

Electrospun conductive polymer fiber substrates to study the effect of electrical stimulation on the differentiation of oligodendrocyte progenitor cells

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INTRODUCTION

- In the central nervous system, the myelin producing mature oligodendrocytes are derived from oligodendrocyte progenitor cells (OPCs).
- OPCs respond to mechanical, chemical, and electrical cues from their environment².
- Our aim is to determine whether OPC differentiation is strongly influenced in vitro by the application of a biologically relevant electric field.
- Conductive nanofiber scaffolds which can function as axon surrogates have the potential to enhance the study of remyelination therapies for conditions like multiple sclerosis.

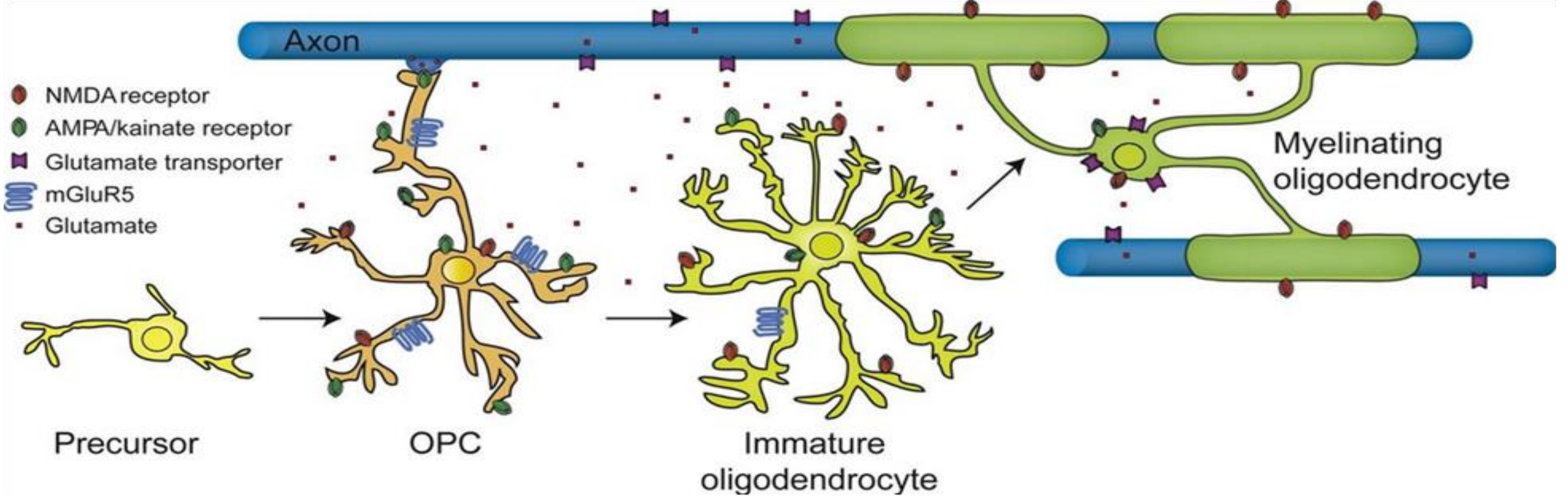


Figure 1. Schematic depiction of a myelinating oligodendrocyte (right) that has differentiated from a mitotic progenitor (oligodendrocyte precursor cell [OPC], left)¹.

