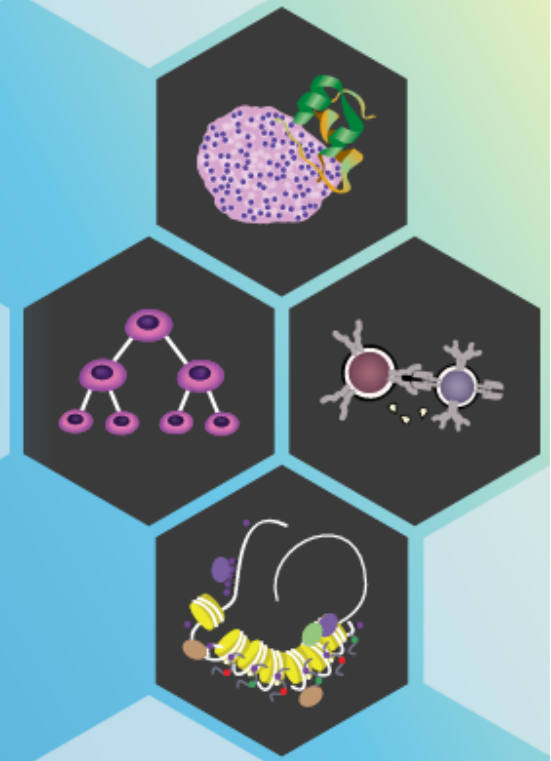
2024 RACHMIEL LEVINE-ARTHUR RIGGS

Diabetes Research Symposium

Encapsulation as a Means for Immune Protection



Alice A. Tomei, PhD

Miami Engineering Career Development Associate Professor, Department of Biomedical Engineering

Director, Islet Immunoengineering Lab, Diabetes Research Institute

University of Miami

Disclosures

- Consultant for ISLA Pharmaceuticals.
- Royalties from Sernova Corp.

This presentation and/or comments will be free of any bias toward or promotion of the above referenced companies or their product(s) and/or other business interests.

This presentation and/or comments will provide a balanced, non-promotional, and evidence-based approach to all diagnostic, therapeutic and/or research related content.

This presentation has been peer-reviewed and no conflicts were noted.

Cultural Linguistic Competency (CLC) & Implicit Bias (IB)

STATE LAW:

The California legislature has passed Assembly Bill (AB) 1195, which states that as of July 1, 2006, all Category 1 CME activities that relate to patient care must include a cultural diversity/linguistics component. It has also passed AB 241, which states that as of January 1, 2022, all continuing education courses for a physician and surgeon **must** contain curriculum that includes specified instruction in the understanding of implicit bias in medical treatment.

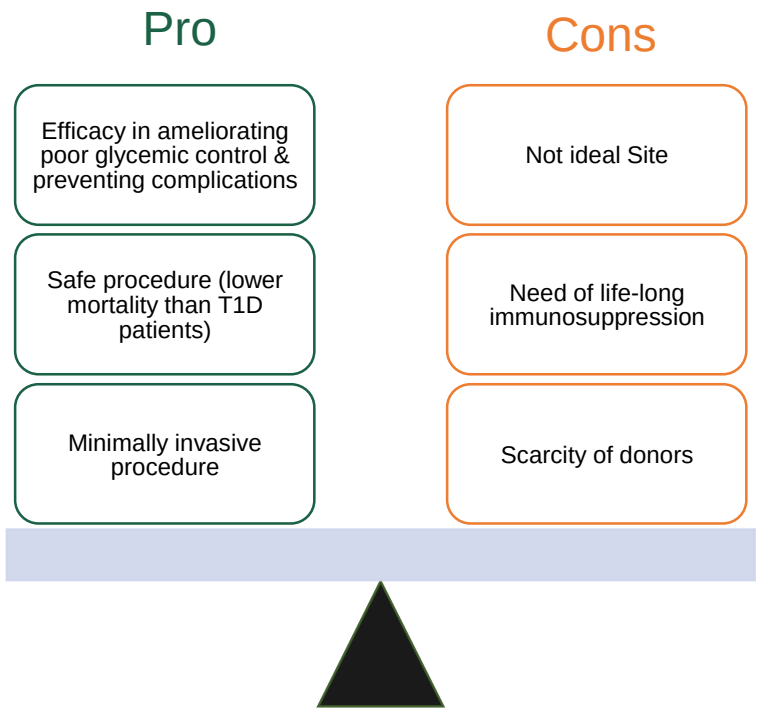
The cultural and linguistic competency (CLC) and implicit bias (IB) definitions reiterate how patients' diverse backgrounds may impact their access to care.

EXEMPTION:

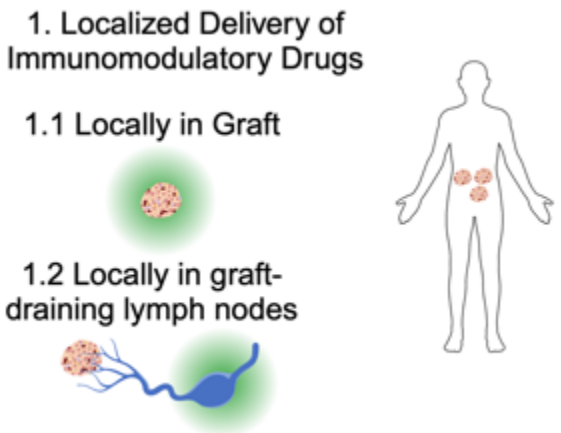
Business and Professions Code 2190.1 exempts activities which are dedicated solely to research or other issues that do not contain a direct patient care component.

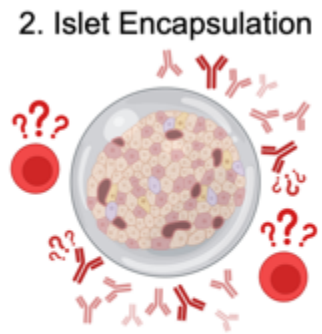
This presentation is dedicated solely to research or other issues that do not contain a direct patient care component.

Addressing Challenges of Beta Cell Replacement for T1D Treatment

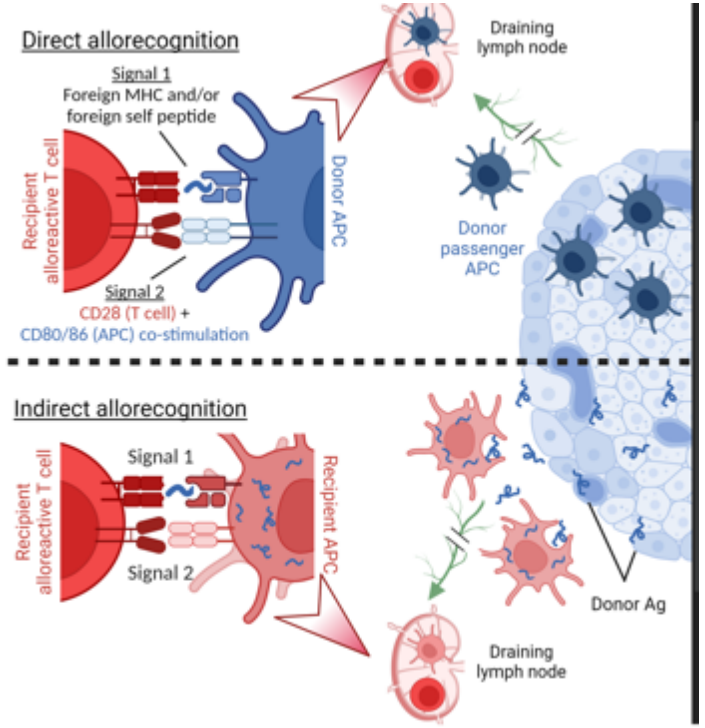


Cell immunoisolation (Encapsulation) and/or Local Immunomodulation Through Biomaterials





Mechanisms of Allogeneic Islet Rejection and Anti-Rejection Therapy Evolution



Azathioprine

Anti-lymphocyte antisera

OKT3

Anti-thymocyte globulin

Basiliximab

Infliximab

Teplizumab

Belatacept

1950 1960 1970 1980 1990 2000 2010

Steroids

Cyclosporin A

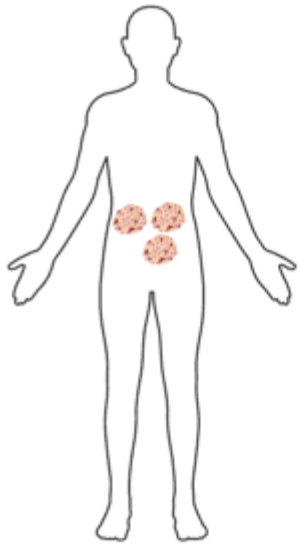
Tacrolimus

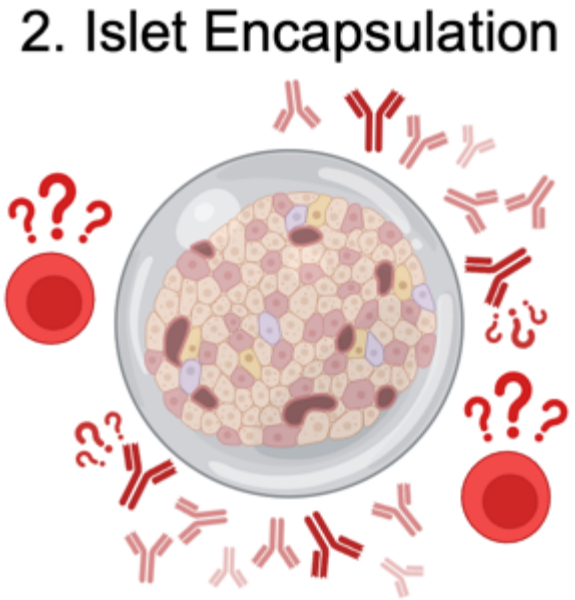
FTY720

Sirolimus

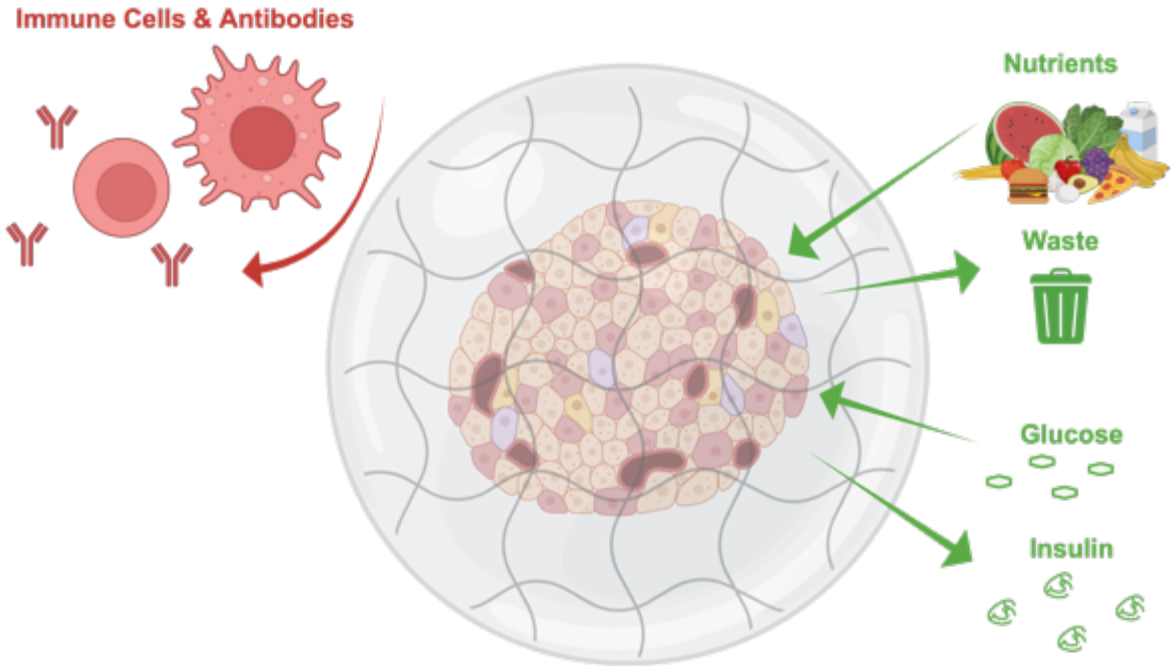
Mycophenolate

Cell immunoisolation (Encapsulation) for Beta Cell Replacement Without Chronic Systemic Immunosuppression





Protecting Insulin-Secreting Cells from Rejection through Biomaterial Encapsulation



