University of Central Florida

NanoScience Technology Center

Dr. Warren Casey

Director, NICEATM

11th of July, 2018

Dear Dr. Casey,

This letter is to introduce our functional neuromuscular junction (hNMJ) system composed of human cells that could be used for drug evaluations or toxicity testing in vitro, as a response to the call for Information on Technologies for Detection and Measurement of Botulinum Neurotoxin.

In this phenotypic human neuromuscular junction model, human myotubes and motoneurons derived from stem cells were cultured in a serum-free medium in a BioMEMS construct. The system is composed of two chambers linked by microtunnels to enable axonal outgrowth to the muscle chamber that allows separate stimulation of each component. The muscle's contractions, induced by motoneuron activation or direct electrical stimulation, were monitored by image subtraction video recording for both frequency and amplitude. Bungarotoxin, Botulinum Neurotoxin (BOTOX® analyzed in this system) and curare dose response curves were generated to demonstrate pharmacological relevance of the phenotypic screening device. A detailed description of this model and its application for dosage testing of NMJ-related drugs can be found in our publication in Biomaterials 2018 [1].

This is a human cell-based assay, thus the result would be more applicable to human system than those from animal studies. It utilizes stem cells as the source, so bypasses any quantity limitation and would be suitable for large scale high throughput analysis. In addition, the readout is cell function (NMJ function in particular) instead of viability which has been widely used in toxicity study, therefore more sensitive than those systems.

We would be happy to welcome any suggestions or opportunities for its application. Some detailed information about Botulinum Neurotoxin testing in our NMJ system is attached in next page. Please don't hesitate to contact me if further information is needed.

Sincerely,

Professor James J. Hickman, Ph.D.
Professor of NanoScience Technology, Chemistry, Biomolecular Science and Electrical Engineering
Head, Hybrid Systems Laboratory
12424 Research Parkway, Suite 400
Orlando, FL 32826
407-823-1925

Some detailed information about dose testing of BOTOX® in our NMJ system

BOTOX® blocks vesicle fusion at the presynaptic terminal. For testing the effects of BOTOX®, 20 µl of drug, diluted in culture medium, was added to the muscle side. After a wait time of 10 min. to allow for diffusion and drug action, the motoneuron chamber was stimulated at different frequencies. Each testing frequency was preceded by a 2s of high frequency stimulation (10 Hz) in order to deplete the readily releasable pool from synaptic terminal, so that the effect of drug can be uncovered. A minimum of 4 chambers were tested for each drug. Placebo experiments were conducted by adding culture medium at similar volumes followed by wait times and stimulation patterns similar to those applied in the presence of drugs. BOTOX® (Allergan, Irvine, CA), was applied to the muscle side at the following concentrations: 0, 6.3 mU, 18.8 mU, 43.8 mU, 93.8 mU, 193.8 mU, 393.8 mU, 593.8mU, 793.8 mU and 993.8 mU [mU = milli Units of BOTOX® obtained at 100 Units/vial]. The effect of the drug is quantified by the NMJ functional testing. Specifically, MNs were stimulated at different frequencies while myotube contractions were monitored by videotape recordings of pixel differentials. This pixel differential was co-plotted with the stimulation pulse, allowing the identification of any correlation (or lack thereof) between the electrical stimulation and cell response to that stimuli.

Quantification of the NMJ function after series drug dosing indicated that BOTOX® caused a simple, monophasic dose-response as muscle contractions were attenuated (Figure 1). The half-maximum inhibitory response was IC_{50} = 0.54±0.16 U (h_c = 1.2) when fitted (R^2 = 0.893).

At present, there are as many as four different commercially available formulations of BoNT A [2]. The biological activity of each batch varies considerably as is the case with highly active biological formulations and requires dose standardization [3]. 1 Unit of activity of a BoNT preparation is defined as the dose required to kill 50% of mice of a given weight [4]. An ex vivo alternative to this ethically controversial conventional LD50 assay has been developed by Huber et al. [5] to determine the potency of the botulinum toxin preparations. Their assay, termed the Intercostal NMJ assay, records the decay of force from electrically stimulated rat muscle tissue sections in response to increasing doses of BoNT A. Their study utilized the commercially available BoNT A preparation Dysport® (Ipsen Ltd, Slough, UK) and their dose response was over the range of 10-60 LD50 units. A comparison of the pharmacokinetic properties of different formulations of BoNT A has shown that the clinical potencies of BOTOX® and Dysport® are relatively in the same range. Our dose response curve for the *in vitro* human NMJs lies in the range of 0.01 – 1 LD50 Units of BOTOX®, thus exhibiting the potential for a much more sensitive assay for the potency testing of BOTOX® preparations.

