



Abstract

AdoShell is an easily implantable and fully retrievable matrix for islet transplantation aiming to cure diabetes by cell therapy without immunosuppression. It features a hydrogel allowing insulin diffusion while preventing immune cells and antibody exposure. It is reinforced with a surgical mesh and silicon sheets. It has demonstrated immunoprotection and islet functionality *in vitro* and *in vivo*. AdoShell was scaled up for clinical application to enable the loading of a therapeutically relevant quantity of islets. A surgical procedure for implantation by laparoscopy was developed.

AdoShell® Islets showed robust *in vivo* insulin secretion in immunocompetent rats transplanted with allogenic islets (N=4, n=25 rats, 40 days), and in immunodeficient mice with human islets (N=4, n=20 mice, 12 weeks) or sc-islets (N=2, n=15 mice, 19-25 weeks).

AdoShell was optimized with a multi-compartment design accommodating up to 150 kIEQ. Its shape and size allow transplantation of multiple devices for a dose up to 750 kIEQ. Furthermore, the implant was designed for laparoscopic implantation and stapling at the implantation site, the reinforcement frame ensuring high resistance to folding.

AdoShell islets can be manufactured at the islet isolation unit from ready-to-use GMP-compliant reagents. Islets are homogeneously distributed intra and inter compartments (CV=9%) and preserve viability and function throughout the manufacturing process.

A surgical procedure with a protective sleeve was developed to deliver the implant while maintaining its integrity.

In addition to the established immunoprotection and survival of human islets in AdoShell®, the development towards First in Human was completed by the manufacturing process and surgical procedure.

Introduction & Background

- Requirement for immunosuppression after pancreatic islet transplantation makes the benefit/risk balance unfavorable for most patients (Holmes-Walked DJ, *et al.*, Transplantation. 2017).
- AdoShell® is an encapsulation system for islets to achieve transplantation without immunosuppression, which consists of (Figure 1):
 - A silicon ring sandwiching a surgical mesh
 - A hydrogel formed by click-chemistry cross-linking of biocompatible precursors encapsulating islets. It is permissive for nutrients / hormones diffusion but prevents antibody and immune cell penetration.

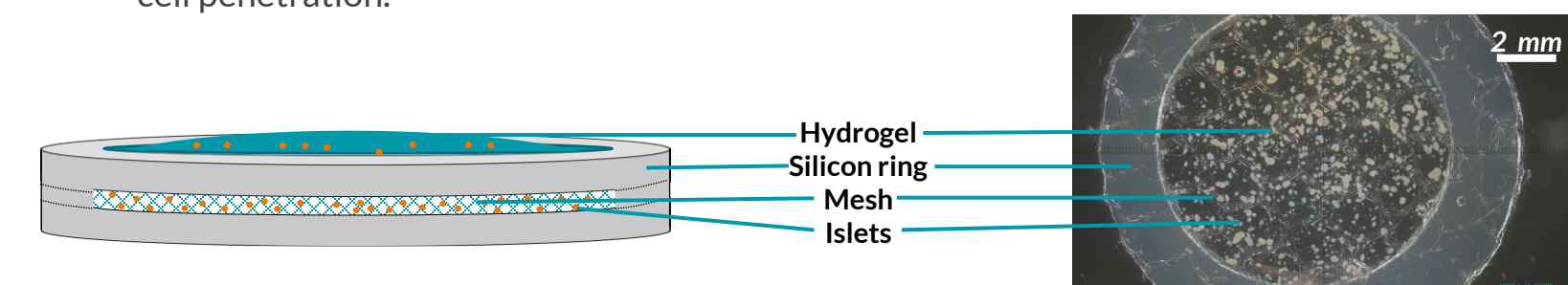


Figure 1: AdoShell® Islets description (left) and prototype for rodent studies (right)

- Encapsulation in the hydrogel maintains islet morphology, as well as insulin (INS) and glucagon (GLC) expression (Figure 2).