Biomaterial coatings need <u>selective permeability:</u>

Yes: nutrients, glucose, insulin

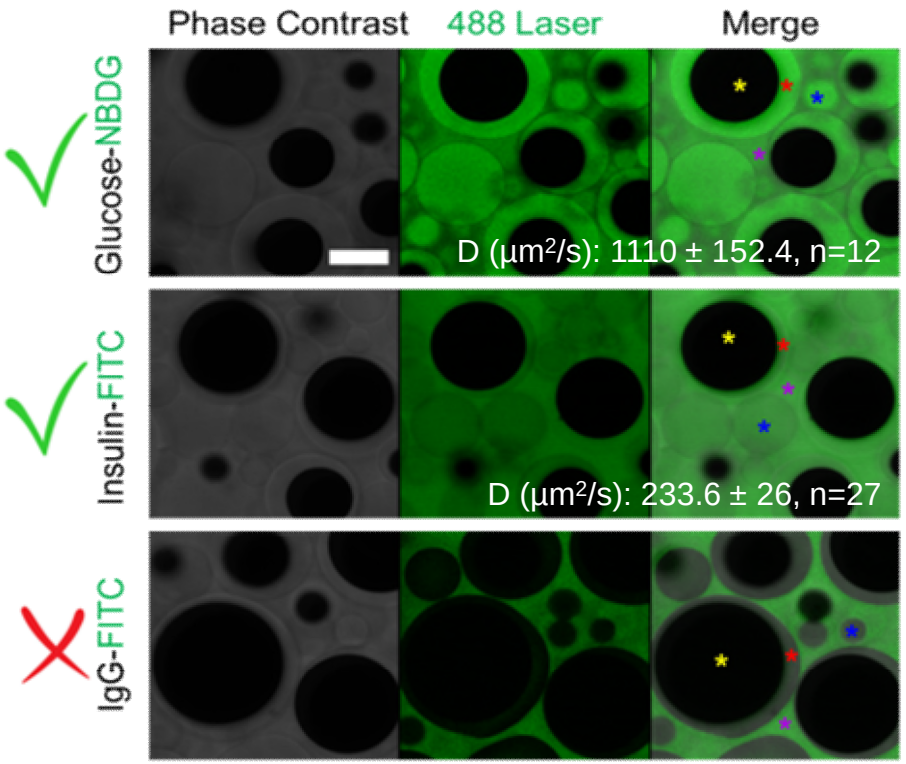
No: cells, antibodies (IgG)

Selective permeability can be achieved using:

- Nanoporous membranes
- Hydrogels with controllable permselectivity

Biomaterial Capsule Requirement: Stable selective permeability for immunoisolation

CC selective permeability

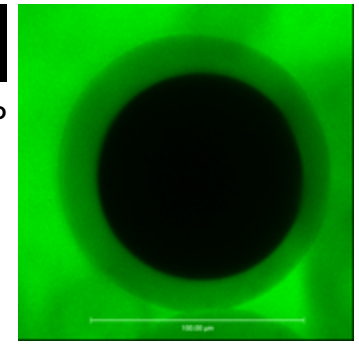


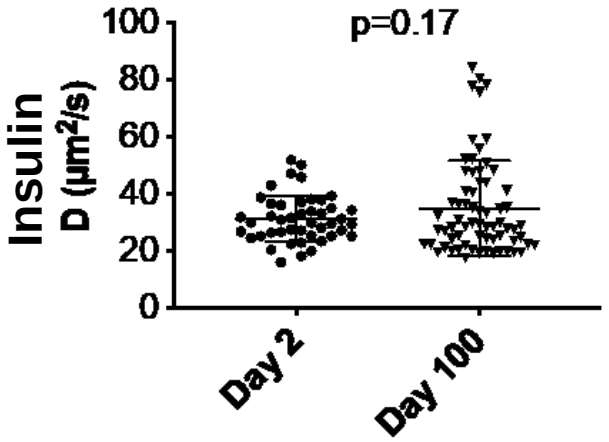
Impermeable to IgG

Long-term stability of CC permeability properties

FITC Insulin

Method: FRAP



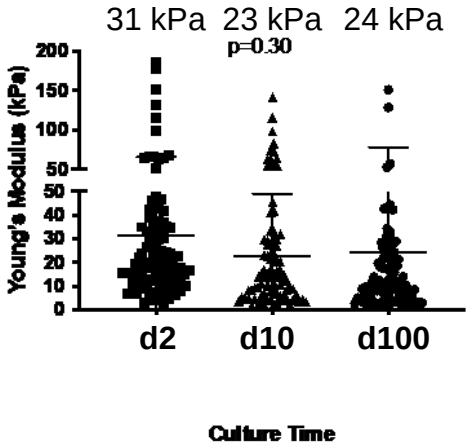


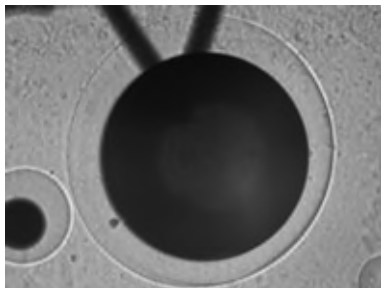
Stock et al., *Science Advances* (2022)

Unpublished

Biomaterial Capsule Requirement: Stable mechanical properties for immunoisolation

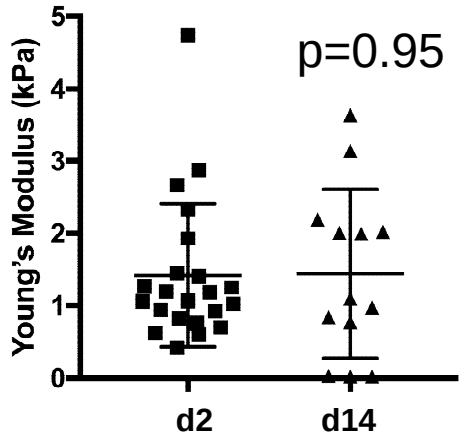
Stability of CC mechanical properties on model beads

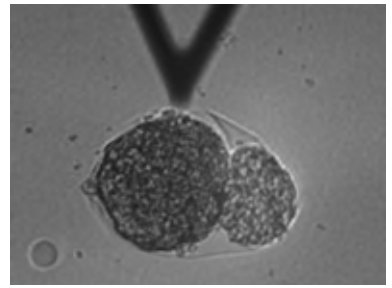




Method: Atomic Force Microscopy

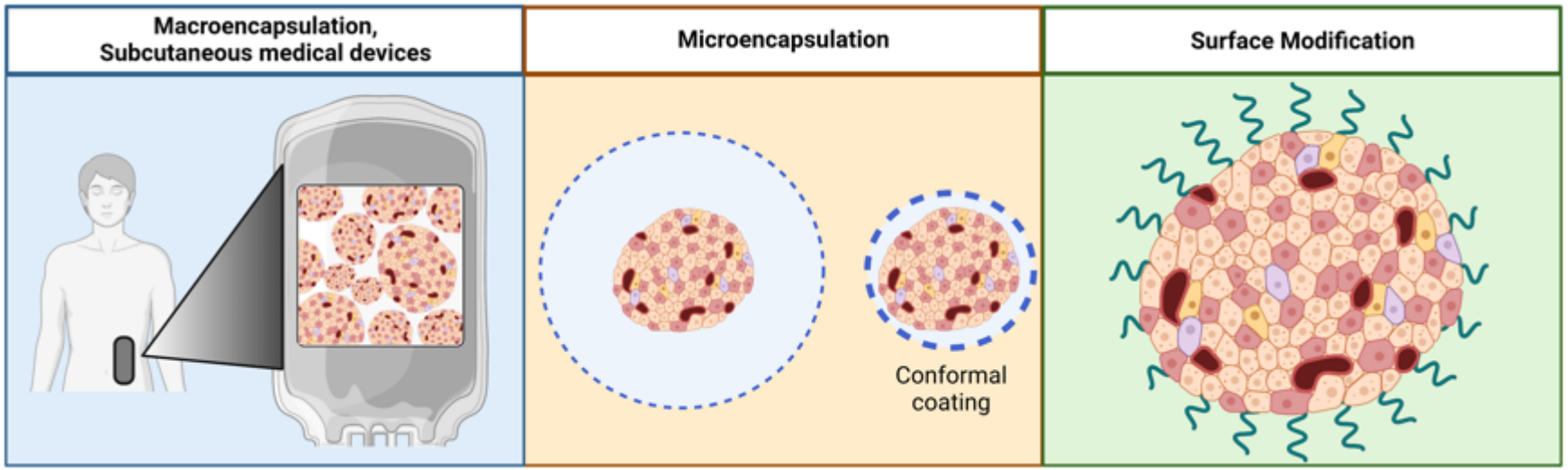
Stability of CC mechanical properties on rat islets





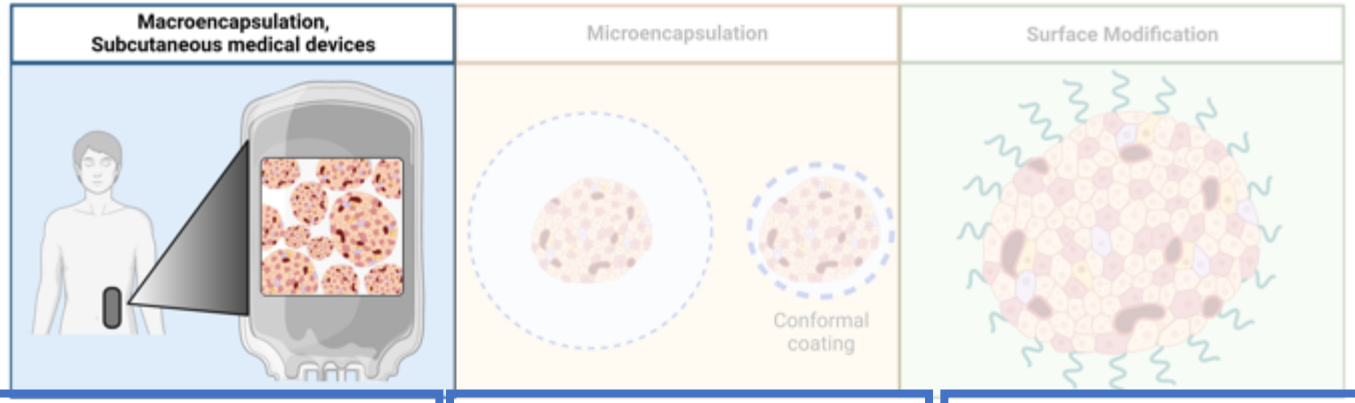
Unpublished

Islet Encapsulation Strategies – Different Designs



Adapted from SoRelle *et al.* Intech. 2011

Islet Encapsulation Strategies - Macro



Adapted from SoRelle *et al.* Intech. 2011

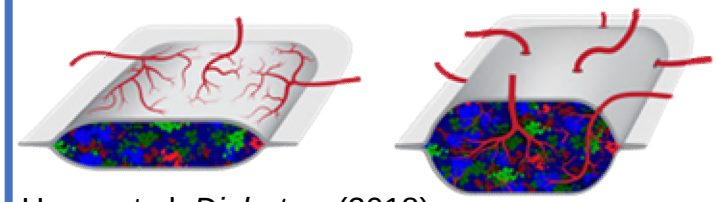
Viacyte Trials

PEC-Encap (VC-01) No immunosuppression

NCT02239354

PEC-Direct (VC-02) With immunosuppression

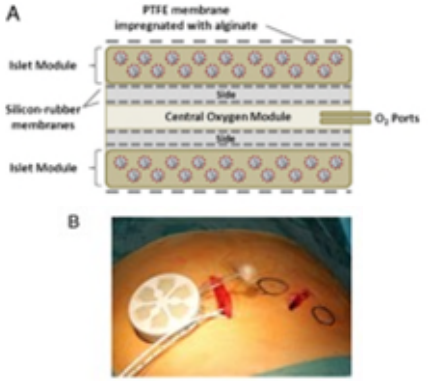
NCT03162926



Henry et al. *Diabetes.* (2018)

Ramzy et al. *Cell Stem Cell.* (2021)

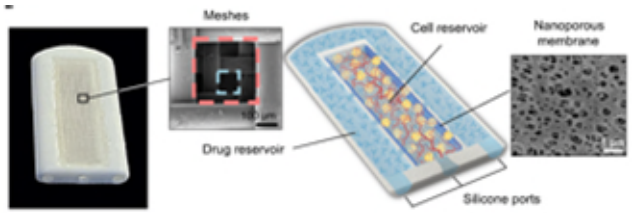
Beta-O2 Oxygenated Subcutaneous Site



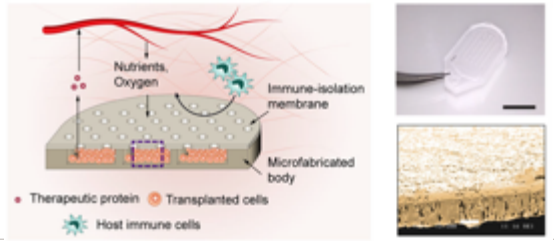
NCT02064309

Carlsson et al. *Am J Transplant.* (2018)

