

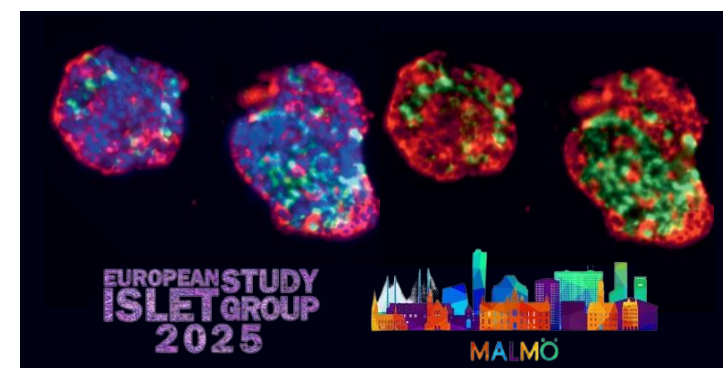


ADOSHELL®: A PERMSELECTIVE AND NON-FIBROTIC SCAFFOLD FOR HUMAN ISLET TRANSPLANTATION

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Abstract

AdoShell® is a fully retrievable, implantable scaffold for islet transplantation as a potential diabetes cure without immunosuppression. Its permselective hydrogel allows insulin diffusion while blocking immune cell invasion and antibody diffusion. Allogenic rat islet transplants in immunocompetent diabetic rats showed prolonged islet survival, insulin secretion, and glycemic control, demonstrating immuno-protection.

We aim to demonstrate AdoShell® compatibility with human primary islets and Stem Cell-Derived Islets (SCDI), its maintenance of long-term islets functionality, viability, and immunoprotective properties *in vitro* and *in vivo*.

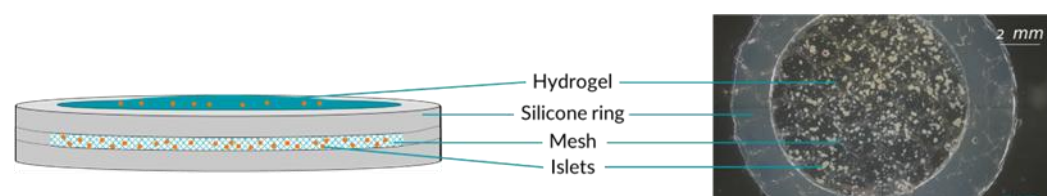
Human islet viability, assessed via LIVE/DEAD and TUNEL assays, was maintained after encapsulation. Morphology and architecture were preserved, as confirmed by immunohistochemistry of α - and β -cells. AdoShell® showed glucose-responsive insulin secretion with a slight 2-minute delay compared to naked islets (perfusion experiments). Functionality was maintained for up to 4 months *in vitro*. To confirm immunoisolation of encapsulated human primary islets, they were co-cultured with IL-2-activated allogeneic PBMCs for 4 days. They showed sustained viability, IEQ number, and total insulin content compared to naked islets exposed to the same conditions. *In vivo*, AdoShell® human Islets in immunodeficient mice exhibited stable C-peptide secretion for more than 2 months (n=10 mice, 2 studies). *In vitro* secretion indexes of explants were similar to pre-implantation levels. Similarly, encapsulated SCDI matured *in vivo* and demonstrated functionality for at least 2 months.

AdoShell® supports primary human islet immunoprotection, long-term survival and functionality, and enables the *in vivo* maturation and functionality of SCDI. AdoShell® is therefore a well-suited platform for cell delivery without immunosuppression.

Introduction & Background

- Cell therapy is a promising treatment for many pathologies (diabetes, hemophilia, liver failure,...). However, the requirement for immunosuppression makes the benefit/risk balance unfavorable for most patients (Sandra Petrus-Reurer, et al., Communications biology. 2021).
- AdoShell® is an encapsulation system for cell therapy 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 cells. It is permselective for nutrients / hormones diffusion but prevents antibody and immune cell penetration.
- AdoShell® Islets preparation
 - Human or rat islets, isolated from deceased donor or allogeneic rat pancreases respectively, were encapsulated by simple mixing with the hydrogel precursor solutions.