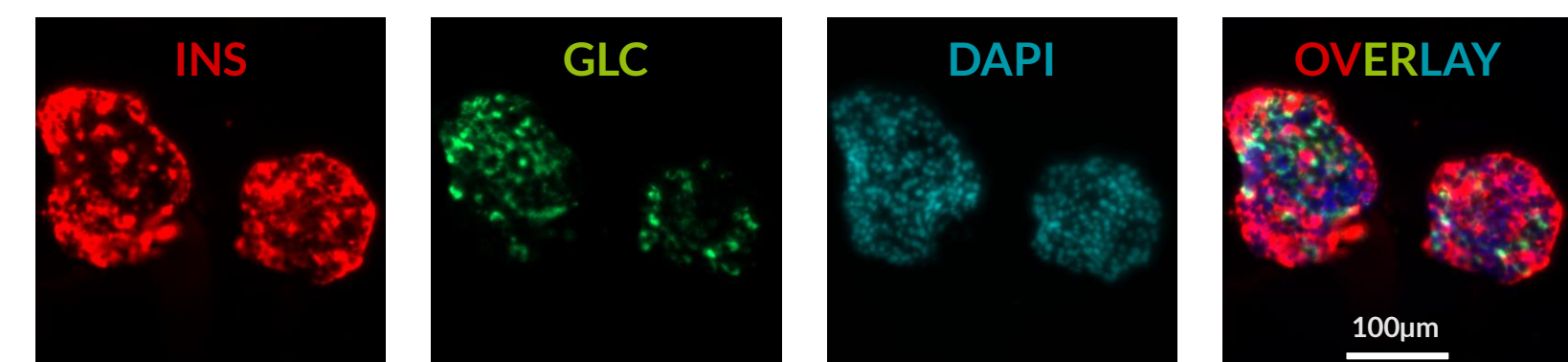


Figure 2 – Immunofluorescence of human islets encapsulated in AdoShell®

AdoShell® *in vitro* immunoprotection

- Naked and encapsulated human islets were incubated for 4 days in the presence of activated human peripheral blood mononuclear cells (PBMCs).
- Contrary to naked islets, encapsulated islets remain fully viable (Figure 3).

- AdoShell allows immunoprotection of primary human islets versus PBMC-mediated death

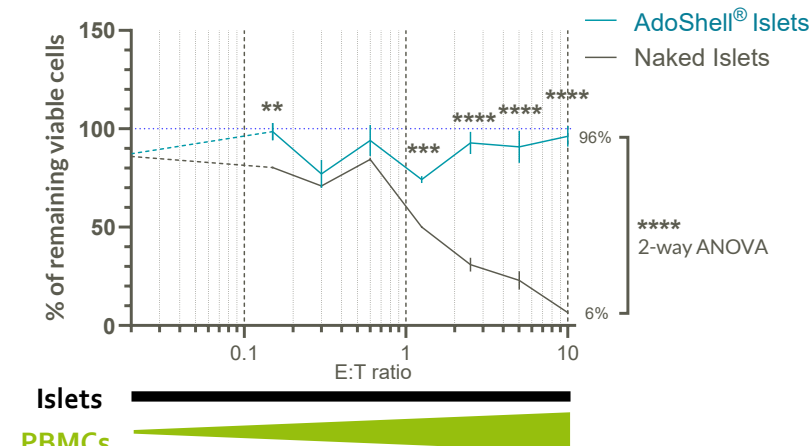


Figure 3 – AdoShell® Immunoprotection of human islets. (bars represent SEM, 2-way ANOVA)

Development toward clinical application of AdoShell®, an encapsulation system for pancreatic islet transplantation

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AdoShell® *in vitro* functionality