Nanoporous Membrane + Localized Immunomodulation

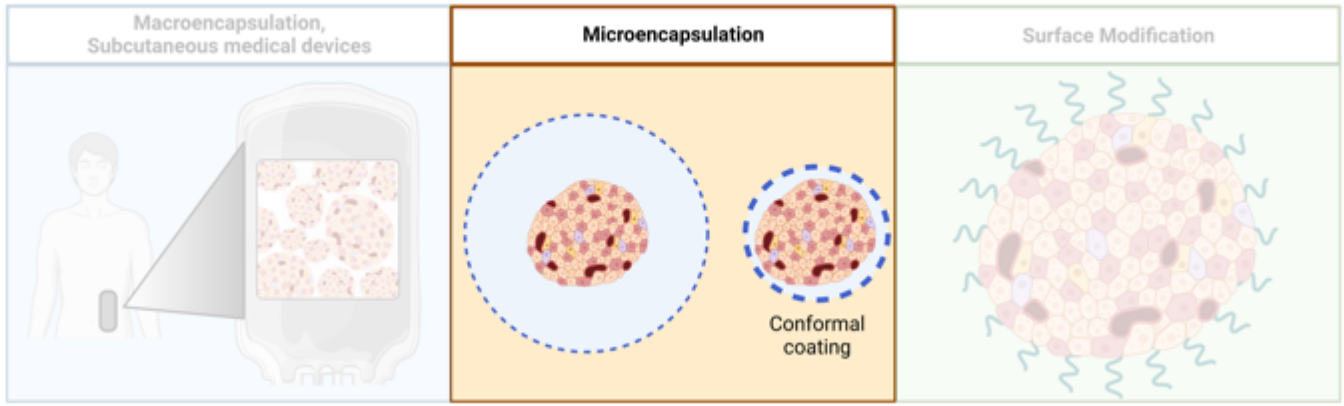


Grattoni's Lab, Nat Commun. (2022)



Anderson/Langer's Lab, Nat Biomed Eng. (2021)

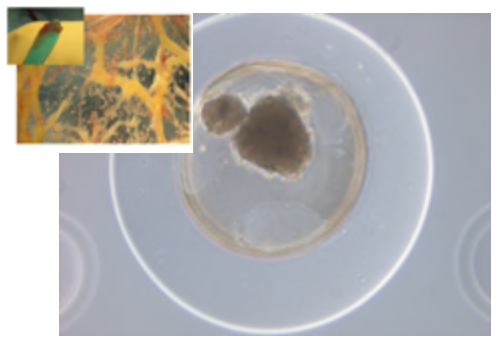
Islet Encapsulation Strategies - Micro



Adapted from SoRelle *et al*. Intech. 2011

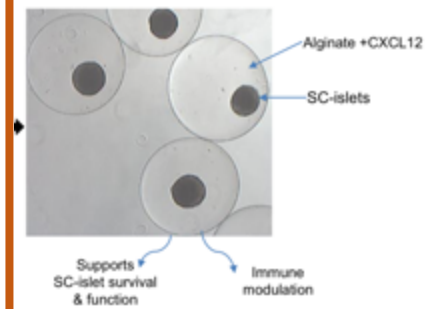
Microencapsulated islets → non-human primate

Double Alginate Capsule



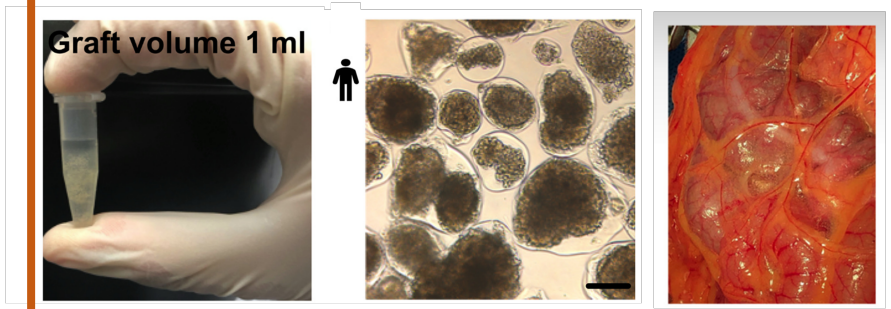
Weber's Lab,
Xenotransplantation (2018)

Vicapsys CXCL12 Alginate



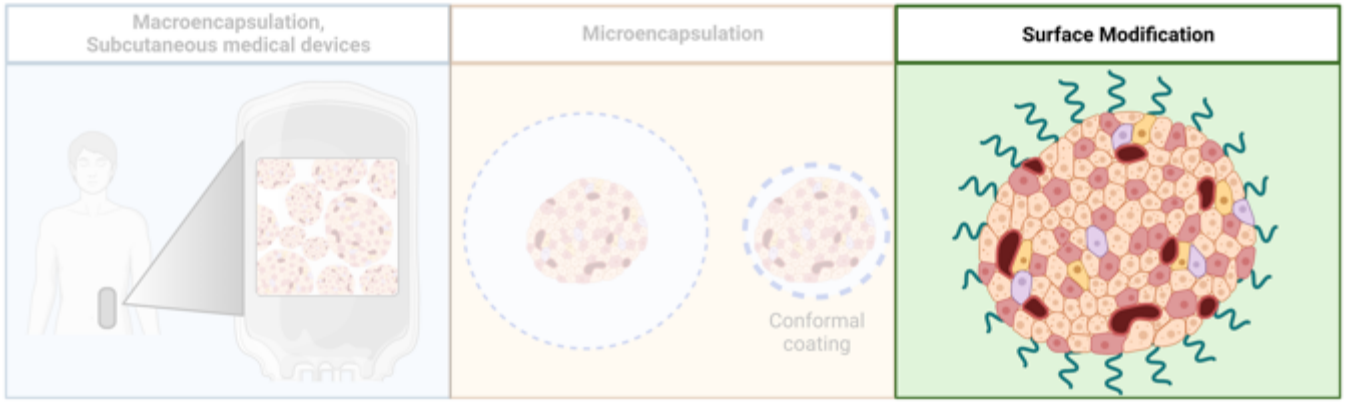
Poznansky's Lab,
Xenotransplantation (2023),
Am J Transplant (2019)

PEG Conformal Coating



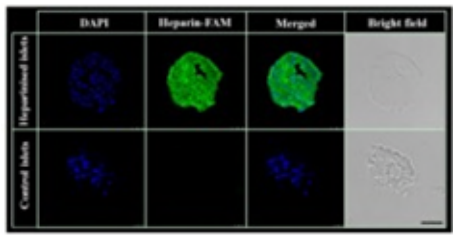
Tomei Lab,
Am J Transpl (2018), Stem Cell Reports (2020),
Sci Adv (2022)

Islet Encapsulation Strategies - Nano



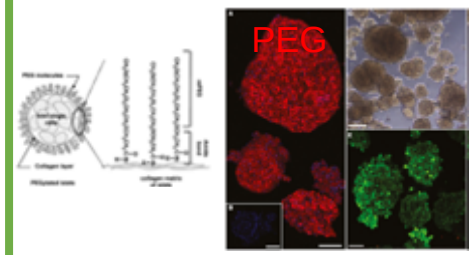
Adapted from SoRelle *et al*. Intech. 2011

Islet surface modification with heparinized PEG



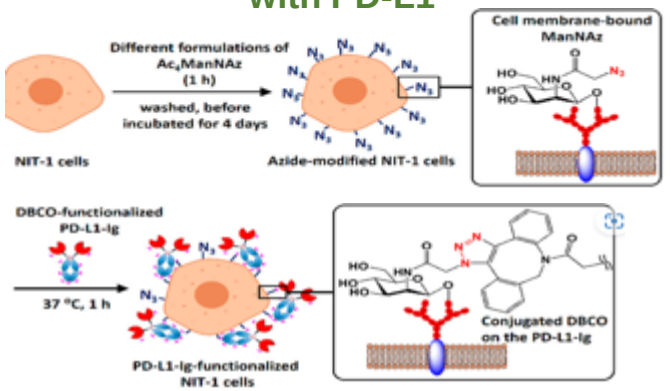
Lou *et al*. Mater Sci Eng. (2017)

Islet surface modification with PEG (PEGylation)



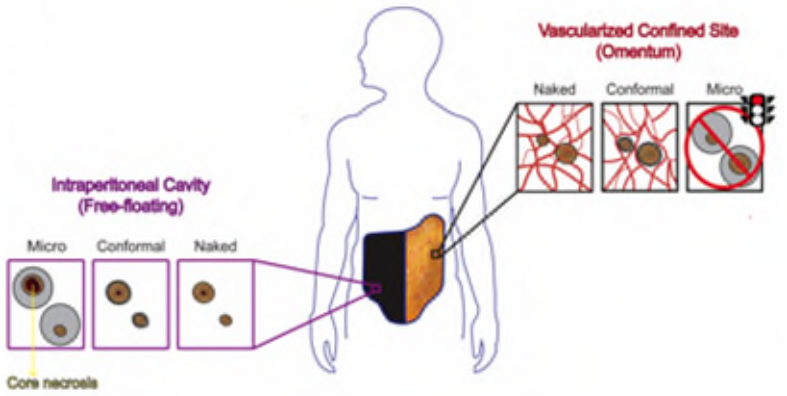
Stabler Lab,
Am J Transplant (2020)

Islet metabolic surface engineering with PD-L1

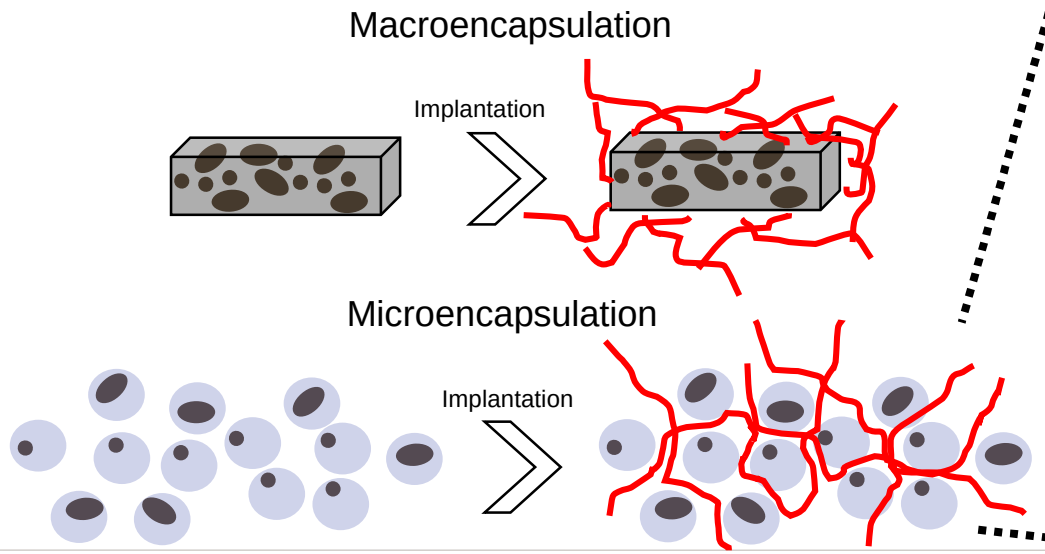


Wang's Lab, ACS Nano. (2021)

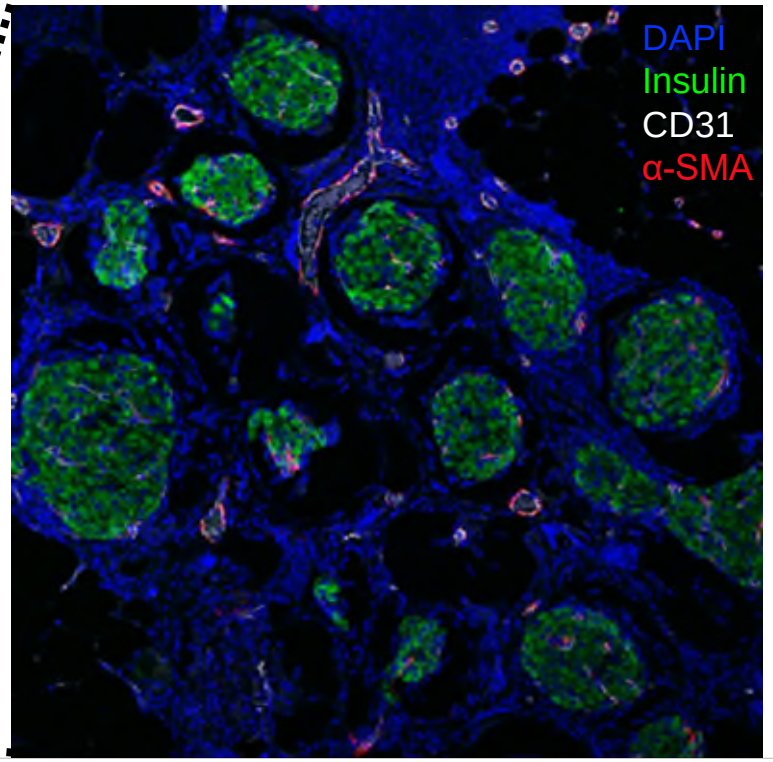
The issue of the transplant site and the importance of vascularization in confined sites



Transplantation in Confined Vascularized Sites



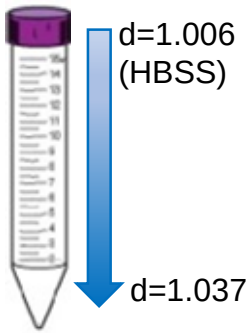
Conformal coated islet allografts in fat pad



