) less than those for islet only controls at both time points.

**Fig. 6.**
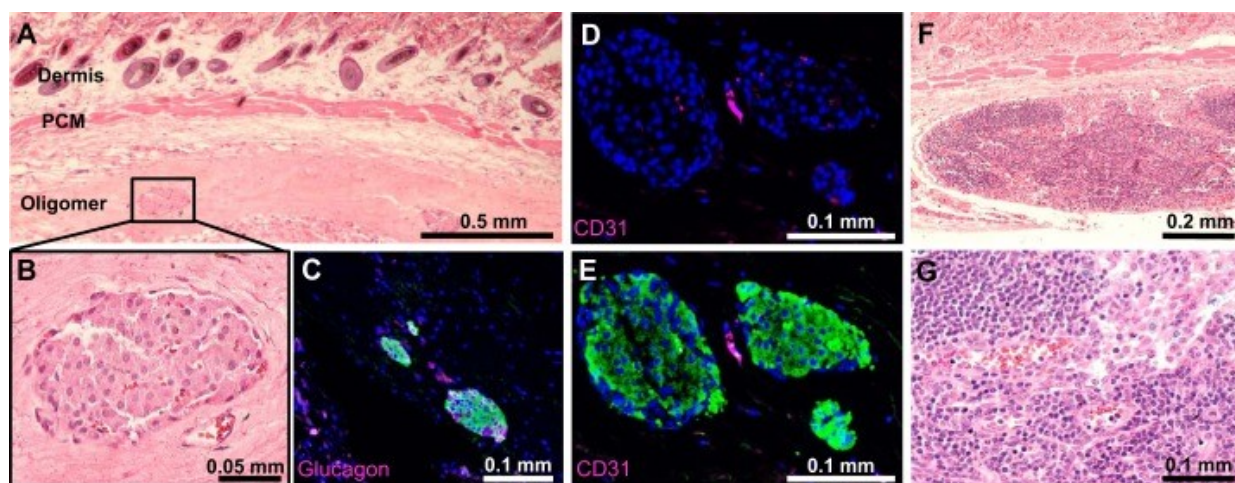


Histopathological analysis of Oligomer-encapsulated (3 mg/ml), syngeneic islets 90 days following subcutaneous transplantation within diabetic mice. *A*, *B*: H&E-stained cross-section showing Oligomer encapsulated islets within the subcutaneous space below the panniculus carnosus muscle (PCM). The self-assembled Oligomer matrix persisted and integrated with surrounding host tissues, with evidence of functional revascularization. *C*: encapsulated islets stained positive for insulin (green) and glucagon (purple). *D, E*: CD31 (purple) staining confirmed the presence of endothelial cells near islets costained to visualize insulin (green) and nuclei (blue). *F, G*: histopathological analysis of explant 90 days following subcutaneous transplantation of islets only within diabetic mice showed evidence of inflammatory-mediated destruction and necrosis of islets.

**Fig. 7.**



*A*: nonfasting blood glucose levels (means ± SD) following subcutaneous in situ macroencapsulation of allogeneic CD1 mouse islets in Oligomer concentrations of 3.0 mg/ml ($n = 3$) and 4.2 mg/ml ($n = 5$) within diabetic C57BL/6J mice. *B–F*: histopathological analysis of Oligomer-encapsulated (4.2 mg/ml), allogeneic islets 60 days following subcutaneous transplantation. *B, C*: H&E-stained cross-sections showing encapsulated islets within the subcutaneous space below the panniculus carnosus muscle. *D*: islets stained positive for insulin (green) and glucagon (purple). *E, F*: CD31 (purple) staining confirmed the presence of endothelial cells near islets costained for insulin (green) and nuclei (blue).