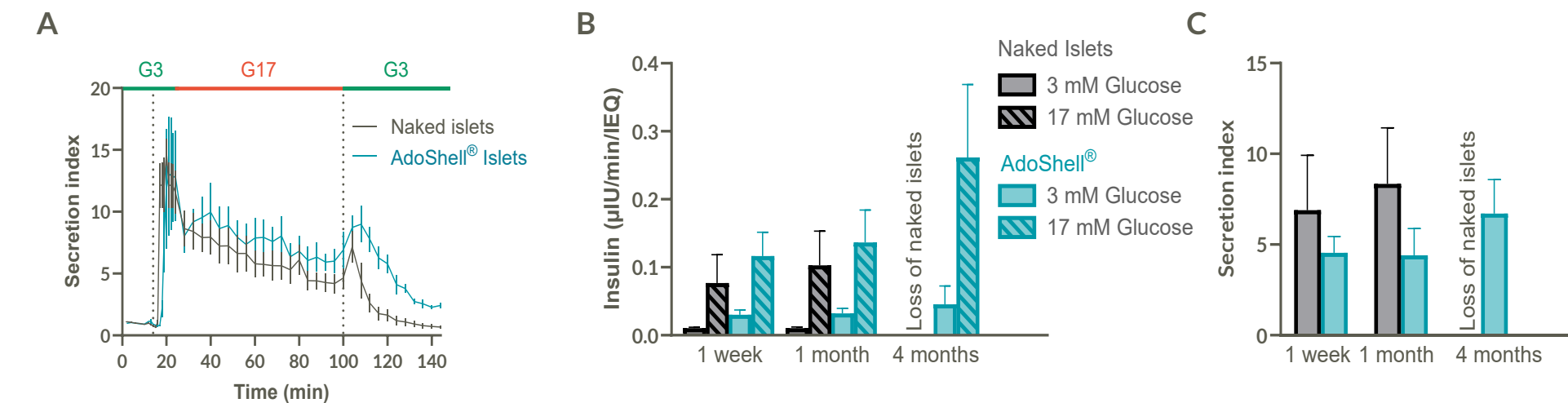


Figure 4 – *In vitro* insulin secretion (bars represent SEM)

- Encapsulation in AdoShell® preserves glucose-stimulated insulin secretion, with a ~2-minute delay in peak response, likely reflecting diffusion through the hydrogel. Return to baseline is also delayed (~12 minutes) (Figure 4 – A).
- Insulin secretion levels are comparable between naked and encapsulated islets, with a slight increase of basal insulin secretion (Figure 4 – B).
- Functionality of encapsulated islets is maintained for 4 months, while naked islets are lost after 1 month in culture (Figure 4 – B and C).
- These results demonstrate that AdoShell® maintains long term survival and functionality of encapsulated human islets.

Methods

AdoShell® Islets in rodents

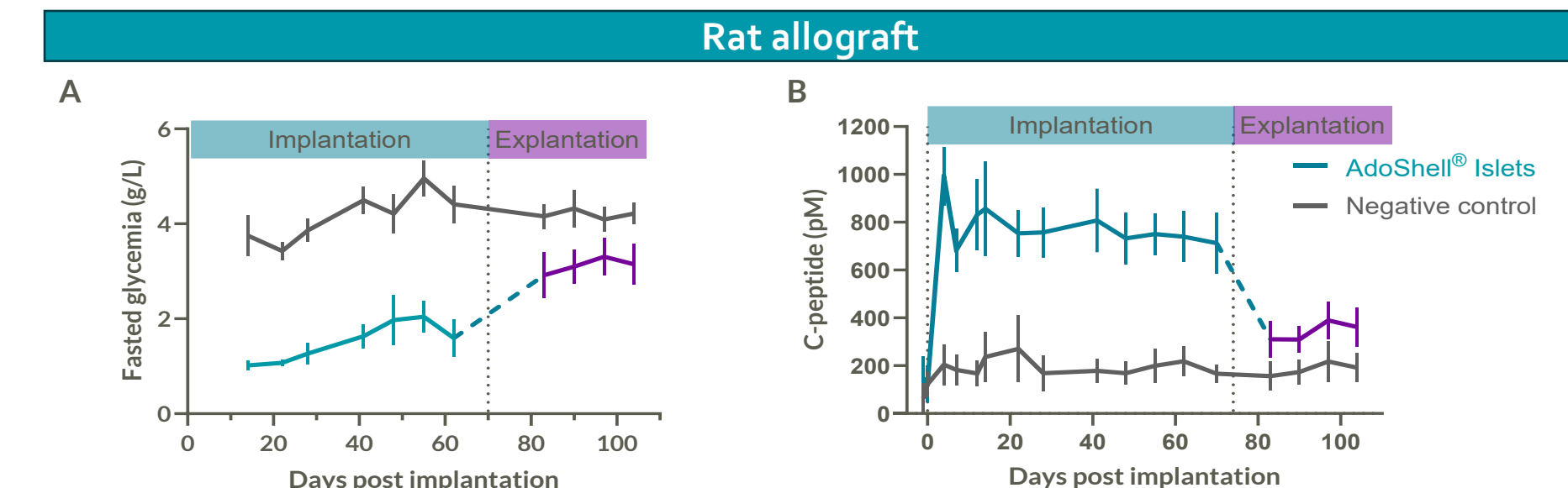
- Rodents (immunocompetent diabetic rats induced with streptozotocin or immunodeficient mice) were intraperitoneally transplanted with AdoShell® containing rat islets, human islets or stem cell derived islets. AdoShell® Islets function was assessed by quantifying rat or human C-peptide plasma concentrations overtime (species-specific ELISA methods).
- Rodents were explanted and analyzed by static GSIS.
- Explant functionality was compared with *in vitro* control implants similarly analyzed on the day of implantation.