Purification of cell-free capsules to reduce the volume of therapeutic material

1. Continuous gradient to determine coated islet density

V=15 mL



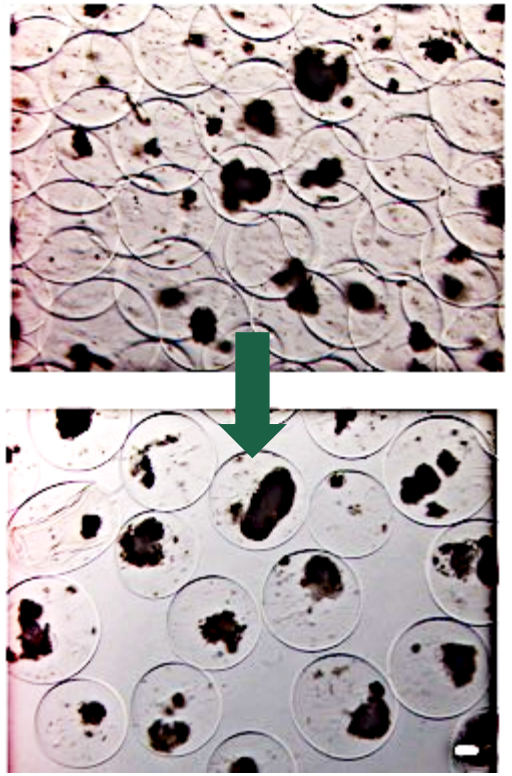
2. Gradient centrifugation to separate coated islets from empties

V=15 mL

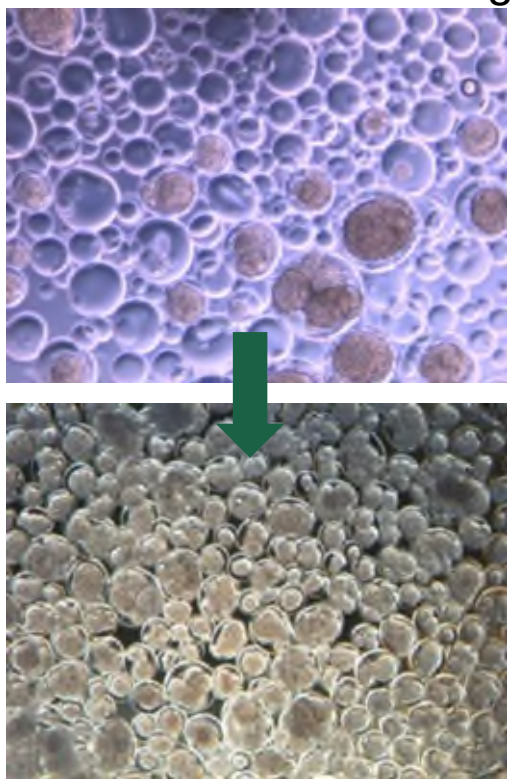
Centrifugation
600g for 5min

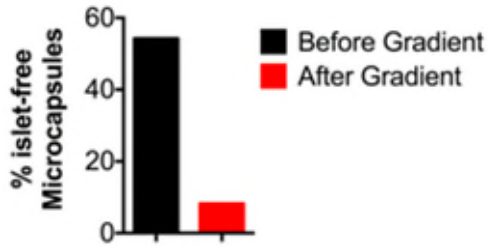
D=1.037 for CC
D=1.027 for microcapsules

Alginate Microcapsules



PEG Conformal Coating



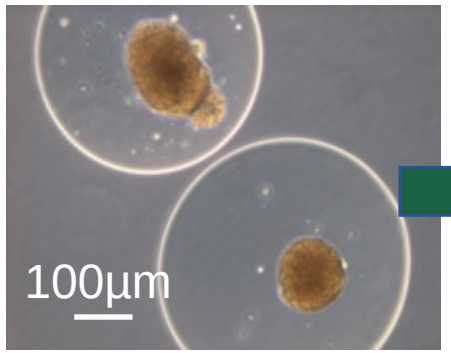


98% reduction in empty capsules

Minimizing Capsule Size for physiological GSIS

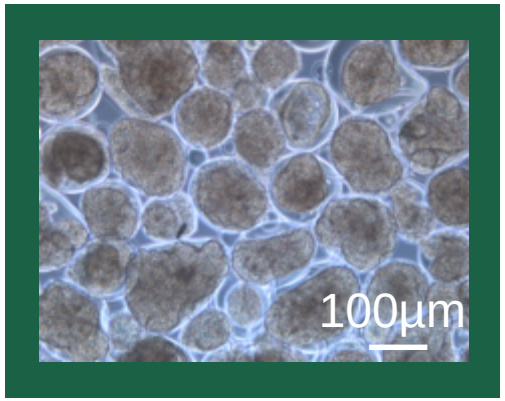
Traditional Microcapsules

One size fits all

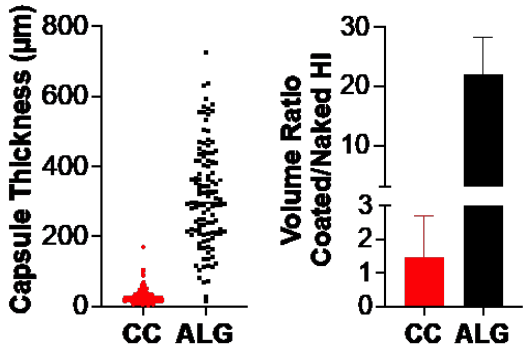


Conformal capsules

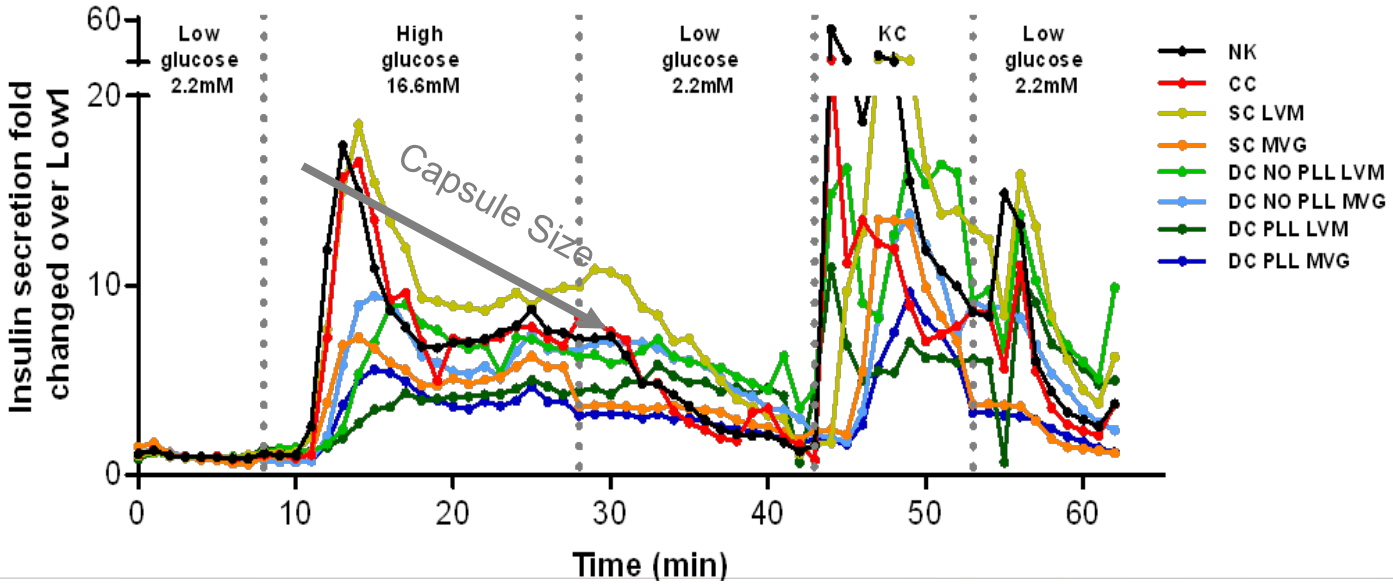
conform to the islet size & shape



Minimized Capsule Thickness & Graft Volumes



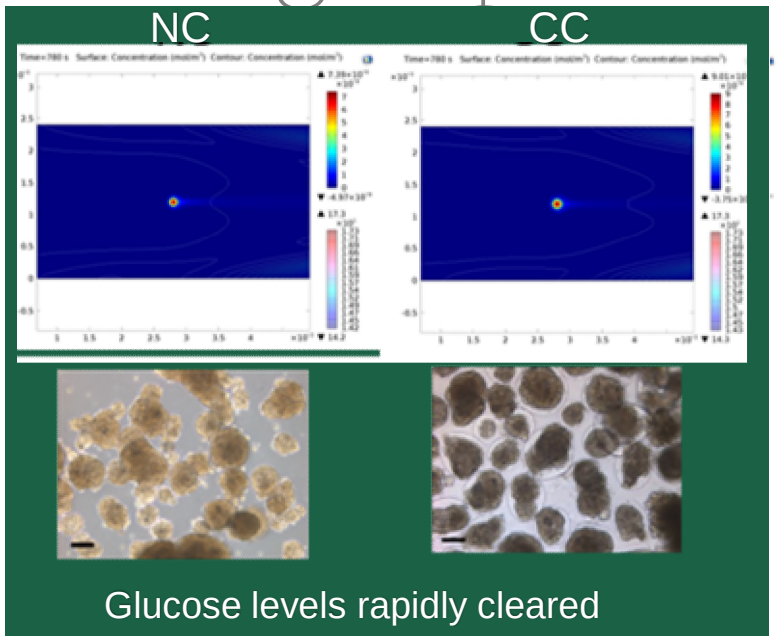
Physiological Glucose-Stimulated Insulin Secretion



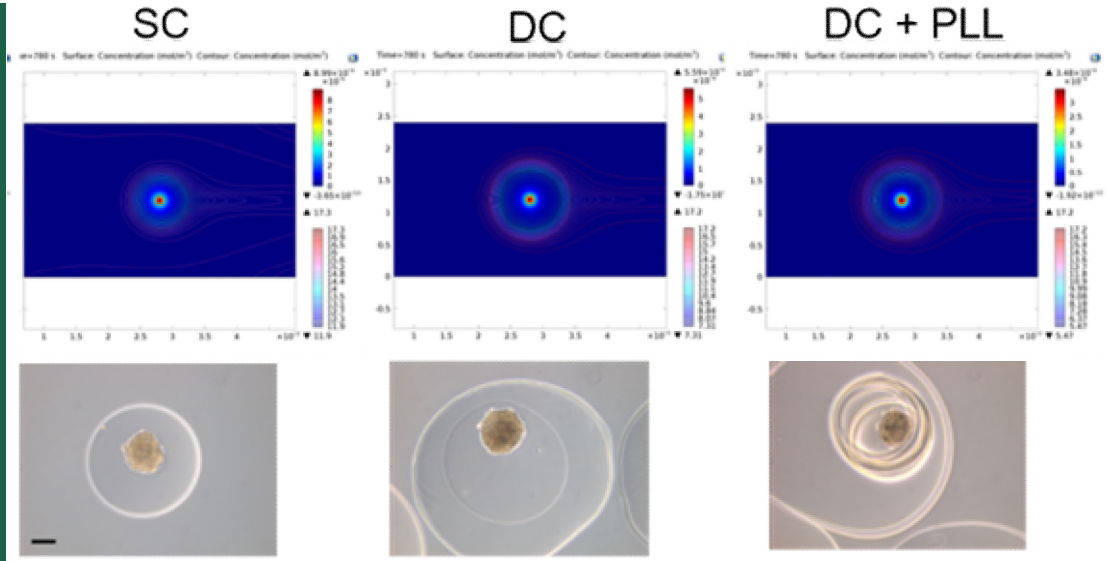
De Toni et al., *Frontiers in Bioeng & Biotech* (2022)