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

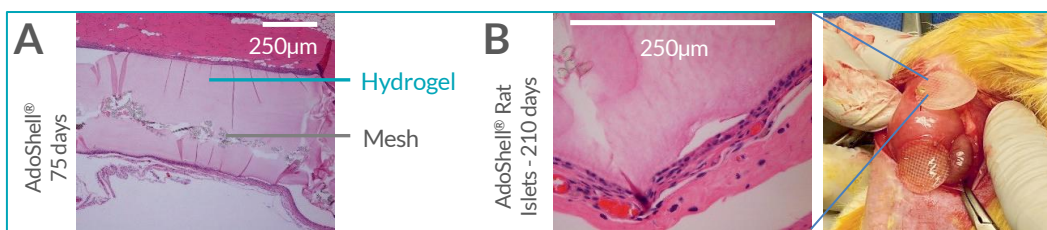


AdoShell® Islets tolerance studies

- AdoShell® Islets Tolerance studies
 - AdoShell® with or without pancreatic islets was implanted and sutured in the peritoneal cavity of immunocompetent rats.

Study	Cells	Receiver	Duration
A	None	Wistar rats	75 days
B	Rat islets	Wistar rats	210 days

Figure 2 - Local tolerance of AdoShell® and AdoShell® Islets in immunocompetent rats

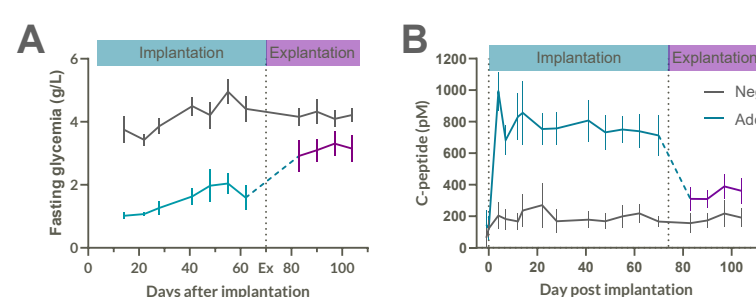


- AdoShell® showed good local tolerance after 75 days of implantation in the peritoneal cavity of immunocompetent rats. (Figure 2 - A).
- Similar results were obtained for AdoShell® containing allogeneic rat islets after 210 days (Figure 2 - B) of implantation in the peritoneal cavity.
- These results demonstrate that the tolerance of AdoShell® containing allogeneic material is compatible with long term graft survival *in vivo*.

AdoShell® for allogenic islet delivery in rat

- AdoShell® Rat Islets in immunocompetent rats
 - Immunocompetent rats were intraperitoneally transplanted with AdoShell® rat Islets (21kIEQ/rat). AdoShell® Islets function was assessed by quantifying C-peptide concentration in rat plasma (ELISA method).

Figure 3 - AdoShell® Rat Islets survival and function in immunocompetent diabetic rats (bars represent SEM)



- Allogenic rat islets encapsulated in AdoShell® and implanted in immunocompetent diabetic rats led to a reduction of hyperglycemia compared to untreated controls (Figure 3 - A). This reduction was linked to an increase in rat C-peptide during the implantation period compared to both before implantation and after explantation (Figure 3 - B).
- AdoShell® ensured good engraftment, metabolic survival, and immunoprotection of encapsulated allogenic rat islets for at least two months *in vivo*.