Scaled-for-Human AdoShell®

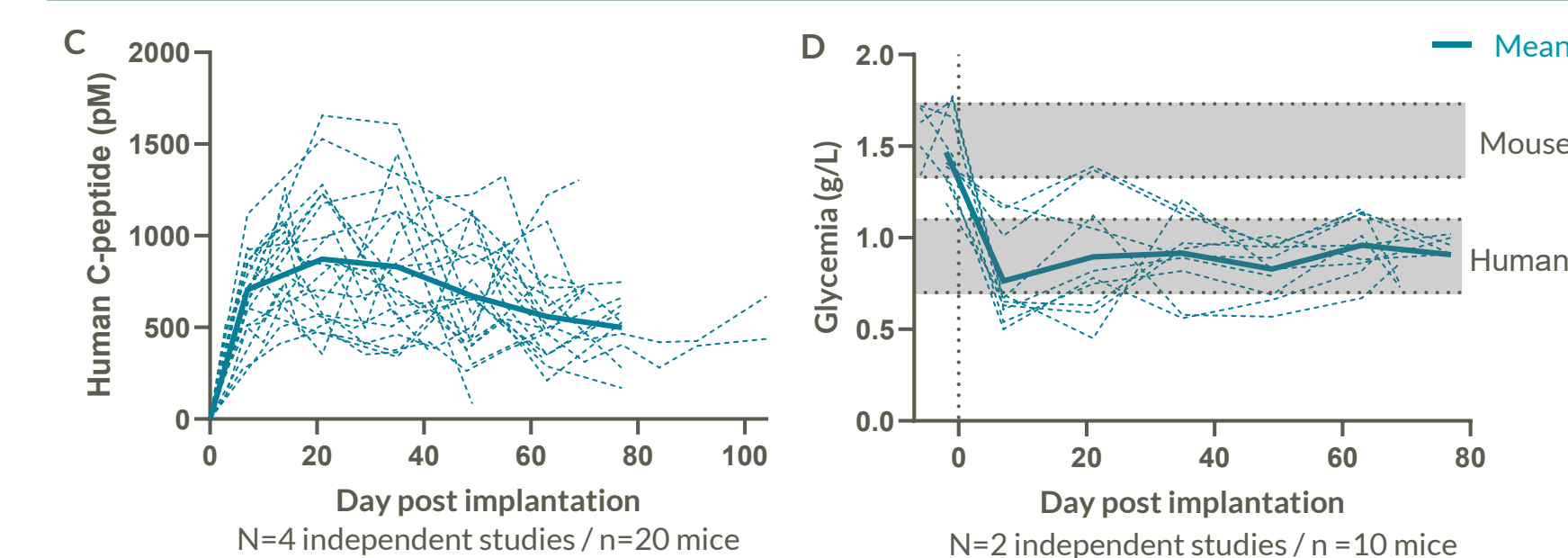
- Manufacturing of human scale AdoShell® Islets was realized at mid scale using the same process but reduced volumes (330µL) or human scale (1mL per compartment) and analyzed by GSIS and imaging methods.
- Human scale AdoShell® was implanted in pig by laparoscopy in a preperitoneal site. It was immobilized with sutures or clips.