Minimizing Capsule Size for In Vivo Function

In silico

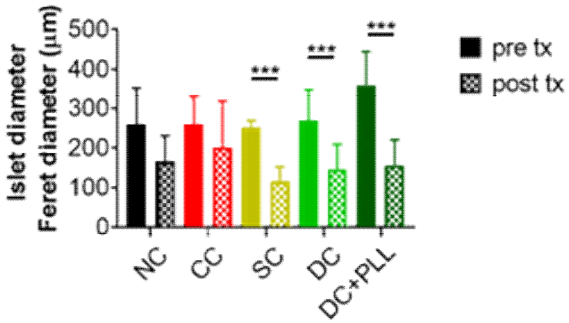


Glucose levels rapidly cleared

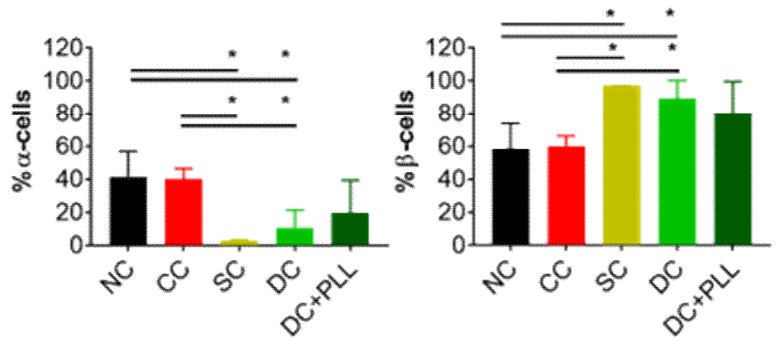


Glucose levels remain elevated for longer times in larger capsules

In vivo



Islet diameter decreases post TX in larger capsules



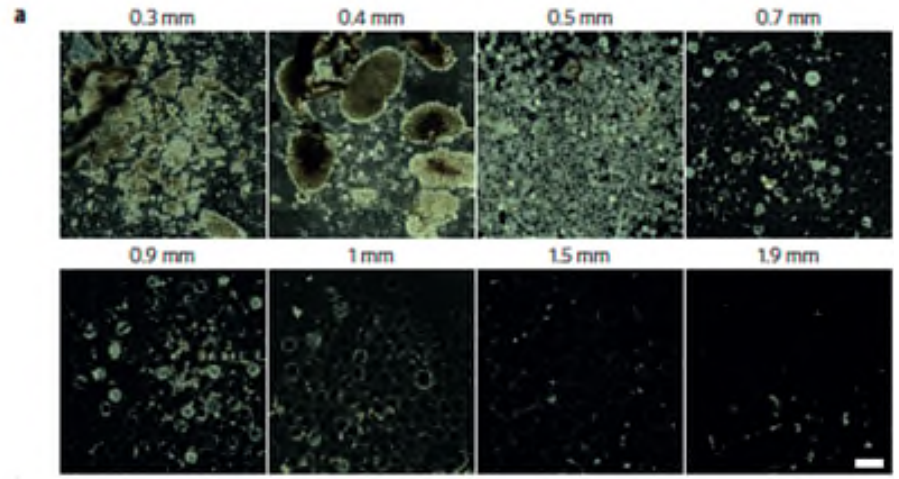
Change in islet composition in larger capsules

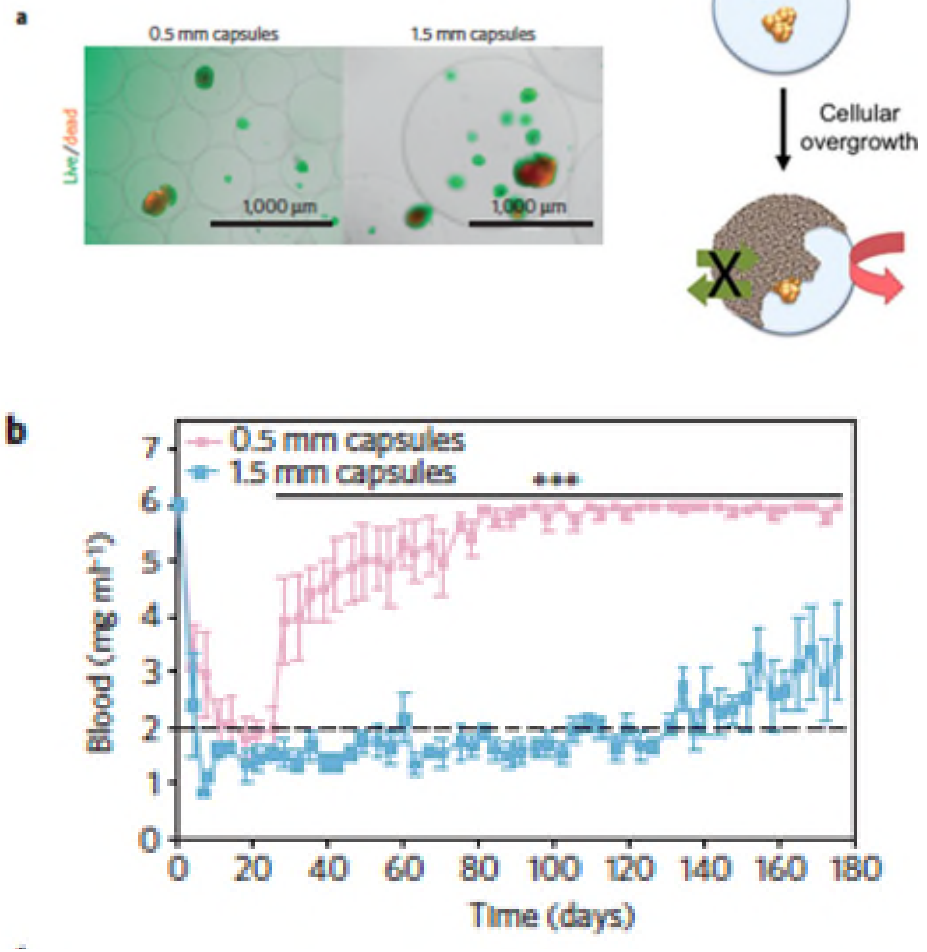
BG	SC 2k	SC 1k	DC 2k	DC 1k	DC+PL L 2k	DC+PL L 1k
Avg	96	72	43	53	44	105
SD	41	11	25	15	12	42

Mice receiving larger capsules experience hypoglycemia

De Toni T. et al. Front. Bioeng. Biotechnol., 2022

But…small capsules increase foreign body immune responses decreasing functionality

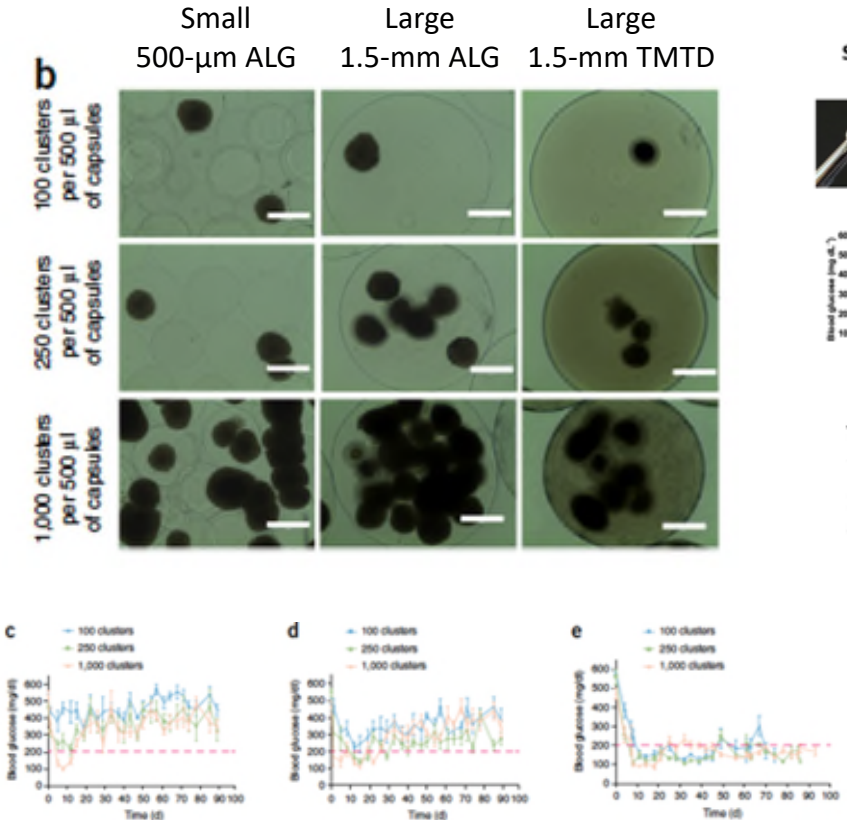




Veiseh O, Nat Mat vol. 14 (2015)

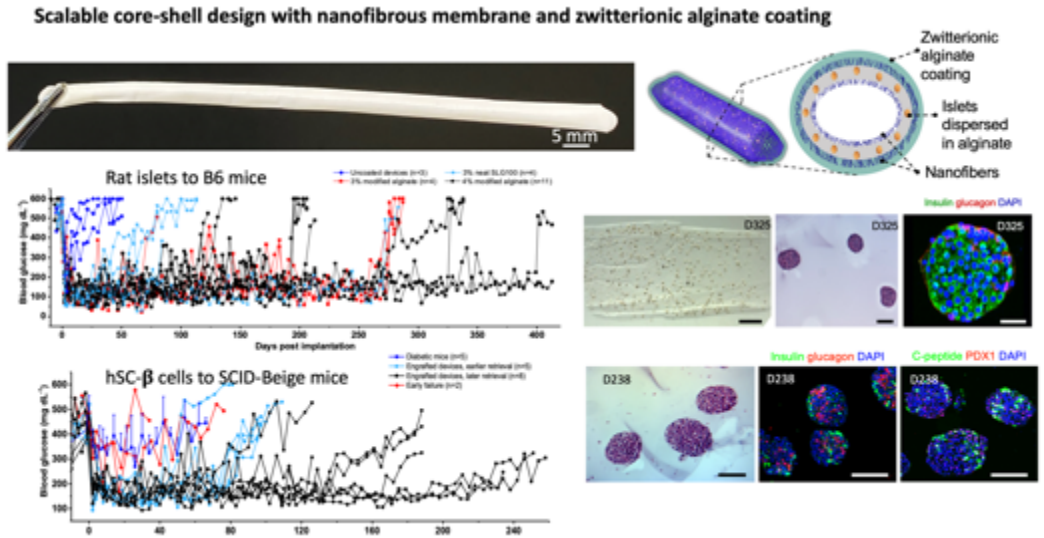
Biomaterial modifications can reduce foreign body immune responses

SC-β cells + TMTD alginate -> C57BL/6J mice



Anderson/Langer, Nature Medicine 2016

Rat islets in modified macrodevice -> B6 mice
SC-β cells -> SCID-beige mice.

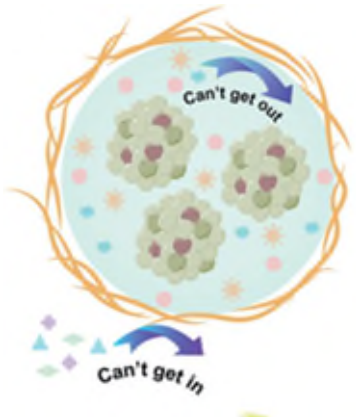


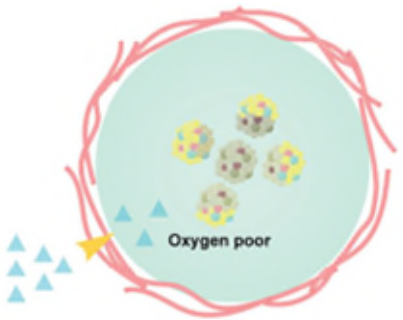
Ma, Small, 2022

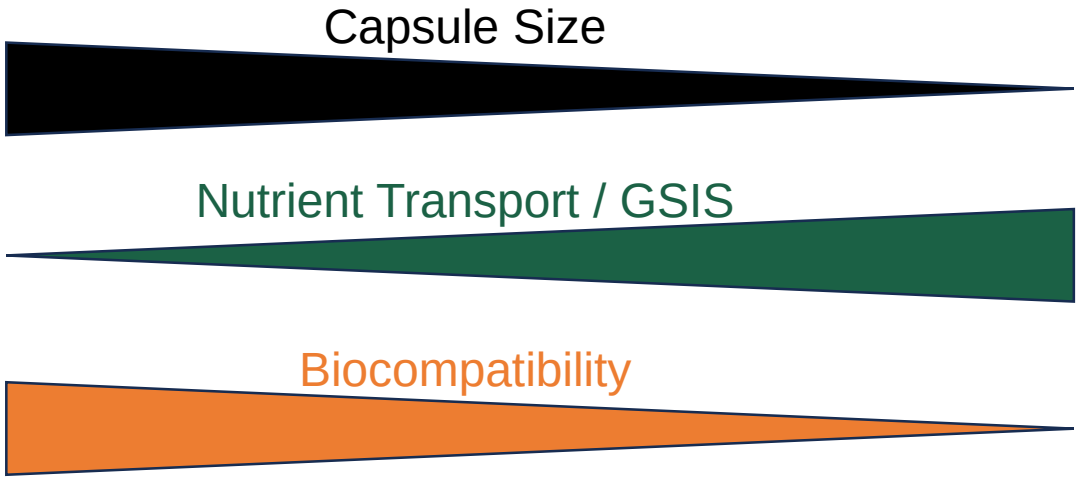
Protecting Insulin-Secreting Cells from Rejection through Biomaterial Encapsulation

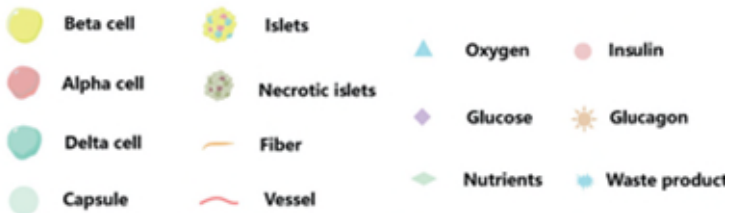
Encapsulation Issues

- Biomaterial suboptimal *biocompatibility* may lead to fibrotic capsule formation
  - large is better
- Large capsule size even when vascularized may lead to *core hypoxia and delayed GSIS*



