The use of a suspended carbon fiber culture to model myelination by human Schwann cells

Antonio Merolli[1,2], Yong Mao[1], Joachim Kohn[1]
[1]New Jersey Center for Biomaterials, Rutgers University, 145 Bevier Rd., Piscataway, NJ 08854
[2]Policlinico Gemelli, Universita' Cattolica del Sacro Cuore, largo Gemelli 8, 00168 Rome, Italy

Statement of Purpose: Understanding of myelination-remyelination process is essential to guide tissue engineering for nerve regeneration. In vitro models currently used are limited to cell population studies and have difficulty dissecting individual contributions to the process. In a limited number of reports, engineered fibers have been used to evaluate myelination in the absence of axons in vitro; however, by far, Oligodendrocytes but not Schwann cells from human (or other species) have been used (1-5). We established a novel model that does not include axons or neuronal factors to address, for the first time, the contribution of biophysical properties of axonal structure to the myelination process by human Schwann cells (HSCs). The single wire construct allowed us to create a map to locate every single cell precisely and it was easy to track individual cells day-by-day.

Methods: Our model uses a single carbon fiber, suspended in culture media, to provide an elongated structure of defined diameter with 360-degree of surface available for HSCs cells to wrap around. On a PTFE ring-scaffold (outer diameter 14 mm), notches were made on the top surface at the opposite points across the diameter. A single carbon fiber (Fibre Glast, Brookville, OH, USA) (6.7 µm diameter) was placed in the notches, tightened and fixed in place with cyanoacrylate glue. The sterilized scaffold was carefully positioned on the bottom of wells of a non-tissue culture treated 24 –well plate. HSCs (ScienCell Research Laboratories, Carlsbad, CA) were cultured in Schwann cell medium (SCM, ScienCell Research Laboratories) on a polylysine coated dish till 80% confluence. The medium was then removed and a fresh serum-free SCM containing 10 µM of CellTracker Green CMFDA (C2925) (Thermofisher) was added to the cells. Cells were resuspended at $3x10^5$/ml in SCM. 1 ml of cells was then added to each well with a scaffold placed at the bottom of the well. Microscopic observation was performed by phase-contrast and fluorescence analysis (Zeiss Observer D1) once a day for a period of nine days. On the last day, cell viability was evaluated by Live staining.

After fixation in 4% paraformaldehyde, scaffolds were incubated with Mouse anti-MAG antibody (ab89780 AbCam) and rabbit anti-MBP antibody (ab124439 AbCam) then secondary antibodies, anti-rabbit IgG-Alexa 555 and anti-mouse IgG-Alexa 488 were added.

Results: Immediately after cell seeding, rounded HSCs were detected on the suspended CF as individual cells or in aggregates. However, after 2-4 hours of incubation and medium change, aggregates were rarely detectable on the fiber. The cells that remained on the fiber were strongly adhering to the fiber as evidenced by the fact that they could not be washed away. *Cell elongation* along the wire became obvious starting on day 2. We also observed cells, which apparently wrapped around the fiber completely and *changed morphology* during the following days, exhibiting *outfolding* (blebbing) in a timely fashion (Figure 1). Outfoldings occurring far from the cell body have been associated with the initial stages of myelination, when cytoplasm flows into spiraling channels between enwrapping layers of the Schwann cell membrane. We noticed the *fading of the fluorescent cell tracker* after prolonged culture. In order to rule out the possibility that cells are dying, we stained the cells with calcein AM (live staining) and recorded that *HSCs on the CF remained alive*. The expression of myelin basic protein (MBP), a fundamental specific molecule in the process of myelination, was evaluated at Day 5 and Day 9. MBP was detected not only in the body but also in the elongations and blebs of cells along the fiber. The expression of myelin associated glycoprotein (MAG) was evaluated at Day 9. MBP and MAG are not completely co-localized and *distinct MBP or MAG staining* was observed along the fiber.

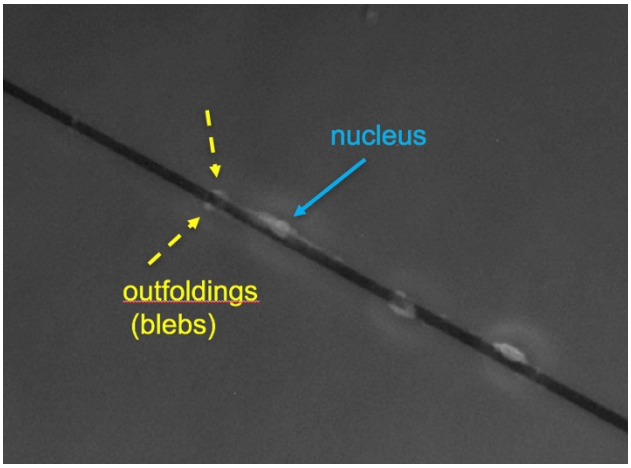

Figure 1. Elongated cell on day 4.

Conclusions: We observed cell attachment, elongation and enwrapment around a fiber over a period of 9 days. Cells are alive and express Myelin Basic Protein and Myelin Associated Glycoprotein as expected. This model enables us to spatially and temporally track the process of myelination by individual human Schwann cells along a single fiber. Natural/artificial molecules and external physical factors (e.g., pulsed electrical current) may be tested as possible regulators of myelination by this new model.

References:

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