Engineering the Microenvironment of Human Schwann Cells by Providing In Vitro Electrical Firing Artificial Axons

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Objectives. Myelin basic protein (MBP) keeps the cytoplasmic sides of the overlapping Schwann Cells (SC) membrane close together. It is still an unmet clinical need to restore a disrupted myelination process caused by traumas or diseases. SC receive signals from axons to coordinate normal myelination and most of these signals are molecular factors. There is evidence, however, that non-molecular factors in the form of patterned electrical impulses (PEI) play an important role. PEI have been investigated in co-cultures where both neurons and myelinating cells were present. This co-culturing prevents an uncoupling of the direct effect of PEI on myelinating cells from the indirect effect mediated by neurons. To uncouple these effects, we developed an in vitro model where an electroconductive carbon fiber (CF) acts as an artificial axon, with suitable electronics delivering PEI. (1)

Methods. The CF provides the biophysical characteristics of an axon but does not contribute any molecular signaling. In our "suspended wire model" (SWIM), the CF is suspended in a liquid media supported by a 3D printed polycaprolactone scaffold, with PEI generated by using an Arduino 101 microcontroller. Characteristics of the pattern were completely defined via software. The voltage was set and the resistance was varied in the circuit to generate variable amperage. A pulsed electrical current and its pattern were shaped according to the following underlined characteristics: 1)-the stimulus is constituted by a single impulse or multiple impulses; 2)-this stimulus is followed by an interval, a period between two stimuli where no impulse is present; 3)-the sequence of stimulus and interval can be repeated a number of times and this give us a frequency of stimulus firing and can be regarded as a tonic stimulation; 4)-after a cycle of tonic stimulation, a rest pause follows where no stimulus is generated. The PEI apparatus was created using an Arduino 101 microcontroller board which is based on the 32 bit, 32 MHz Intel Curie module and includes onboard Bluetooth Low Energy (BLE) capabilities. The embedded software which generates the PEI and communicates over BLE, was written using the Arduino IDE. A visual programming environment, MIT App Inventor was used to develop an application for controlling the PEI apparatus wirelessly over blue tooth low energy. Validation of the output was conducted using a 1 GS/s 50 MHz 4-channel Rigol DS1054Z Digital Oscilloscope.

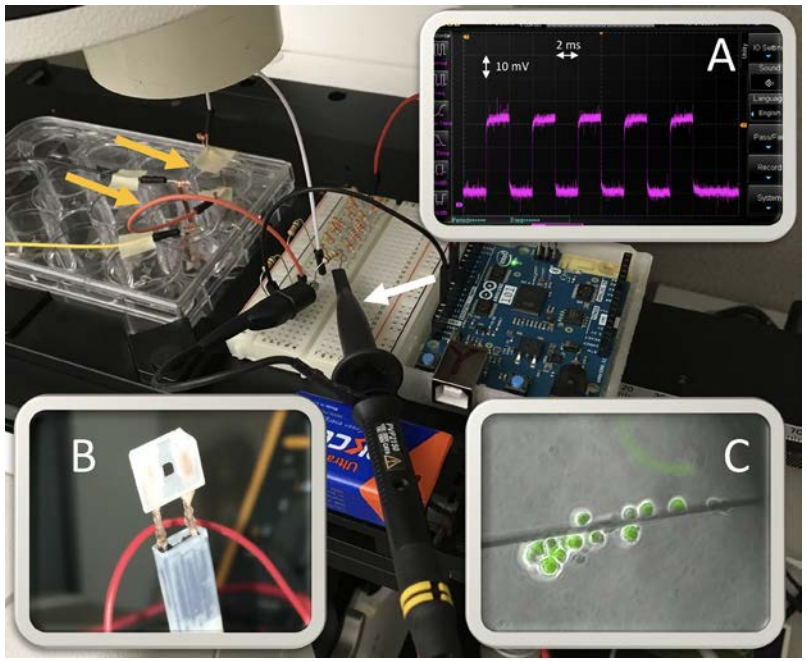
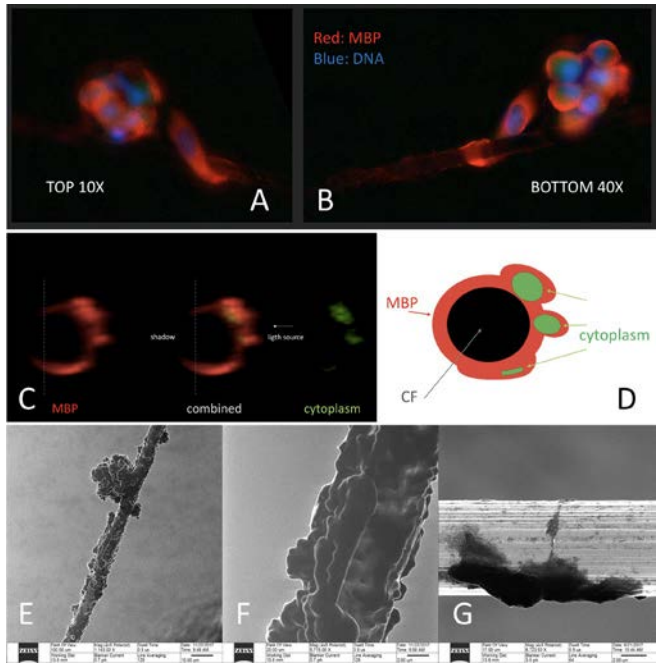