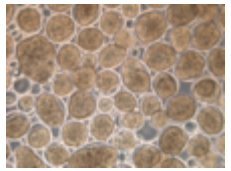




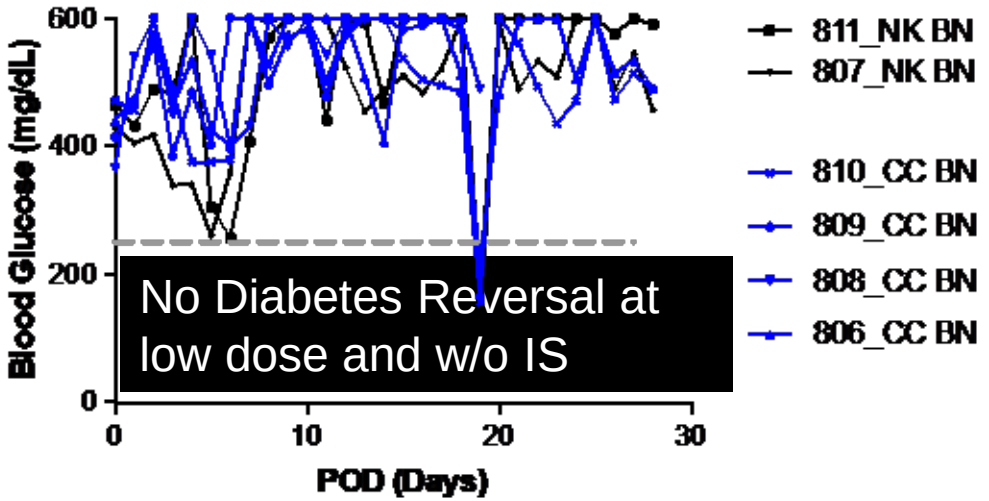


Zhang et al. Frontiers in Immunology 2022

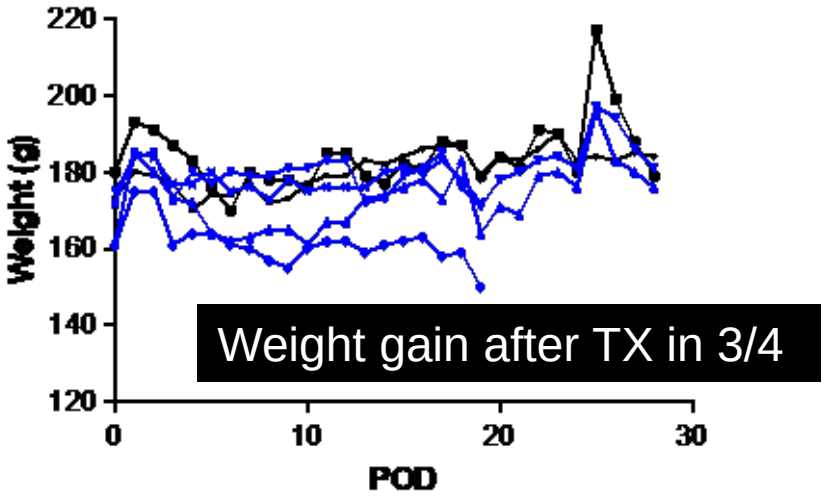
Pilot Testing of Conformal Coating in Sernova Pre-Vascularized Subcutaneous Cell Pouch in Allogeneic Rat Islet Transplant Models Indicates Only Partial Islet Allograft Protection



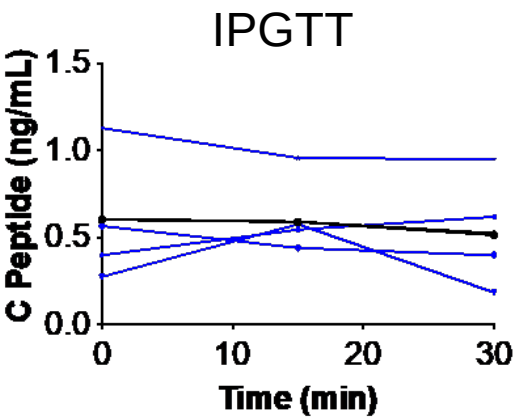
Dose 3k IEQ/rat
Allogeneic TX (BN islets->LW rats) w/o IM



No Diabetes Reversal at low dose and w/o IS

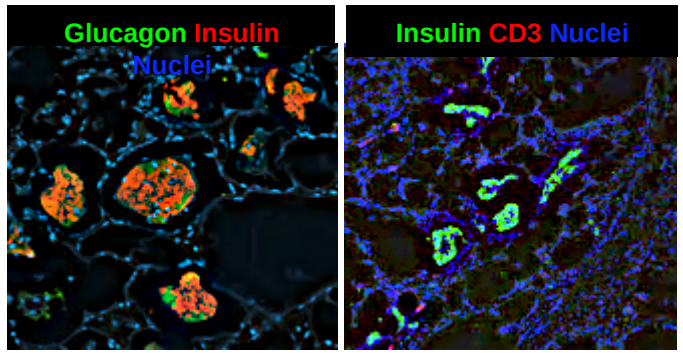


Weight gain after TX in 3/4



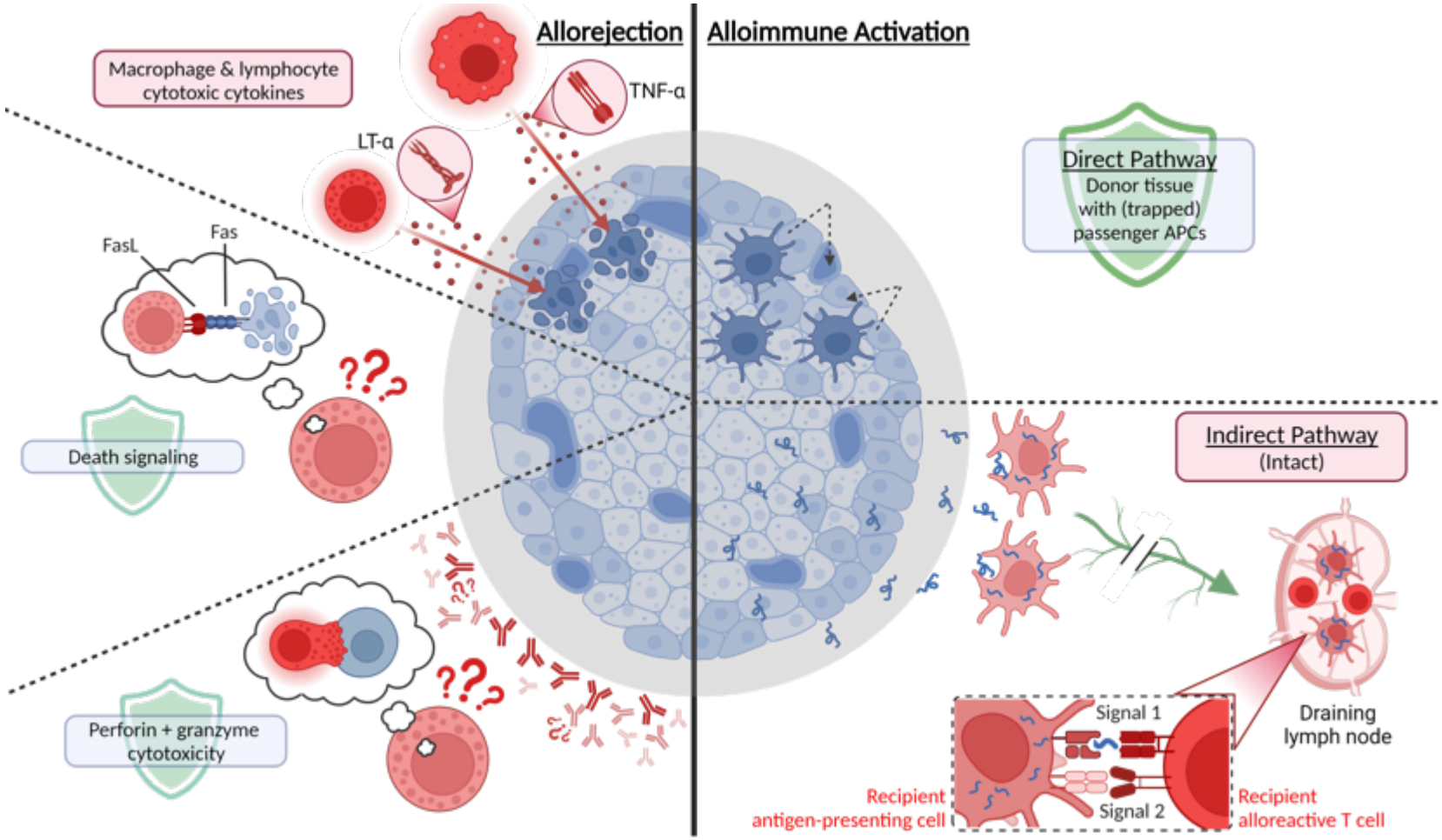
Detectable Cpep

CC islets in Cell Pouch ™

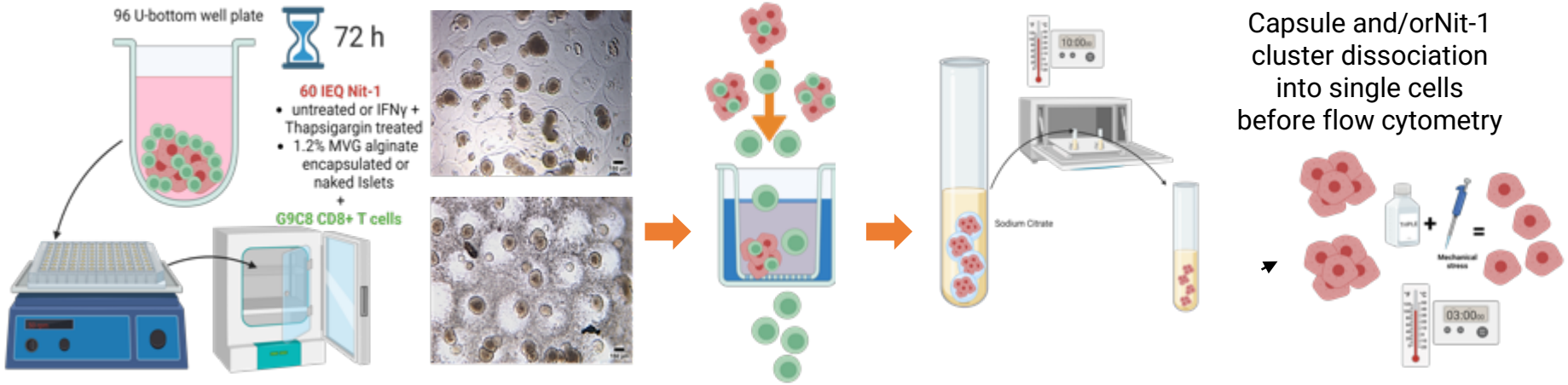


Partial CC allograft islet function w/o IM

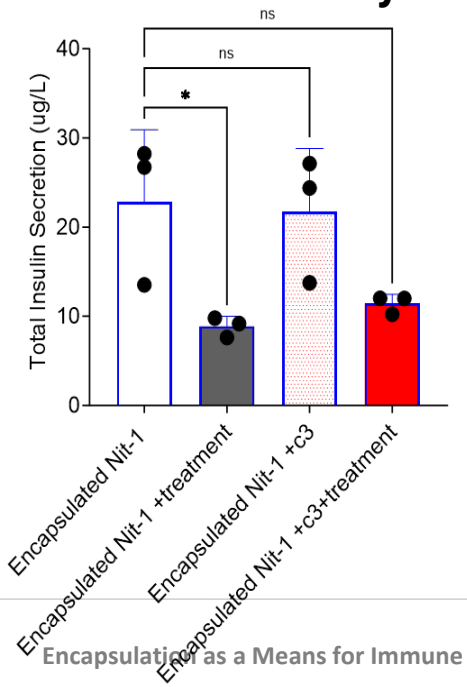
What encapsulation does (and doesn't do) when encapsulated islets are transplanted in confined well-vascularized sites

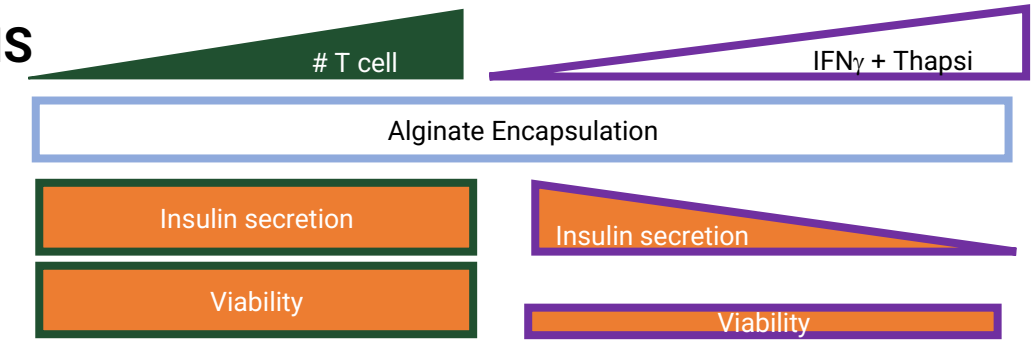


In vitro model to evaluate the degree of protection by encapsulation on islet immune attacks in localized sites

