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Sonication of PFFs for use in vitro

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Generation and sonication of α-synuclein fibrils

Vijay Singh[1], Marta Castellana-Cruz[2], Nunilo Cremades[3], Laura Volpicelli-Daley[1]

[1]Department of Neurology, Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham, Birmingham, AL, 35294, USA.;
[2]Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, CB2 1EW, UK.;
[3]Institute for Biocomputation and Physics of Complex Systems (BIFI)-Joint Unit BIFI-IQFR (CSIC), University of Zaragoza, Zaragoza 50018, Spain.

ASAP Collaborative Rese… | Maria's Workspace

Maria Iuliano
Biederer Lab, Yale University

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We use this protocol and it's working

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Abstract

Animal models that accurately recapitulate the accumulation of alpha-synuclein (α-syn) inclusions, progressive neurodegeneration of the nigrostriatal system and motor deficits can be useful tools for Parkinson's disease (PD) research. The preformed fibril (PFF) synucleinopathy model in rodents generally displays these PD-relevant features, however, the magnitude and predictability of these events is far from established. We therefore have optimized the synthesis generation of α-syn fibrils to ensure reliable, robust results. These fibrils can be added to neurons in culture, differentiated iPSCs, or injected into mice or rats. The protocol includes steps for fibril synthesis as well as sonication for fibril fragmentaion which is a critical step for inducing formation of α-syn inclusions.

This updated protocol has been specified for use in vitro, and has been confirmed for use in both rat and mouse primary cultures.

Attachments

DOCX

fibril_prep_protocol...

5.1MB

Guidelines

This protocol is a modification from previously published manuscripts (Patterson et al., 2019; Polinski et al., 2018; Stoyka et al., 2020; Volpicelli-Daley, Luk, & Lee, 2014).

For safe handling of fibrils please read Bousset L et al. (2016) An Efficient Procedure for Removal and Inactivation of alpha-Synuclein Assemblies from Laboratory Materials *J Parkinsons Dis.*6:143-51
https://pubmed.ncbi.nlm.nih.gov/26639448/

When opening tubes and pipetting, perform in a BSL2 safety hood to prevent contamination.
References:

CITATION

Patterson, J. R., Polinski, N. K., Duffy, M. F., Kemp, C. J., Luk, K. C., Volpicelli-Daley, L. A., . . . Sortwell, C. E. (2019). Generation of Alpha-Synuclein Preformed Fibrils from Monomers and Use In Vivo. J Vis Exp(148).
LINK
10.3791/59758

CITATION

Polinski, N. K., Volpicelli-Daley, L. A., Sortwell, C. E., Luk, K. C., Cremades, N., Gottler, L. M., . . . Dave, K. D. (2018). Best Practices for Generating and Using Alpha-Synuclein Pre-Formed Fibrils to Model Parkinson's Disease in Rodents. J Parkinsons Dis, 8(2), 303-322.
LINK
10.3233/JPD-171248

CITATION

Stoyka, L. E., Arrant, A. E., Thrasher, D. R., Russell, D. L., Freire, J., Mahoney, C. L., . . . Volpicelli-Daley, L. A. (2020). Behavioral defects associated with amygdala and cortical dysfunction in mice with seeded alpha-synuclein inclusions. Neurobiol Dis, 134, 104708.
LINK
10.1016/j.nbd.2019.104708

CITATION

Volpicelli-Daley, L. A., Luk, K. C., & Lee, V. M. (2014). Addition of exogenous alpha-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous alpha-synuclein to Lewy body and Lewy neurite-like aggregates. Nat Protoc, 9(9), 2135-2146.
LINK
10.1038/nprot.2014.143

Materials

Equipment:

- -80 °C freezer
- BSL2 Culture Hood
- CO_2 Incubator

Materials:

- Cultured neurons
- 1x PBS
- 1% SDS
- Preformed α-synuclein fibrils (Note: for rat or mouse cultures, ms PFFs work best)
- Polystyrene Sonication Tubes **Active Motif Catalog #**53071

Protocol materials

Polystyrene Sonication Tubes **Active Motif Catalog #**53071 Materials, Step 5

Safety warnings

Please see the Safety Data Sheet (SDS) for safety warnings and hazards before start.

When opening tubes and pipetting, perform in a BSL2 safety hood to prevent contamination.