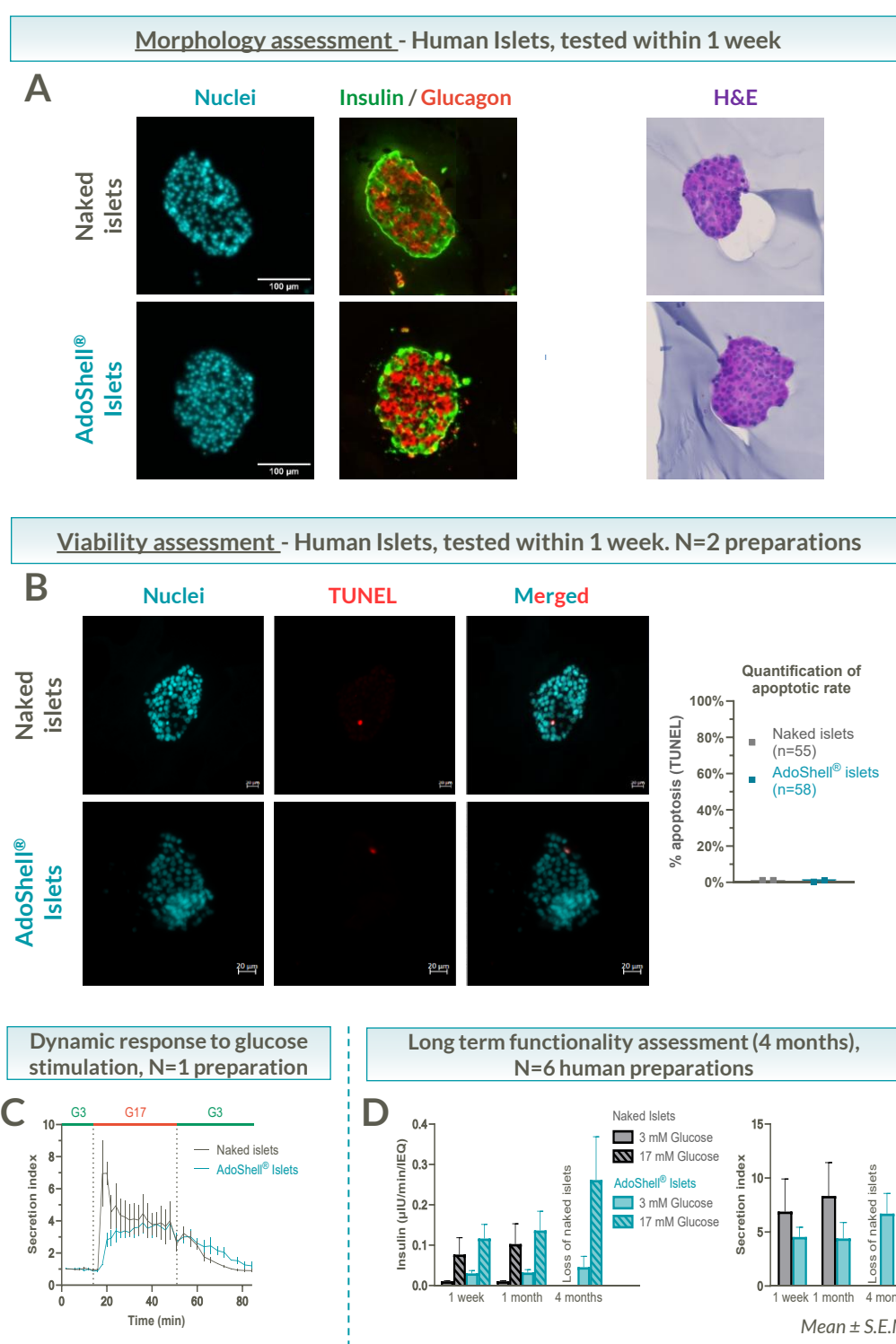
Methods

- AdoShell® Islets *in vitro* functionality
 - Hydrogel disks containing a total of ~200 islets equivalent (IEQ) were evaluated in dynamic or static glucose stimulated secretion assay and compared to naked islets.
- Islet architecture and viability
 - Tissue sections were stained with Hematoxylin and Eosin (H&E) to assess general cellular and structural morphology.
 - Immunohistochemistry was performed using anti-insulin and anti-glucagon antibodies to evaluate islet cell distribution, with DAPI counterstaining used to visualize cell nuclei.
 - Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) was used to detect apoptotic cells and assess tissue viability.
- AdoShell® immunoprotection
 - Human peripheral blood mononuclear cells were obtained from the blood of donors (PBMCs) and activated by incubation with rIL-2.
 - Naked and encapsulated human islets were exposed to increasing doses of PBMCs.
 - Islets were counted and their viability was measured by Live/Dead staining.
 - Remaining islets were lysed, and total insulin content was determined by ELISA method.
- AdoShell® Human Islets in immunodeficient mice
 - Immunodeficient NXG mice were intraperitoneally transplanted with AdoShell® Human Islets (2.2 to 3.1 kIEQ/mouse) in 3 independent studies. AdoShell® Human Islets function was assessed by quantifying human C-peptide concentration in mouse plasma (specific ELISA method).
 - Mice were explanted between D44 and D84 and analyzed by static GSIS and histology.
 - Explant functionality was compared with one of *in vitro* control implants similarly analyzed on the day of implantation.