Results

AdoShell® *in vivo* functionality



Human islets in NXG mice



Human stem cell derived islets in NXG mice

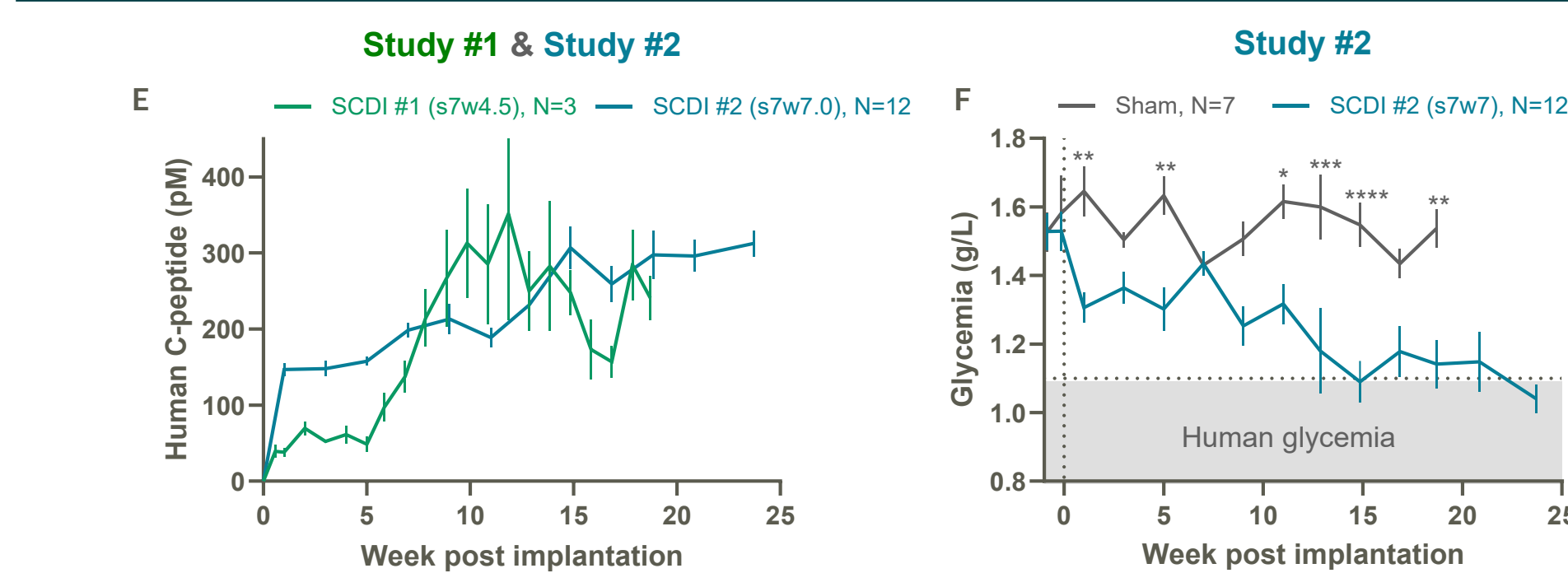


Figure 5 – AdoShell® Islets in rodents (bars represent SEM)

Human islets explants

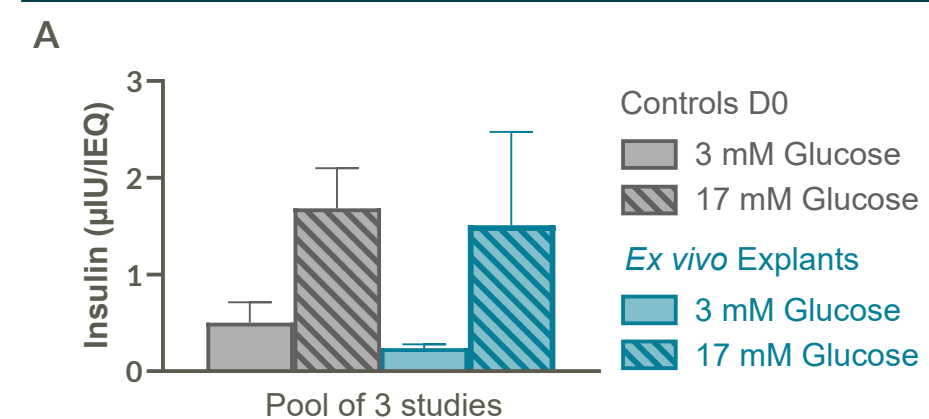
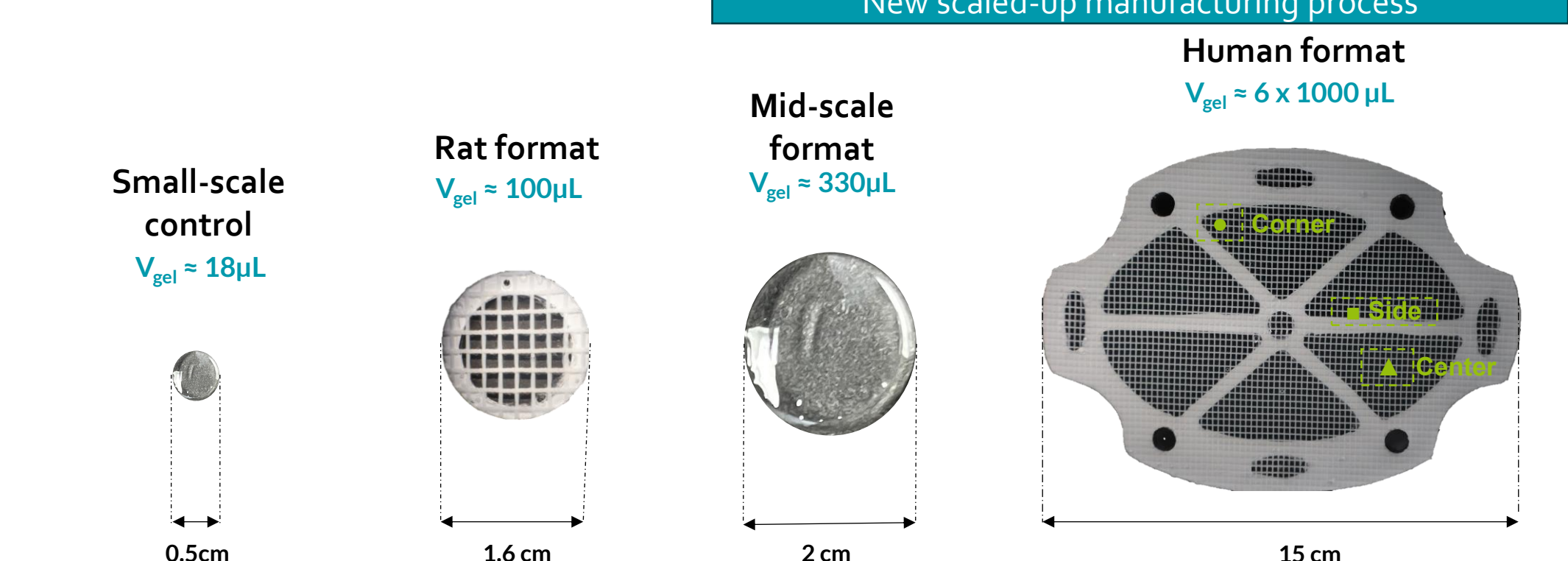


