

Standardizing seeding experiments using the Bioruptor® for the understanding of the neuronal alpha-synuclein pathology

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INTRODUCTION

Dr. Kelvin Luk's lab at the University of Pennsylvania focuses on understanding Parkinson's disease (PD), a progressive neurodegenerative condition for which there is currently no cure. Their current efforts focus on the biology of midbrain dopamine neurons, PD drug discovery, and the role of protein misfolding in PD. The neuronal proteins α -synuclein and tau are prone to pathological misfolding and aggregation in certain neurodegenerative diseases. Tau-containing neurofibrillary tangles characterize Alzheimer's disease whereas Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are characterized by neuronal inclusions composed of α -synuclein (1). The recently developed α -synuclein fibril seeding model has allowed researchers to induce intracellular inclusions that are biochemically and morphologically similar to those found in PD and DLB brains and study their effects (2,3). This model is unique in that inclusion formation can be induced in cultured neurons by exposure to recombinant α -synuclein fibrils. In addition to examining downstream effects of these inclusions, this model can be used to screen putative drugs that modify α -synuclein inclusion formation ahead of downstream, time- and cost-intensive *in vivo* models.

An established approach used in developing this model relies on the sonication of α -synuclein fibrils to create seeds that serve to nucleate inclusions in neurons that internalize them. Sonicated fibrils have a greater ability to seed inclusion formation from either unaggregated protein or crude fibril preparations that can contain fibrils that are bundled together (4). The sonication process achieves this by breaking α -synuclein fibers into smaller units, thus opening up more ends for fibril elongation and templating.

However, standardizing input seeding material can be difficult. Conventional sonication with an immersed probe may produce variable results due to poor control of temperature and energy that is transferred to the sample. The Diagenode Bioruptor®, on the other hand, utilizes a sonication bath-based rotor in which tubes are rotated through an ultrasound field, allowing for consistent exposure of energy. In addition, the combination of the Bioruptor's ultrasound and isothermal processing maximizes sample recovery. Here, we show that the Bioruptor allows better control of the sonication time and temperature enabling consistent energy to be passed into the α -synuclein sample leading to reproducible seeding. Additionally, the Bioruptor is also a closed system using a highly-controlled ultrasonic energy with ACT (Adaptive Cavitation Technology), a non-contact, parallel-processing and isothermal technology, ensuring greater safety, reducing cross-contamination and producing more reproducible results. Standardization of α -synuclein size will be beneficial in the reproducibility of many aspects of research where multiple batches of input material are required, such as high-throughput screening and in animal models.