Before start

Sonicating Fibril

Proper sonication is a key step for the fibril model to work. For all *in vivo* work which involved injecting fibrils into mice or rats, we use the QSonica 700 sonicator with cup horn and tube rack for 1.5 mL polypropylene tubes with a chiller at 16°C. The cup horn sonication produces short fragments which maintain their morphology for 6-8 hours (at least) and can be stored in dry ice overnight, thawed and maintained at room temperature, and therefore remain active after overnight shipments.We found that over time, the heat generated by a probe tip sonicator causes the fibrils to form amorphous aggregates (Figure 1). This is a problem because stereotaxic surgeries can take several hours and the amorphous aggregates that form while the fibrils sit on the bench causes variability and reduces the concentration of seeding competent fragments. Another advantage of using the cup horn sonicator over probe tip is that 25 µL of fibrils can be sonicated, reducing the volume needed. This is also a closed tube system which increases safety. For neuron or cell culture work in which the fibrils are added to media and then the cells immediately after sonication, a probe tip sonicator is okay. Again, this should be performed in a BSL2 hood with all proper PPE (nanoparticle respirator, goggles, gloves etc.). The volume of fibrils to be sonicated cannot be less than 100 µL.

In all cases, we wear PPE when working with fibrils. We clean any spills with 1% SDS.

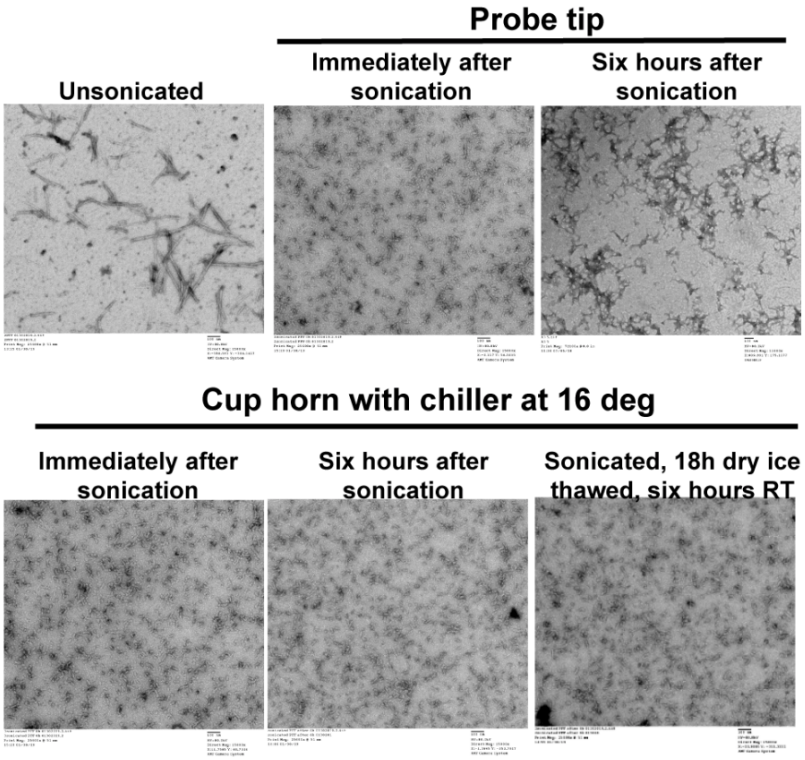

Figure 1: Transmission electron microscopy of α-synuclein fibrils. Immediately after probe tip or cup horn sonication, long fibrils are broken into small fragments. However, after six hours at room temperature, probe tip sonicated fibrils begin to form amorphous aggregates. With cup horn sonication performed at 16°C, the fragments after 6 hours appear similar in morphology compared to immediately after sonication. When the sonicated fibrils are placed in dry ice overnight, thawed and left at room temperature for 6 hours, the fragments appear similar to immediately after sonication, indicating that overnight shipments will maintain active fragments.

PFF Sonication

15m 6s

1 Sonicator Info:

Equipment	
Q700 Sonicator	NAME
Sonicator	TYPE
QSonica	BRAND
Q700	SKU
https://www.sonicator.com/products/q700-sonicator	LINK
Q700 Sonicator with sound enclosure, cup horn, and recirculating chiller	SPECIFICATIONS

2 Fill Qsonica water reservoir with about 900 mL water .

Note

Make sure reservoir water level height is 7 cm.