- AdoShell® SCDI in immunodeficient mice
 - Stem cells were differentiated *in vitro* into pancreatic islets following the protocol of Barsby et al. 2022, STAR Protocols.
 - SCDI were encapsulated similarly to human and rat islets.
 - Immunodeficient NXG mice were intraperitoneally transplanted with AdoShell® SCDI or with naked SCDI in the kidney capsule (~300 to 700 SCDI/mouse).
 - AdoShell® SCDI function was assessed by quantifying human C-peptide concentration in mouse plasma (specific ELISA method).
 - Hydrogel disks containing SCDI for a total of ~150IEQ were evaluated in static glucose stimulated secretion assay and compared to naked SCDI.

Results

Maintenance of islet viability, function and morphology after encapsulation in AdoShell®

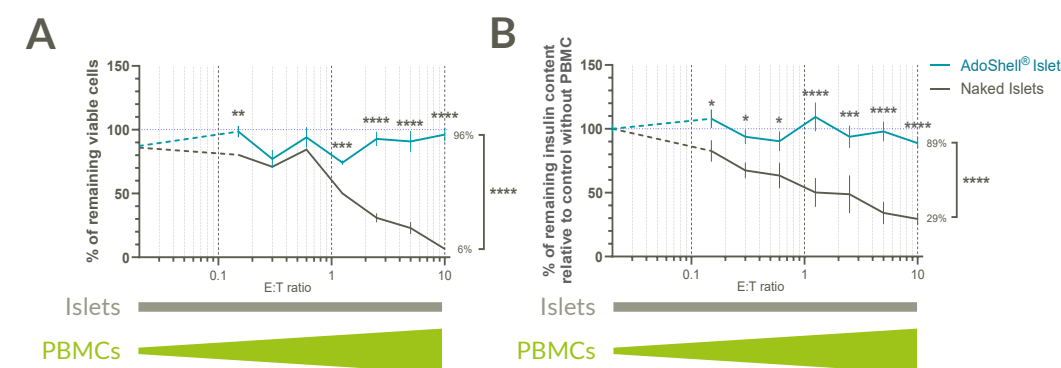
Figure 4 - *In vitro* characterization of AdoShell® Human Islets



- Encapsulation in AdoShell® preserves the structural integrity of human islets and consistently demonstrates high compatibility with islets (Figure 4 - A).
- AdoShell® maintains the viability of encapsulated human primary islets (Figure 4 - B).
- Rapid insulin secretion is maintained, with a 2-minute delay to the first peak and a return to basal levels within 12 minutes (Figure 4 - C).
- Encapsulated human islets demonstrate stable and robust *in vitro* functionality, preserved for at least 4 months post-encapsulation, whereas naked islets died after 1 month (Figure 4 - D).
- In vitro*, AdoShell® ensured good maintenance of morphology and viability of encapsulated islets and a stable metabolic survival for at least 4 months *in vitro*.