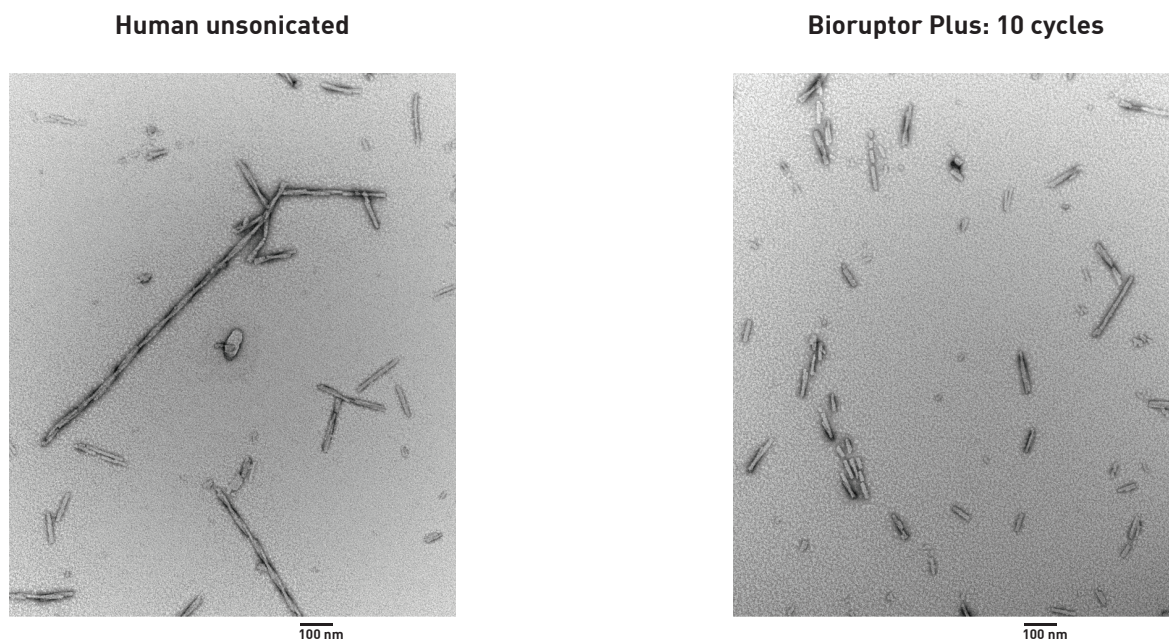
MATERIAL AND METHODS

The generation of fibrillar α -synuclein to seed inclusion formation was previously described [5, 6, 7]. Here we describe the Bioruptor sonication protocol in the creation of seeding material. Fibrils were added to sterile PBS to a final concentration of 0.1 mg/mL. The final volume was 200 μ L of diluted pre-formed fibrils (PFFs) per appropriate Bioruptor 1.5 ml microtube (Diagenode). The fibrils were sonicated using the Bioruptor Pico at 10°C at 30s ON, 30s OFF using 5, 10, 15, and 20 cycles. For comparison, the Bioruptor Plus was also used at HIGH power for 10 cycles, 30s ON, 30s OFF at a constant temperature of 10°C. It is critical to generate fibrils that are 50-100 nm in length, and sonication products can be verified using transmission electron microscopy. It is recommended that such verification be done initially to ensure that the protocol produces fibrils in this size range under the conditions in each individual lab.

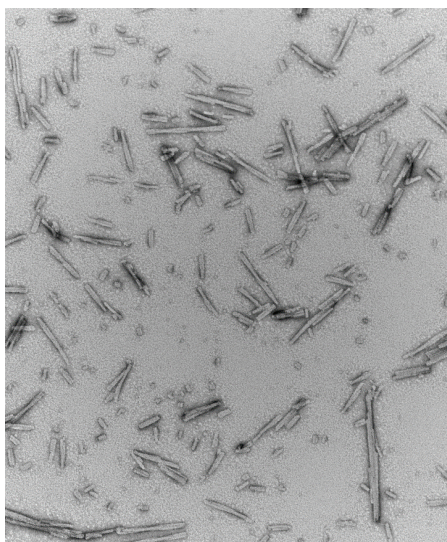
RESULTS

Shown below in Figure 1 are transmission EM images of PFFs before (left) and after (right) sonication in the Bioruptor Pico for the number of cycles indicated (unsonicated and sonicated with the Plus (10 cycles), or Pico with 5, 10, 15, or 20 cycles.). Sonication was performed for 30 s ON and 30 s OFF at 10°C.