3 Turn on sonicator and recirculating chiller. Set the temperature at 10 °C and allow to cool.

4 Thaw 30 µL aliquots of PFFs at [M] 0.200 µg/µL at Room temperature

Note

Typically, a starting dilution of [M] 0.200 µg/µL in 1x PBS has worked consistently for us in vitro. Ideal sonication volume in our hands is between 20-30 µL . (We chose 30 µL , as it was a typal volume needed for our experiments)

5 Transfer to Polystyrene Sonication Tubes **Active Motif Catalog #**53071 within cell culture hood

Safety information

When working with PFFs, it is best to follow proper safety precautions and wear appropriate PPE, including lab coat, gloves, sleeve guards, safety glasses or face shield, and N95 or FFP2 mask.

Inactivation and disposal of PFFs and PFF-contaminated items can be done using 1% SDS as described in literature.

Collect and dilute solutions into 10vol 1% SDS for 01:00:00 at Room temperature
Discard used tips into primary collection container with 1% SDS
Inactivated liquid can be discarded with liquid waste
Discard inactivated items in primary container into biohazard
Wipe surfaces or contaminated tools down completely with 1% SDS, followed by water, then EtOH

CITATION

Bousset L, Brundin P, Böckmann A, Meier B, Melki R (2016). An Efficient Procedure for Removal and Inactivation of Alpha-Synuclein Assemblies from Laboratory Materials..
LINK
https://doi.org/10.3233/JPD-150691

6 Transfer tube to Qsonica tube holder for placement within the cup horn

Note

The gap between tube bottom surface and Qsonica probe upper surface should be 1 cm.

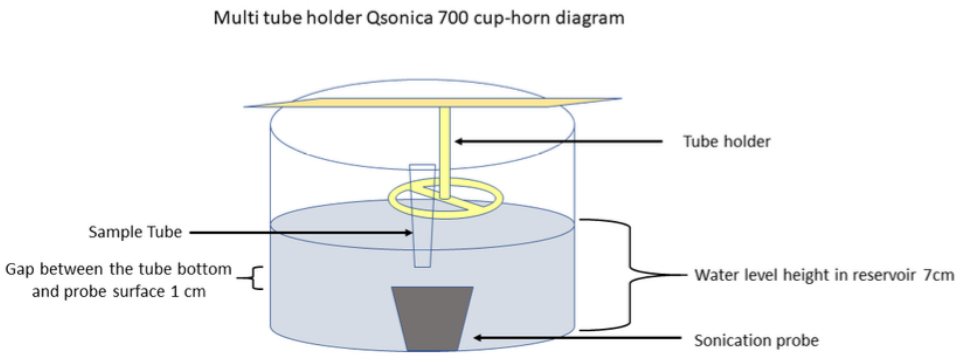

Figure 2. Diagram of Qsonica700 with multi-tube holder

7 Total sonication time 00:15:00 30m

Pulse on 00:00:03 and off 00:00:03

Temperature 10 °C

Amplitude at 45%

Sonication output should be between 90-100kJ

Note

The goal is to consistently obtain fragments of α-synuclein that are on average 50 nm in length (Figure 1, 3). If the fibrils are not sufficiently fragmented, the abundance of α-synuclein inclusions produced can be low and highly variable.

We have found that for in vitro experiments, these parameters and energy output typically result in sufficient PFF fragmentation, though periodic confirmation may be useful.

PFF Treatment 15m 6s

8 In culture hood, dilute PFFs to a volume of 300 µL in 1x PBS for a concentration of [M] 0.02 µg/µL

9 Add 25 µL (0.5 µg) to each well of a 24-well plate. Equal volume 1x PBS was added for control conditions.

Note

0.5 µg/mL is our typical treatment concentration for PFFs across plating configurations. Optimization may be necessary for individual experiments.

10 Age cultures for desired treatment length before fixation or collection.

Note

A 7-day PFF treatment results in robust p-α-syn accumulation. Shorter treatments may also be used, depending on experimental design/objectives.

Citations

Step 5

Bousset L, Brundin P, Böckmann A, Meier B, Melki R. An Efficient Procedure for Removal and Inactivation of Alpha-Synuclein Assemblies from Laboratory Materials.
https://doi.org/10.3233/JPD-150691