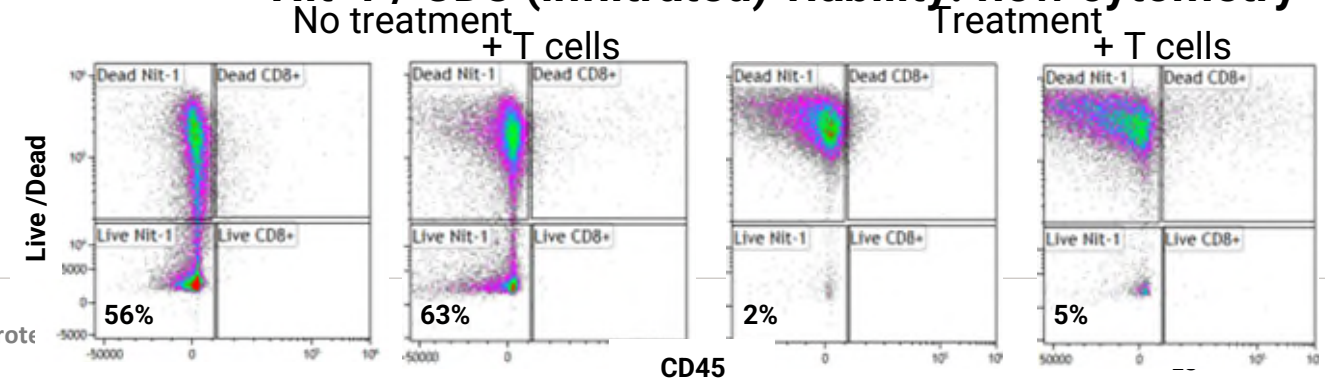


Nit-1 functionality: GSIS





Nit-1 / CD8 (infiltrated) viability: flow cytometry



Decreasing indirect alloreactive T cell activation by blocking costimulation through CTLA4Ig localized delivery

Cytotoxic T lymphocyte antigen 4 (CTLA4)

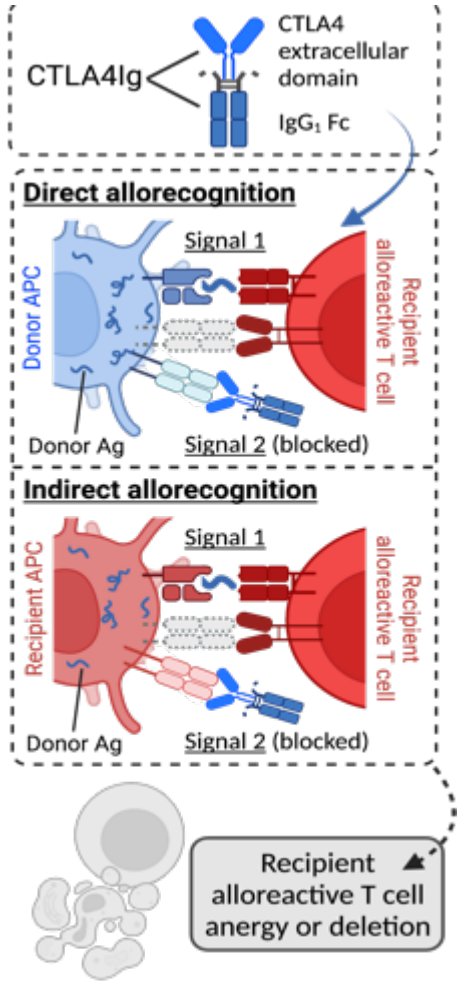
- Immune checkpoint molecule, binds to CD80/86 and interferes with signal 2
- 1991-92: CTLA4 Ig (abatacept) is created and suppresses T cell activation *in vivo*
- 2005: Two amino acid substitution → belatacept, 10x more potent

Approved for

Belatacept	Abatacept
Heart transplant, prophylaxis of organ rejection	Graft-versus-host disease prophylaxis (acute), adult and pediatric
Kidney transplant, prophylaxis of organ rejection	Psoriatic arthritis
Lung transplant, prophylaxis of organ rejection	Rheumatoid arthritis, adult and pediatric

Clinical Trials in Islet TX

- NCT00468403
- NCT00501709



Linsley et al. *J Exp Med.* (1991)
Linsley et al. *Science.* (1992)
Larsen et al. *Am J Transplant.* (2005)

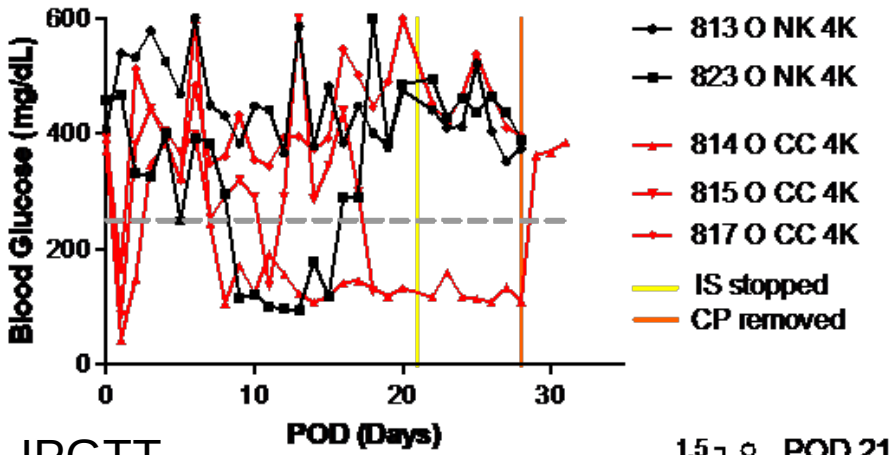
Combination Therapy of Conformal Coating and CTLA4Ig Systemic Treatment in Sernova Subcutaneous Cell Pouch Improves Allogeneic Rat Islet Allograft Function

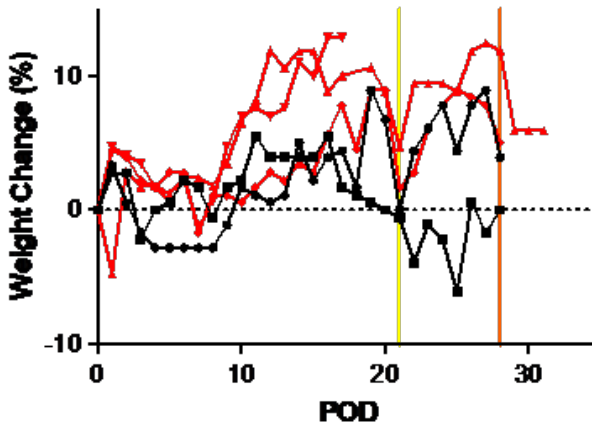
Dose 4k IEQ/rat

Allogeneic TX (BN islets->LW rats) w IM

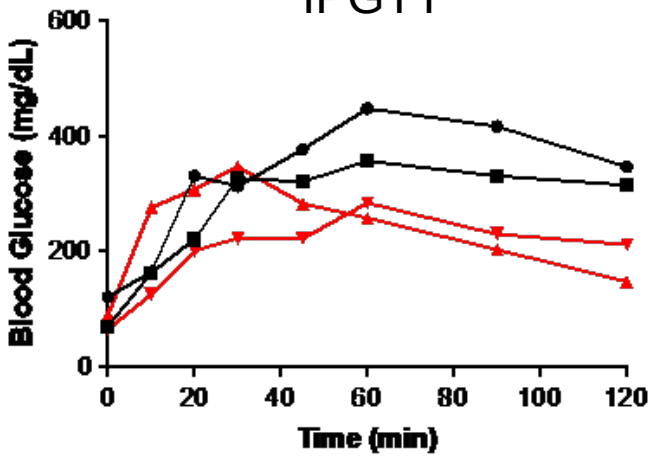
+ Orencia (2mg/rat)

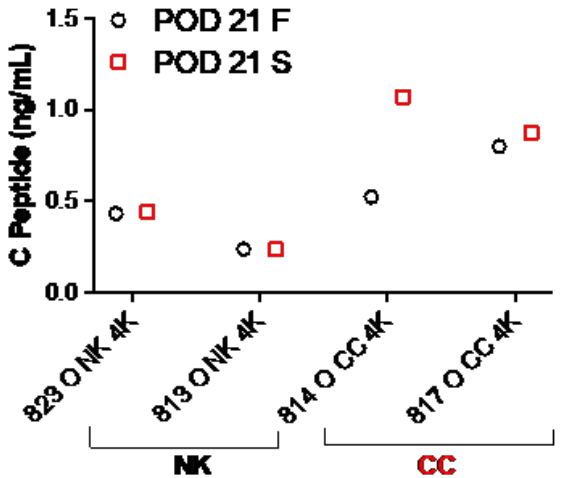
every other day start POD 0 until POD 21





IPGTT



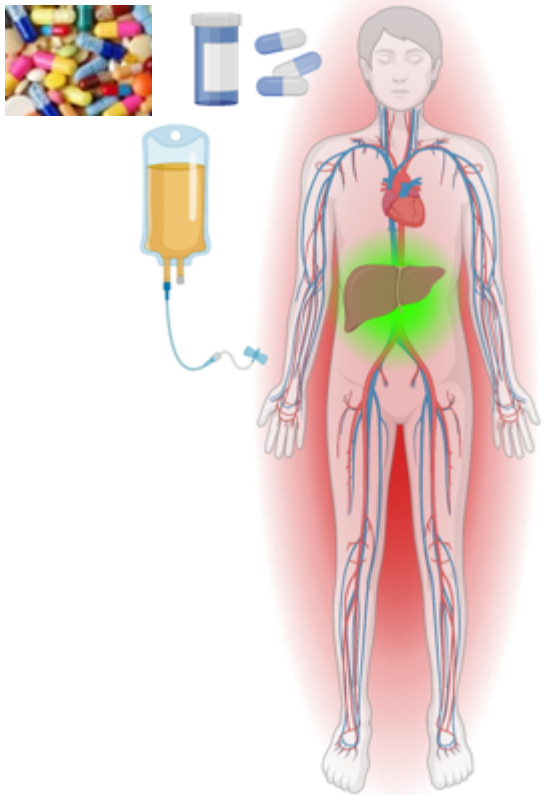


Diabetes Reversal in 2/3 CC and longer duration than NK

Localized immunomodulation to limit systemic effects and increase anti-rejection efficacy in extrahepatic islet transplantation

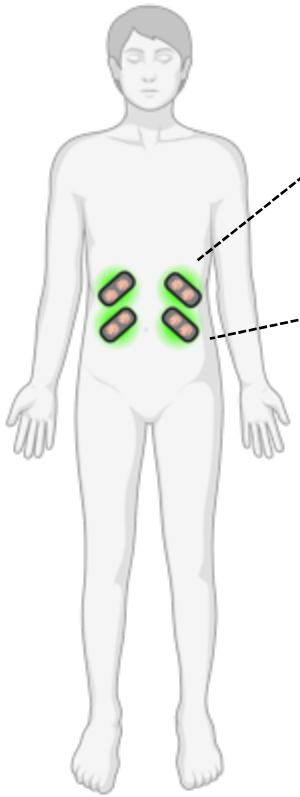
Systemic Delivery

Therapeutic effect within intrahepatic graft



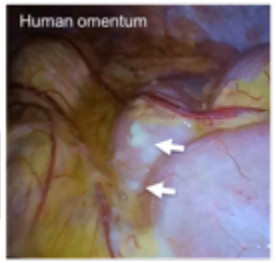
Systemic adverse effects

Localized Co-Delivery of islets and drugs



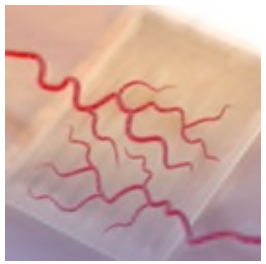
Islet Transplantation in Extrahepatic Sites