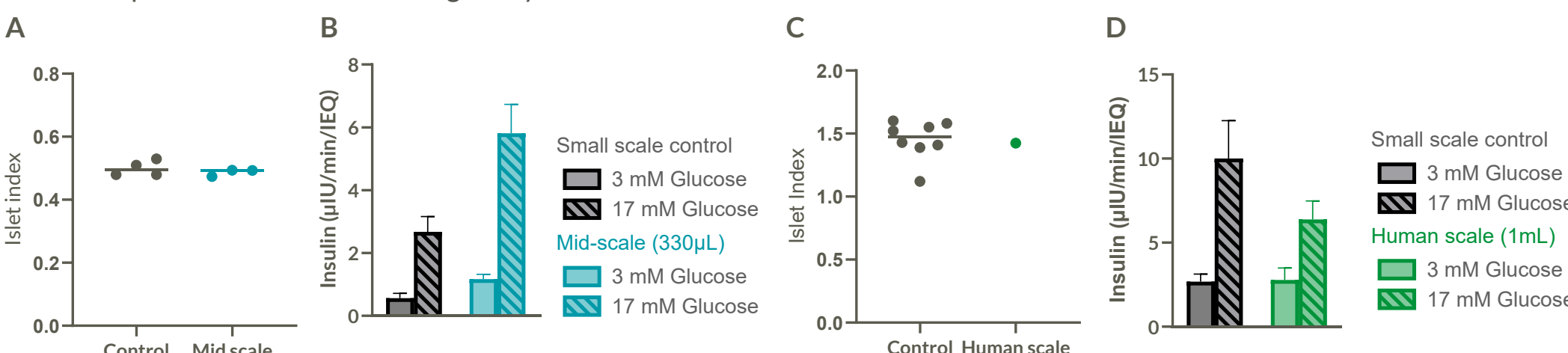
Figure 6 – AdoShell® explants analysis after implantation in rodents (bars represent SEM)

- Implantation of rat and human islets encapsulated in AdoShell® into rodents led to an increase of C-peptide concentration followed by a plateau (Figure 5 – A and C). (Figure 5 – E). With stem cell derived islets a progressive increase in C-peptide levels was observed, consistent with *in vivo* maturation
- C-peptide secretions were associated with glycemic regulation (Figure 5 – B, D and F).
- Ex vivo, explants of human islets after implantation in NXG mice were functional, with similar secretion indexes and insulin secretion levels before (D0) and after explantation (D44 to D90) (Figure 6 – A). On the other hand, explants of stem-cell derived islets showed an increase of insulin secretion and functionality, consistent with their *in vivo* maturation (Figure 6 – B).
- These results demonstrate the maintenance of viability and function of transplanted AdoShell® Islets for > than 3 months.