Figure 1: *The experimental set-up on an fluorescent inverted microscope stage. Recording from the circuit where made by a probe (white arrow) and showed a modest "capacitor effect" on the impulse (A). Two wells (yellow arrows) in a 12-well culture plate were equipped with the scaffold (B). Fluorescent green live-tracker shows cells interacting with the CF prior to the delivery of PEI (C).*



Results. When PEI was delivered in vitro (Figure 3), hSC started to adhere to the CF and expand their membrane on the surface of the fiber. The cells continue to wrap around the CF in the presence of PEI, thus reproducing the early steps of the myelination process. Our results showed that hSC can sustain the administration of PEI up to 3 hours (the maximum duration tested so far). Helium-ion scanning microscopy and high definition confocal laser microscopy showed evidence of a complete wrapping of the CF by human Schwann cells in the presence of PEI together with the presence of cytoplasmic channels which spiral around the CF.

<- Figure 2: *up: The simultaneous reading of output generated by 3 boards shows the 3 patterns. The inset of a recording at the lowest Voltage of 32 mV shows the presence of 1 impulse per stimulus in pattern 1 and 5 impulses per stimulus in patterns 2 and 3. Down: a feature of the SWIM system is the possibility to image both the top (A) and the bottom of the CF (B). A transverse section was produced by software at the level of the wrapping of the hSC around the CF; since the fiber blocks the transmission of light, a shadow obscure the entire circumference of the fiber (and that is the reason why a sequential imaging of the top and the bottom is useful). It may be speculated that cytoplasmic channels (green) represent the HDCM image equivalent of the "Schmidt-Lanterman incisures" or of the "bands of Cajal" (D). HIM show the CF fully covered by cell membrane in a field of view of 100 micron (E) and 20 micron (F). In G a comparison is provided with a CF where SC membrane is scantly present; the bare CF is bright and it clearly shows it regular texture of parallel crevices (F).*

Conclusions. In this study, we demonstrated the adherence and enstheathment of human Schwann cells (hSC) to CF in the presence of PEI. We reproduced the early steps in the myelination process. Developing an apparatus to successfully deliver PEI to cells in culture makes it feasible to investigate the response of SC to PEI in vitro.

Reference: (1) Merolli A, Mao Y, Voronin G, Steele JAM, Murthy NS, Kohn J. *A method to deliver patterned electrical impulses to Schwann cells cultured on an artificial axon.* Neural Regen Res. 2019 Jun;14(6):1052-1059

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