Omental Pouch

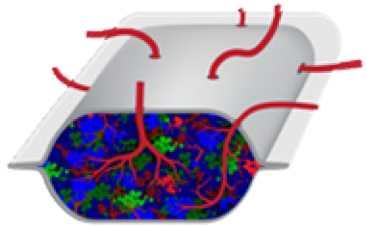


UMiami
NCT02213003

Prevascularized Subcutaneous Sites



Sernova Corp
NCT03513939

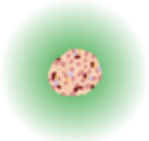


Vertex VC-02
NCT03162926

Could enable localized immunomodulation

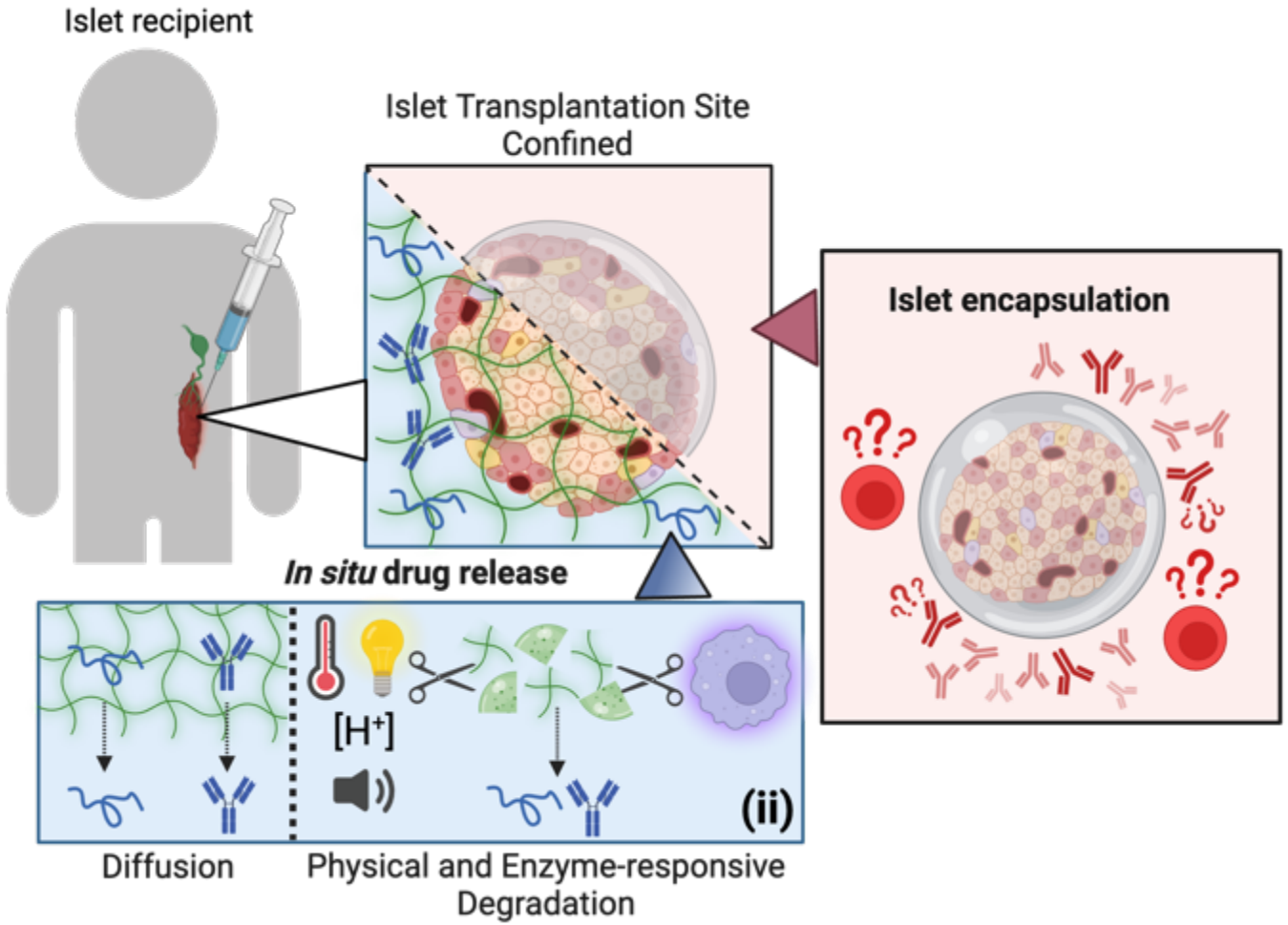
- ✓ Minimize systemic toxicity and immune deficiency
- ✓ Decrease drug dose / frequency
- ✓ Increase drug bioactivity

1.1 Locally in Graft



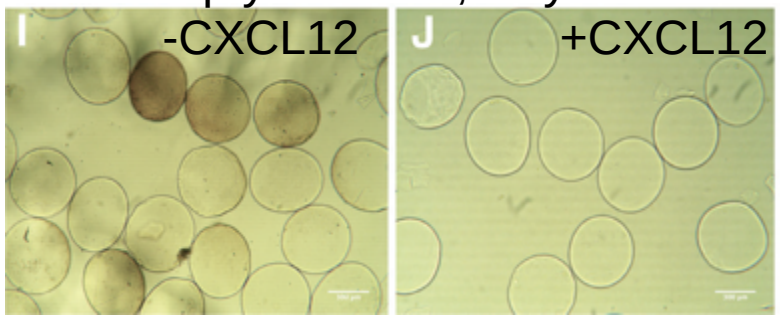
1.2 Locally in graft-draining lymph nodes

Combination of islet encapsulation and local immunomodulation for transplantation in confined sites



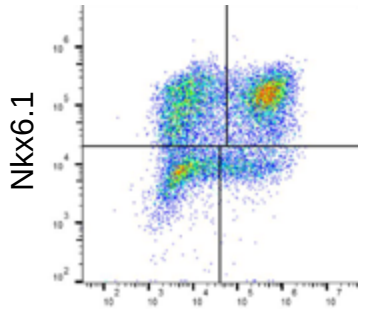
Example: CXCL12 co-delivery with alginate capsules

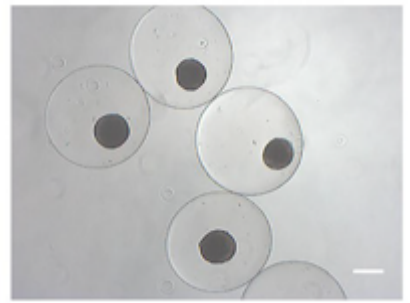
Empty in NHPs; Day 180

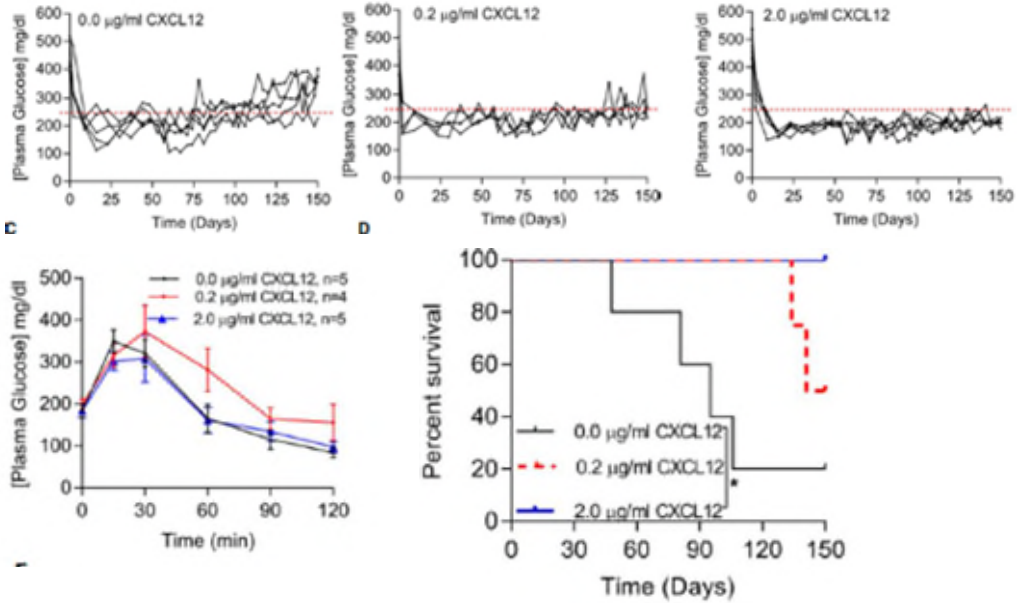


Sremac M et al., Transplantation Direct 2019

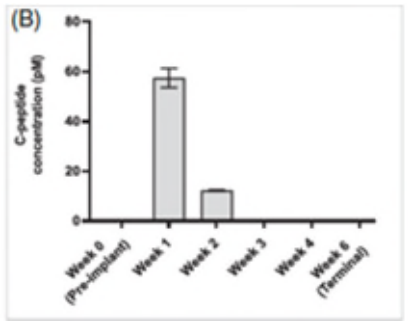
SC-islets -> B6 mice

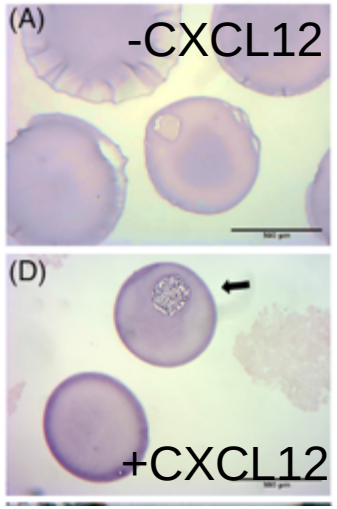






Pig islets in NHPs



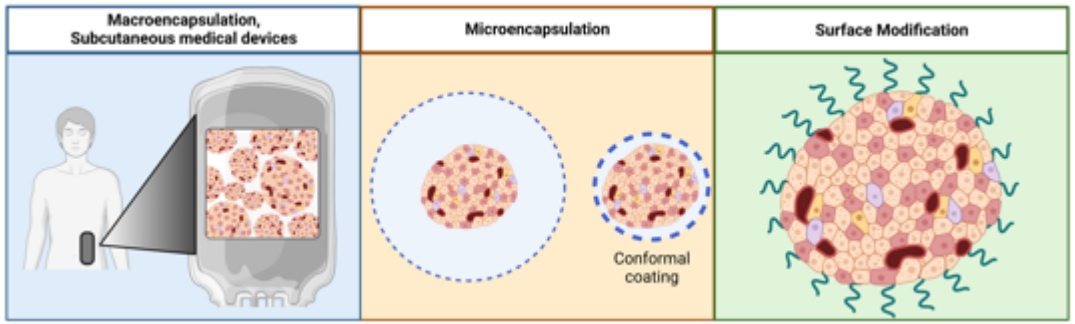


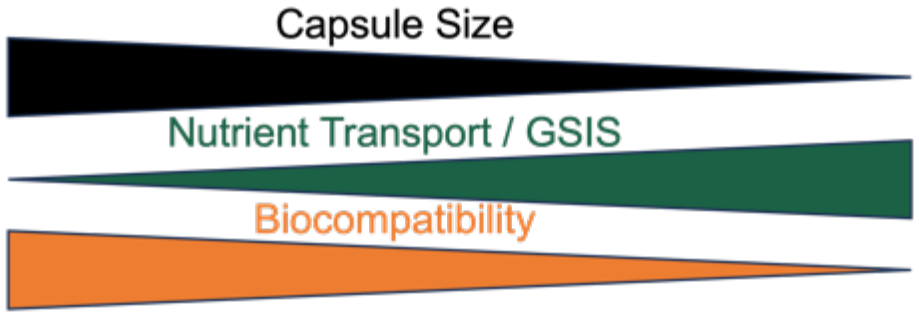
Sremac M et al., Xenotransplantation 2023

Alagpulinsa D et al, , Am J Transplant 2019

Conclusions:

- ✓ Islet immunoisolation through encapsulation requires biomaterials with
- ✓ selective and stable permeability
- ✓ stable mechanical properties
- ✓ Several capsule designs have been tested in small, large animals, and humans characterized by scale (macro / micro / nano) some of these requiring co-administration with immunomodulatory drugs

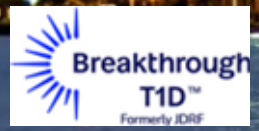




- ✓ Elimination of empty capsules feasible by gradient centrifugation
- ✓ Larger capsules in the peritoneal cavity worse for GSIS and in vivo function but better for biocompatibility
- ✓ Transplantation of islets in minimal thickness coatings (conformal) does not provide protection to indirect alloreactive T cells in rats requiring combination with immunomodulatory drugs which could be delivered locally in the graft
- ✓ Immunomodulatory drugs can be co-delivered with encapsulated islets in localized extrahepatic sites through localized immunomodulation

Acknowledgments:

Funding:
1 R01DK109929
2 R01DK109929
3-SRA-2024-1477-S-B
3-SRA-2017-347-M
2-SRA-2019-780-S-B
5-CDA-2016-171-S-B
Semma Therapeutics
Sernova Corp.



Tomei Lab:
Zach Wilkes
Leonor Teles
Chris Li
Ilaria Pasolini
Michelle Ley
Grisell Gonzalez
Ana Hernandez
Oriana Marrone Mantovani
Dhanashree Surve

Previous members:
Freddy Gonzalez-Badillo
Flavia Zisi Tegou
Maria Abreu
Diana Velluto,
Aaron Stock
Teresa De Toni
Vita Manzoli
Mike Lupp
Ana Claure

Collaborators:
Drs. Bayer, Velluto, Buchwald, Berman, Kenyon, Ricordi Andreopoulos, Ziebarth
University of Miami

Dr. Creusot (*Columbia*)
Dr. Zustiak (*SLU*)
Drs. Weber, Safley (*Emory*)

Figures created with BioRender.com