AdoShell® Human Islet Immunoprotection

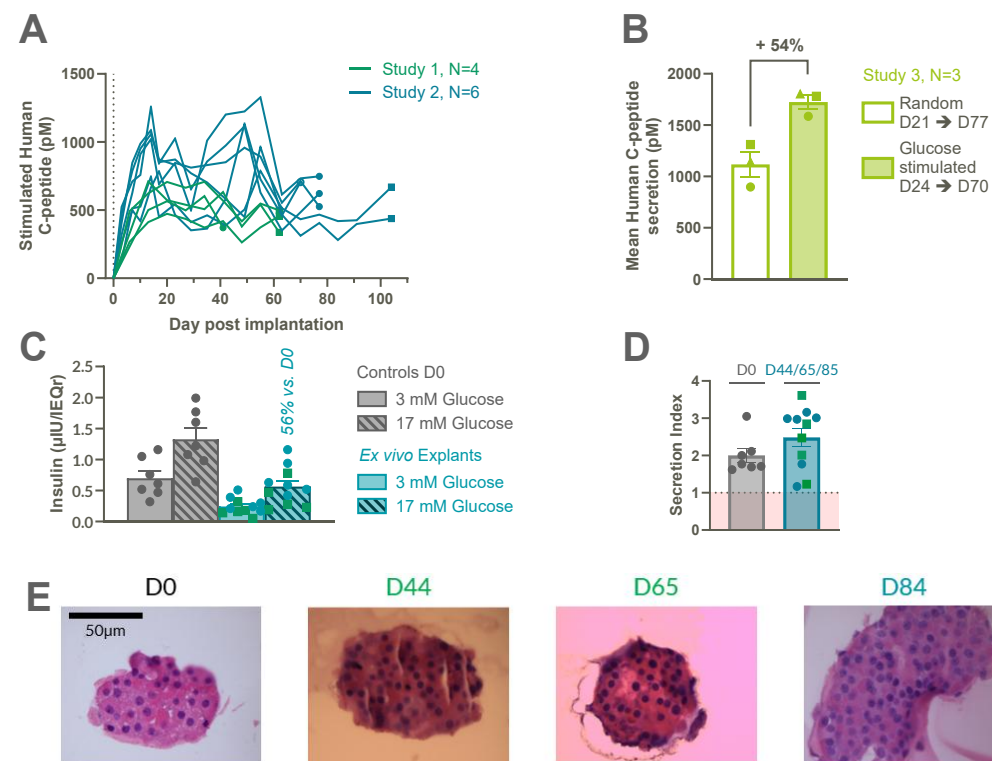
Figure 5 - Immunoprotection of AdoShell® Human Islet from rIL-2 activated PBMCs (bars represent SEM, statistical analysis: two-way Anova)



- Primary human islets were eliminated by exposure to increasing doses of primary human PBMCs. Encapsulation in AdoShell® showed strong protection to cell-mediated death, maintaining most islet cells viable (Figure 5 - A) and their insulin content unaffected by PBMC exposure (Figure 5 - B).
- These results demonstrate the capacity of AdoShell® Human Islets for allogenic immune protection.