METHODS

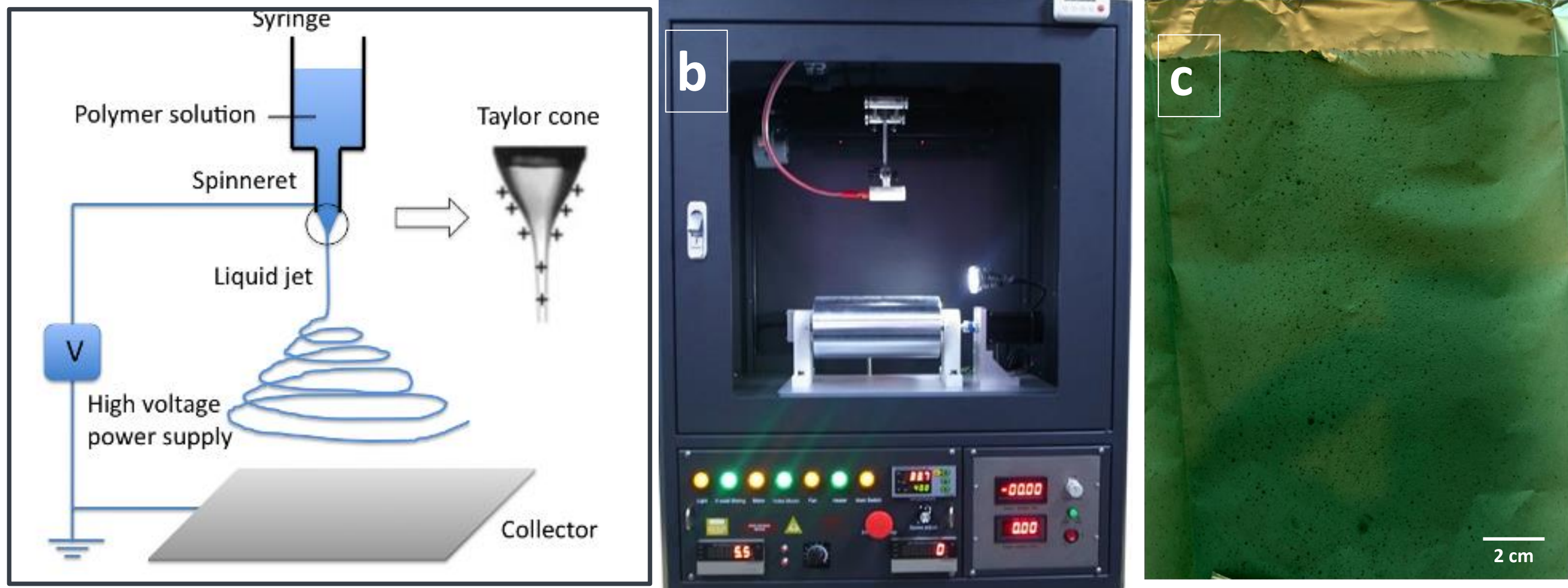


Figure 2. a) Schematic depiction of electrospinning setup b) Electrospinning machine and c) electrospun PANI/PG mat.

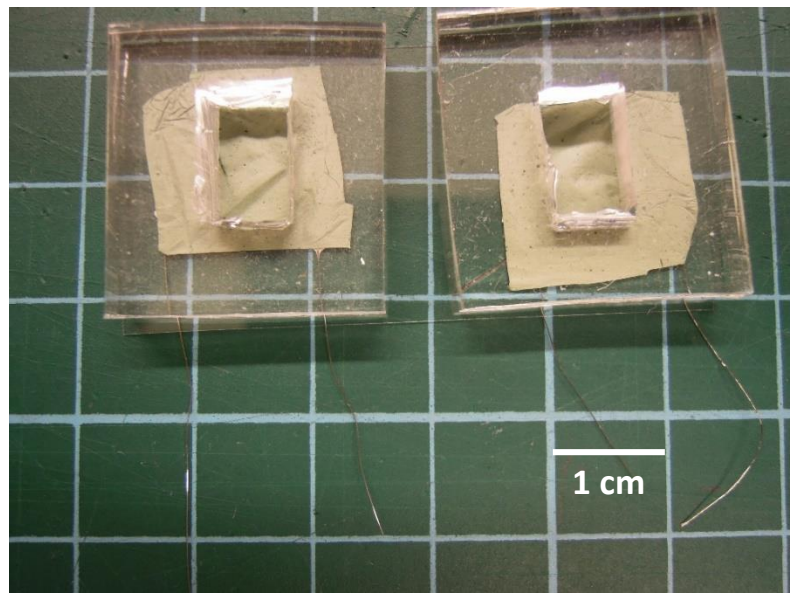
Solution composition

Polyaniline (PANI) emeraldine base, polycaprolactone (PCL), gelatin (G) and 10-camphorsulfonic acid in Hexafluoro-2-propanol (6 wt%)

Process Parameters

- Flow rate = 2 ml/h
- Voltage = 26.1 kV
- Needle gauge = 20G (0.6 mm)
- Collector distance = 15 cm

OPCs seeded on PANI/PG fibers functionalized with fibronectin and cultured at 37° C, 5% CO₂ for 24 hours in proliferation medium. Media was later changed to induce differentiation (DMEM with SATO's modification, sans growth factors).