AdoShell® Islets Scale-up



- AdoShell® is made of GMP compliant reagents and frame. The process can be performed with usual benchtop equipment and is compatible with manufacturing in any clinical islet isolation center.



Inter-compartment homogeneity Mid-scale

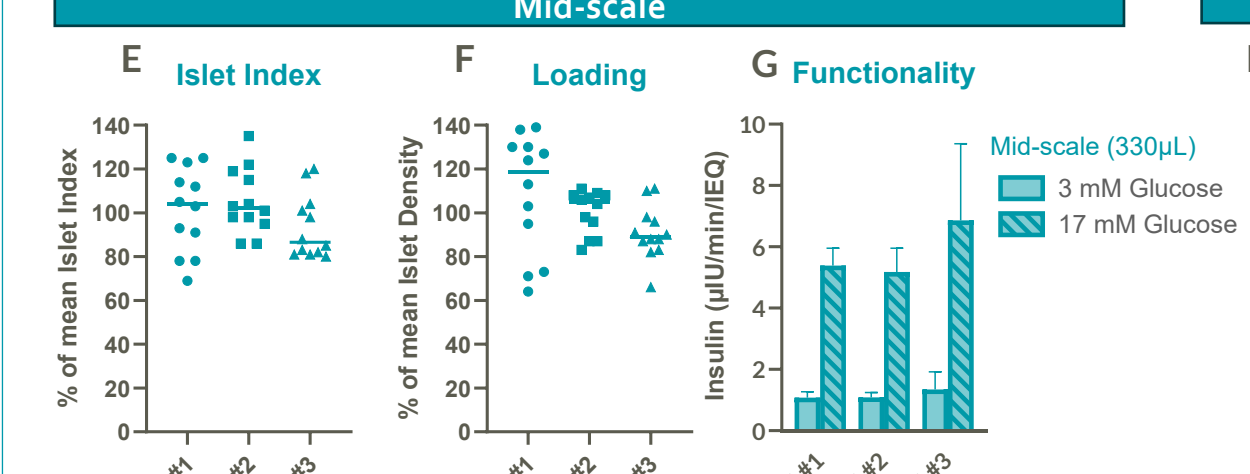


Figure 7 – Maintenance of morphology and functionality over scaling up and intra-inter compartment homogeneity (line represent the mean, bars represent SEM). Each mid scale or human scale implant was sampled for analysis: dot represent individual samples)

- Scaling-up AdoShell® required changes in the manufacturing process to handle larger volumes. These modifications maintain circularity (data not shown), islet index (Figure 7 – A and C) and functionality (Figure 7 – B and D) of encapsulated primary human islets. The overall islet viability is > 90% (determined by Live/Dead staining (not shown)).
- The process is reproducible across replicate compartments and allows to maintain morphology, loading, and functionality. (Figure 7 – E, F, and G).
- The process leads to intra-compartment homogeneity as the circularity (data not shown), islet index, and islet loading are similar in the middle, the sides, or the corners of the human scale implant (Figure 7 – E and F).
- These results demonstrate the conservation of islet properties through the scaled-up manufacturing process and the readiness for clinical deployment.

AdoShell® laparoscopic implantation



Figure 8 – AdoShell® implantation in domestic pig by laparoscopy

- AdoShell® can contain up to 150kIEQ at a density that allowed islet survival in rodent studies.
- Up to 5 implants can be delivered for a total of 750kIEQ which allows the delivery of a therapeutic dose

Conclusions

- AdoShell® provides immunoprotection *in vitro* and *in vivo*.
- AdoShell® ensures long term survival and function of human islets as well as stem cell derived islets.
- Scaling-up AdoShell® to clinical size preserves islet morphology and functionality.
- The manufacturing process ensures homogenous and robust islet distribution and can be performed in a clinical setting with GMP-compliant materials.
- AdoShell® can be implanted by minimally-invasive surgery.
- AdoShell® Islets is a strong candidate for a first-in-human study.