3D Nanofibrillar Cellulose hydrogel platform for long-term human islet culture

Yi-Ju Chen[1], Ben Z. Stanger[1]

[1]University of Pennsylvania

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1 Works for me

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Yi-Ju Chen
University of Pennsylvania

ABSTRACT

It remains a challenge to maintain human islets *ex vivo* to provide a functional and sufficient source for clinical islet transplantation and an *in vitro* model for drug screening or basic biological studies. With a conventional culture method, islets lost their functionality and mass rapidly upon they were isolated from the native environment, which provides both mechanical and biological supports. By merging the advantage of transwell cultures and the mechanical properties provided by the wood-derived nanofibrillar cellulose (NFC) hydrogel, we developed a new islet *ex vivo* platform, which can preserve human islets up to 83 days with intact morphology similar to freshly isolated counterparts. Importantly, these long-term cultured human islets are still potent and can ameliorate hyperglycemia in diabetic mice post-transplantation. The platform is designed to be user friendly and easily adapted to help scientists overcome the difficulties in islet cultures. Furthermore, the application of this platform aims to provide a sufficient window for pre-transplantation islet assessment, a biobanking opportunity of human islets, and an *in vitro* model for human islet studies in drug-screening, stem cell-derived islet (beta)-like organoids maturation, and basic human islet biology.

GUIDELINES

- This protocol was developed by using resources and funding provided by the NIDDK-supported Human Islet Research Network (HIRN, RRID:SCR_014393; https://hirnetwork.org ; UC4 DK104196 to B.Z.S.)
- Human islets were provided by the NIDDK-funded Integrated Islet Distribution Program (IIDP) (http://iidp.coh.org) at City of Hope and Penn Center for Islet Transplantation. The experiments with human islets should be performed in accordance with policies and procedures of the IIDP and the research institution.
- Correspondence should be addressed to Yi-Ju Chen, PhD, yijuchen3@gmail.com

MATERIALS

NAME	CATALOG #	VENDOR
GrowDase enzyme	View	
GrowDex	View	
6.5 mm Transwell® with 8.0 µm Pore Polyester Membrane Insert, Sterile	3464	Corning
CMRL 1066, CIT Modification, 500ml	98-304-CV	Corning
Human Serum Albumin Solution—25%	#800-120	
Heparin sodium salt, 10KU	H3149-10KU	Sigma Aldrich
IGF-1 Recombinant Human Protein, 100µg	PHG0071	Gibco - Thermo Fischer

BEFORE STARTING

- On the day of arrival, 8K to 10K islet equivalent (IEQ) of human islets were transferred to a 50 ml falcon tube in a laminar flow hood and placed on ice for at least 30 min to allow the islets to settle by gravity. Then, remove most of the transport media and leave 5 ml media in the tube without disturbing the pellet. Add 5 ml of CIT culture media into the tube and transfer everything into a 60 mm culture dish (non-treated) for the following islet handpicked procedure.
- The CIT culture media recipe used in this protocol is originally developed by the Clinical Islet Transplantation (CIT) Consortium and

published at CellR4 2014; 2 (3):e981.

Preparation of CIT culture media

1
- Dissolve 10KU Heparin powder with 1 ml of CMRL1066, CIT modification base medium to obtain Heparin solution (10 KU/ml).
- Dissolve 100µg IGF-1 powder in 100µl of sterile 10 mM HCl solution to obtain IGF-1 solution (1 µg/µl)
- Add 500 µl of Heparin solution (10 KU/ml), 50 µl of IGF-1 solution (1 µg/µl), and 10 ml of Human Serum Albumin Solution (25%) into 500 ml of CMRL1066, CIT modification base medium.

The CIT culture media recipe used in this protocol is originally developed by the Clinical Islet Transplantation (CIT) Consortium and published at CellR4 2014; 2 (3):e981. http://www.cellr4.org/article/981
The composition of CIT media is CMRL1066, CIT Modification medium supplemented with 0.5% Human Albumin, 10 U/ml Heparin, and 0.1 mg/ml IGF-1.

Preparation of the nanofibrillar cellulose (NFC) hydrogel

2 Weigh the needed amount of NFC hydrogel (Growdex®) and mix it with CIT culture media at 1:1 ratio by vortexing to make a 0.75% (w/v) hydrogel.

The NFC hydrogel (Growdex®) purchased from UPM Biochemicals is 1.5%.
For example, weigh 0.5g of NFC hydrogel (Growdex®) and mix it with 0.5 ml CIT media by vortexing. This could make 6 replicates of 0.75% hydrogel culture (80 ul for each replicate, see the Step 3).
Low retention pipette tips should be used while handling the hydrogel.

Setup 3D human islet culture with NFC hydrogel

3
- Human islets are handpicked into Eppendorf tubes (50 islets per tube) and kept on the rack to allow the islets to settle by gravity. Remove excess media as much as possible.
- Gently mixed 50 islets with 80 ul of 0.75% (w/v) hydrogel and then dispensed it into an insert of Transwell. It should be avoided to generate air bubbles while mixing and dispensing the samples.
- Placed the NFC hydrogel culture into a humidified incubator (37 ºC, 5% CO_2) for at least 15-30 min.
- Carefully add 80 ul CIT media on top of the NFC hydrogel without disturbing it. Then, add 500 ul of CIT media at the plate well. This 3D NFC culture is maintained in a humidified incubator at 37 ºC with 5% CO_2.

The low retention tips should be used for this step.
After overnight incubation, it should generate a thin layer of media on top of the NFC hydrogel.

Maintain long-term 3D islet NFC culture

4 To maintain a 3D NFC culture in a long-term, 100 ul of CIT media is added to the insert every 3 or 4 days. Replace media at the plat well (bottom) every two weeks.

Retrieve islets from the NFC hydrogel

5
- Remove the media from the plate well
- Mix 50 ul GrowDase enzyme with 200 ul CIT media and then add the mixture (250 ul in total) to an insert of the Transwell.
- Add 300 ul of CIT media at the bottom (plate well).
- Incubate overnight (12-16 hours) in a humidified incubator at 37 ºC with 5% CO_2.
- Once hydrogel is digested, samples are ready to be retrieved for further analysis.

The NFC hydrogel can be digested within 8-12 hours by this setup.

83 days cultured human islets retrieved from 3D NFC hydrogel platform