RESULTS

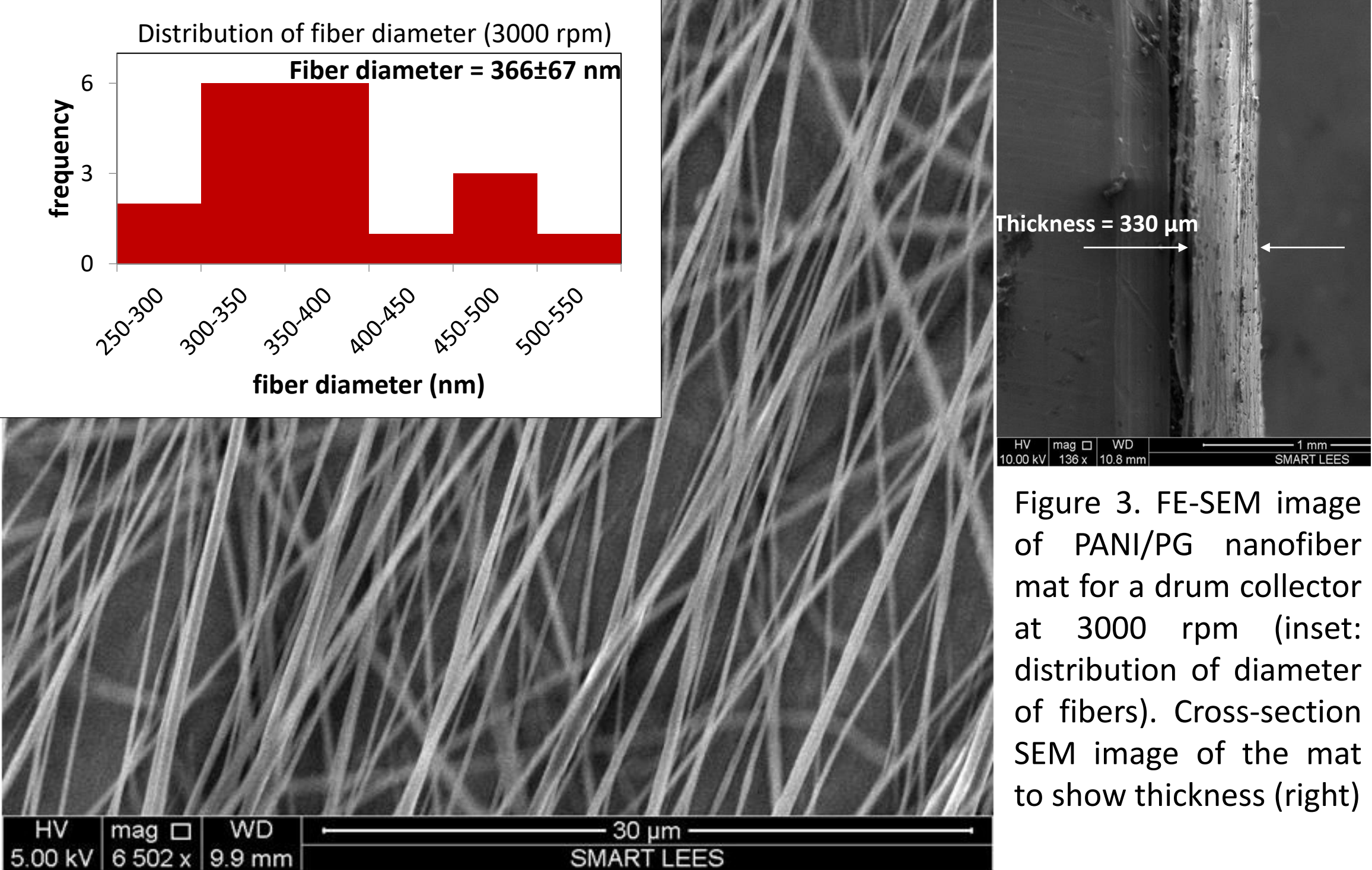


Figure 3. FE-SEM image of PANI/PG nanofiber mat for a drum collector at 3000 rpm (inset: distribution of diameter of fibers). Cross-section SEM image of the mat to show thickness (right)