AdoShell® for human islet delivery in mice

Figure 6 - AdoShell® Human Islets survival and function in immunodeficient mice (bars represent SEM)

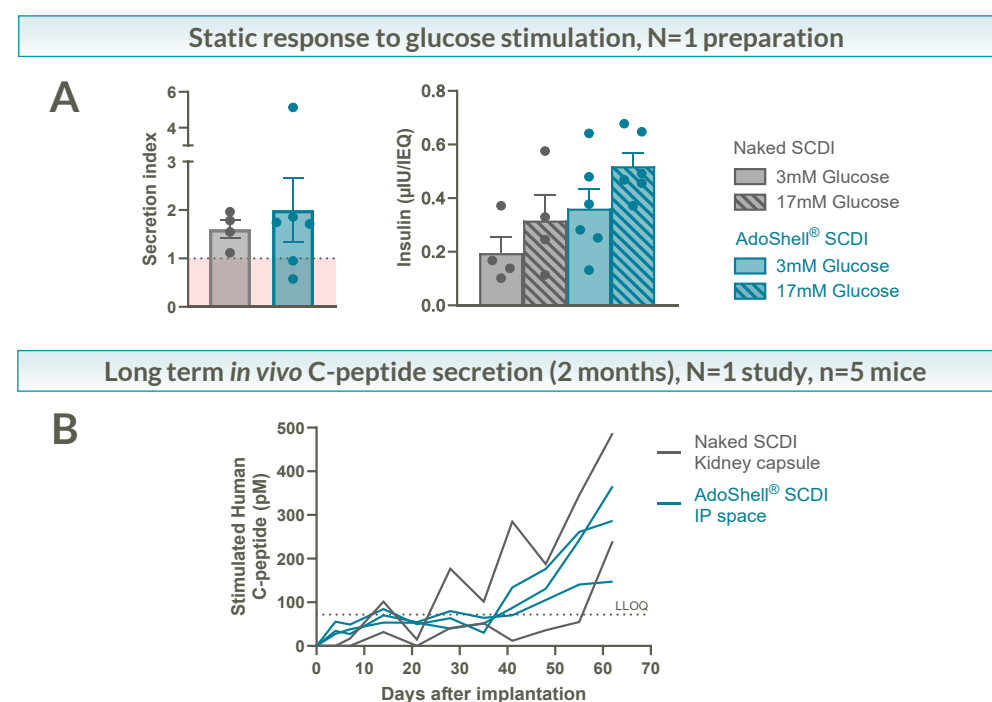


- Human islets encapsulated in AdoShell® and implanted in immunodeficient mice led to mean Human C-peptide concentration of 808.2 ± 280.5 pM at D13/14 followed by a plateau, up to 104 days post-implantation in two different studies (Figure 6 - A).
- In a third study, an increase of 54% of human C-peptide plasmatic concentration 45 min following intraperitoneal glucose administration was observed (Figure 6 - B).
- Ex vivo, explants were functional, with similar secretion indexes before (D0) and after explantation (D44/65/85) (Figure 6 - D). Stimulated insulin secretion levels were 56% of preimplantation values (Figure 6 - C).
- Histological evaluation of the explants showed the preservation of islet structure, thereby confirming islet survival (Figure 6 - E).
- These results demonstrate the maintenance of viability and function of transplanted AdoShell® Human Islets for more than 3 months.

Preliminary data for *in vivo* compatibility of AdoShell® with Stem Cell Derived Islet (SCDI)

- In vitro* characterization followed by xenogeneic transplantation into NXG mice.

Figure 7 - AdoShell® Human Islets survival and function in immunodeficient mice (bars represent SEM)



- Encapsulation in AdoShell® maintains the functionality of SCDI both *in vitro* and after *in vivo* transplantation (Figure 7 - A and B).
- AdoShell® also demonstrates compatibility with the SCDI maturation *in vivo* (Figure 7 - B).
- These results demonstrate that AdoShell® supports the *in vivo* maturation and functionality of SCDI

Conclusion

- AdoShell® is well tolerated *in vivo* without any fibrosis.
- AdoShell® maintains islet viability, function and morphology after encapsulation.
- AdoShell® provides immunoprotection to encapsulated human islets.
- AdoShell® maintains long term *in vivo* function of xenogeneic human primary islets and allogenic rat primary islets.
- Preliminary data suggest that AdoShell® allows *in vivo* maturation of SCDI and maintenance of long-term function.