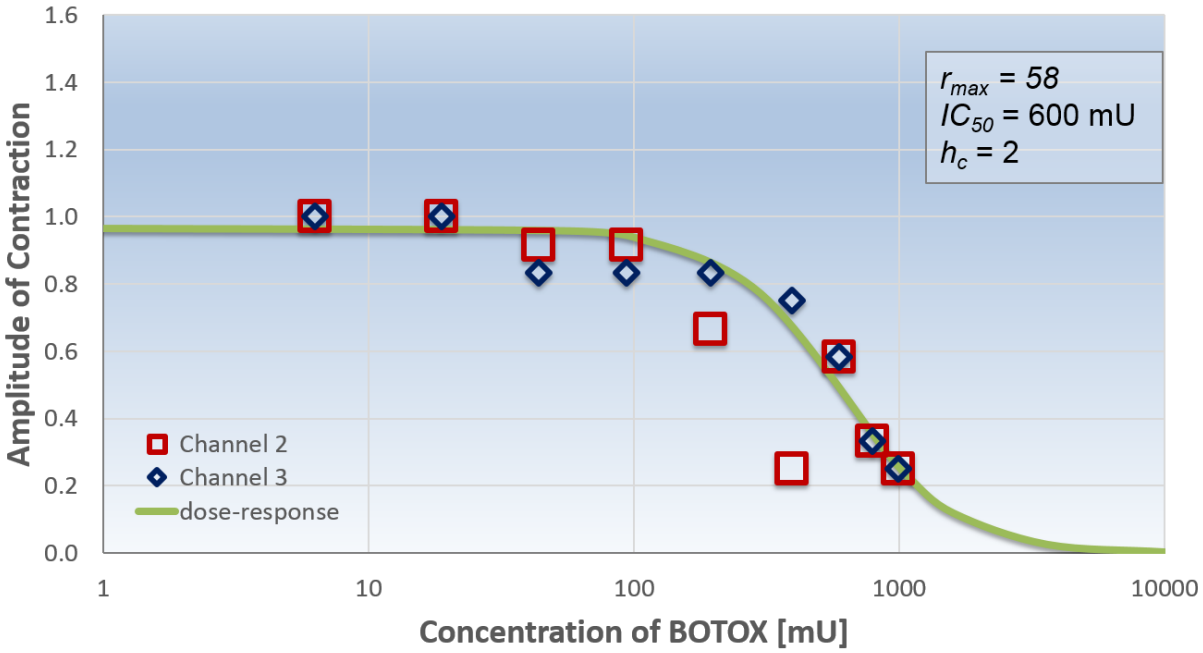

Figure 1. Dose-response curve for BOTOX® at 0.33 Hz. BOTOX® caused a simple, monophasic dose-response in muscle contractions at all frequencies tested. The half-maximum inhibitory response was at IC_{50} = 0.54 U (h_c = 1.2).

References:

1. Santhanam, N., et al., *Stem cell derived phenotypic human neuromuscular junction model for dose response evaluation of therapeutics.* Biomaterials, 2018. **166**: p. 64-78.
2. Jankovic, J., *Botulinum toxin in clinical practice.* Journal of Neurology, Neurosurgery & Psychiatry, 2004. **75**(7): p. 951-957.
3. Wohlfarth, K., K. Kampe, and H. Bigalke, *Pharmacokinetic properties of different formulations of botulinum neurotoxin type A.* Movement disorders, 2004. **19**(S8): p. S65-S67.
4. Appiah-Ankam, J. and J.M. Hunter, *Pharmacology of neuromuscular blocking drugs.* Continuing Education in Anaesthesia, Critical Care & Pain, 2004. **4**(1): p. 2-7.
5. Huber, A., et al., *The Intercostal NMJ Assay: a new alternative to the conventional LD50 assay for the determination of the therapeutic potency of botulinum toxin preparations.* Alternatives to laboratory animals: ATLA, 2008. **36**(2): p. 141-152.