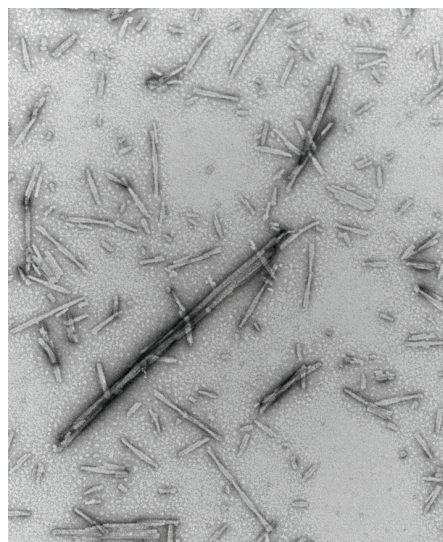
Figure 1. Electron microscopy images



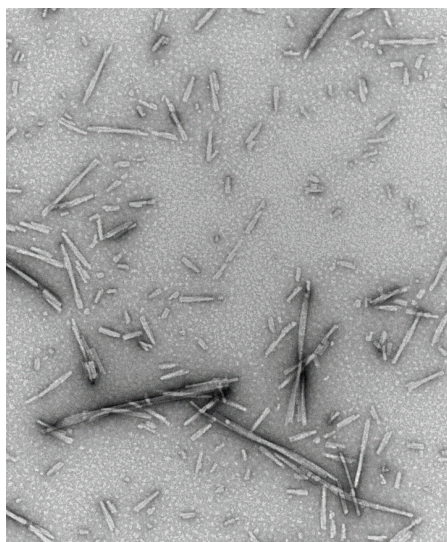
Bioruptor Pico: 5 cycles



Bioruptor Pico: 10 cycles



Bioruptor Pico: 15 cycles



Bioruptor Pico: 20 cycles

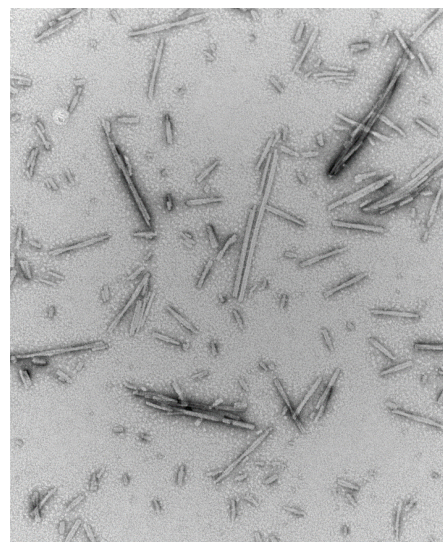
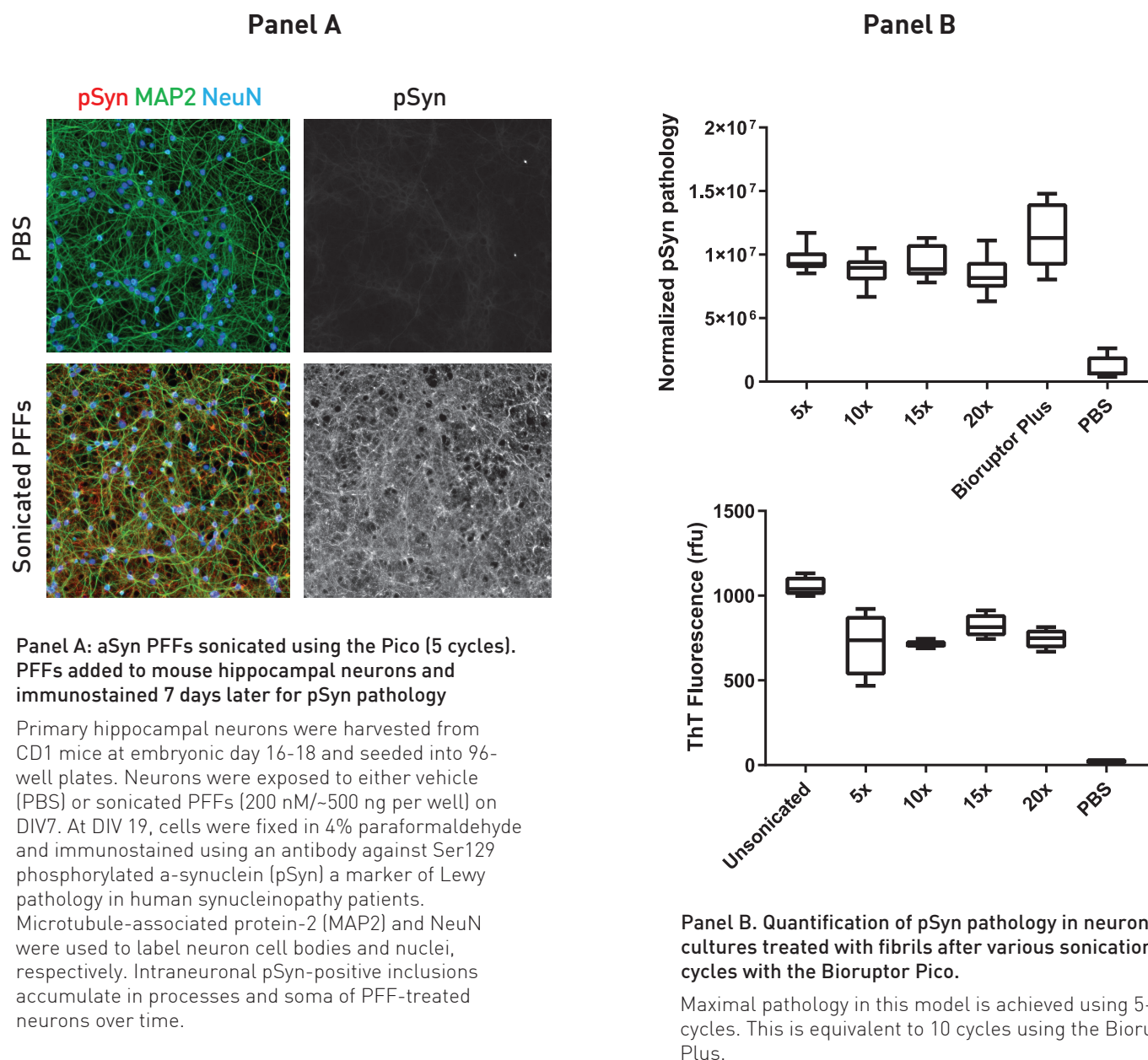


Figure 2.



CONCLUSIONS

We have demonstrated that the Bioruptor ultrasonication system provides a number of benefits in standardizing and creating representative seeding material that can serve to nucleate inclusions in neurons to better understand protein aggregation and misfolding models in α -synuclein. Specifically, we showed the following:

1. The Bioruptor sonication produces seeds of 50-100 nm in length. By generating a high number of seeding-competent fragments, the resulting products provide more surface area that effectively drive aggregation models such as the one shown.
2. Sonication does not alter the amyloid content, thus preserving the functional nature of the fibrils.
3. Unlike probe sonication, the Bioruptor systems allows for better control of the sonication time and temperature, enabling consistent energy to be passed into the sample leading to reproducible seeding.
4. Additionally, the Bioruptor allows for parallel processing of samples in a simple-to-use closed system which ensures greater throughput. This also reduces user-to-user variation, avoids sample cross-contamination, and minimizes exposure to fibril-containing aerosols during the sonication procedure.

By facilitating higher throughput and standardization of α -synuclein seeding will be beneficial for areas of research where multiple batches of input material are required, such as high-throughput screening and in animal models. In addition to α -synuclein, the fibril seeding paradigm has now been demonstrated using other neurodegenerative disease associated proteins (e.g. tau) (8,9), and we anticipate that the utility of the Bioruptor systems will be applicable to these new models of disease pathology as well.

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