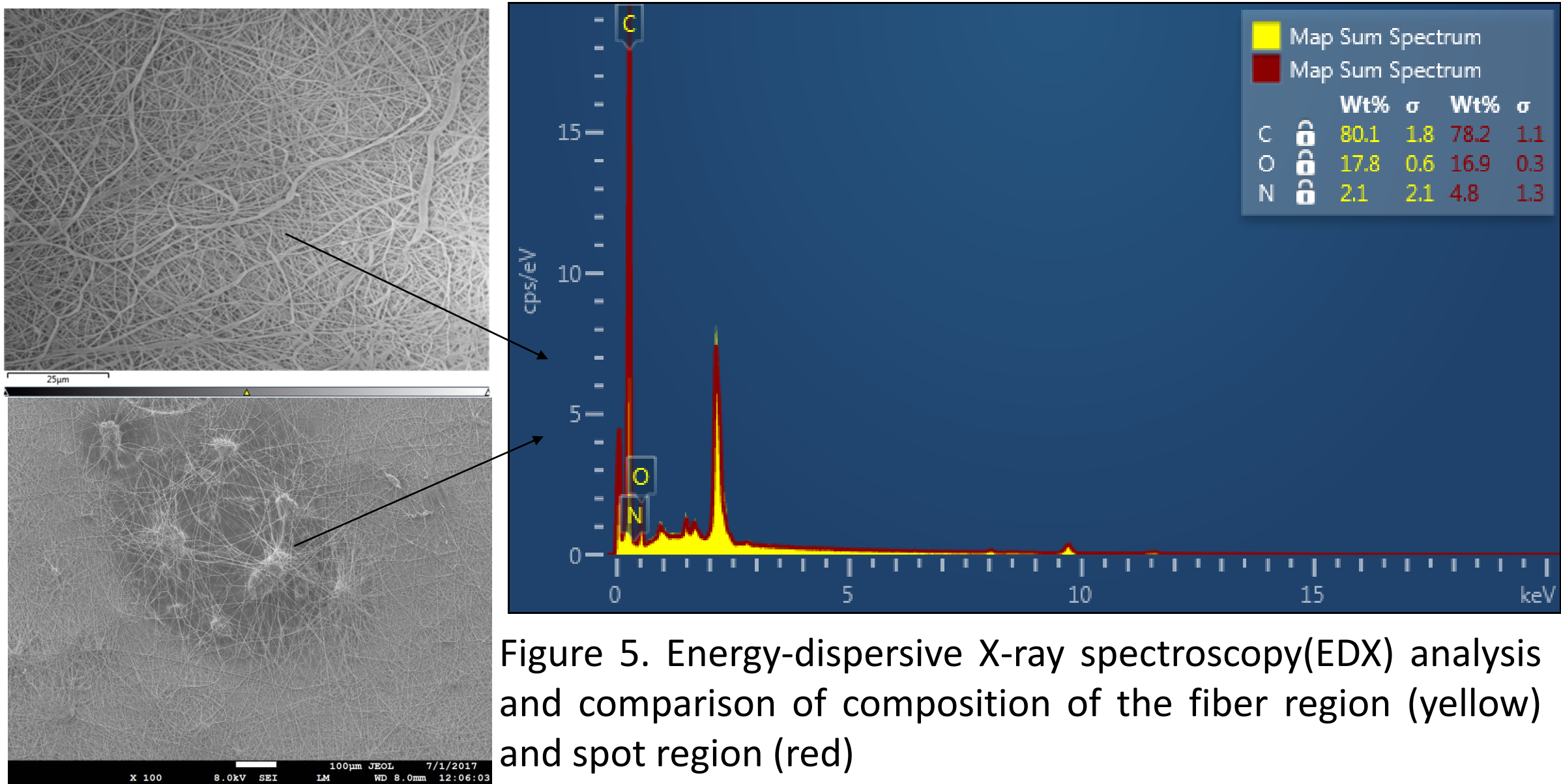
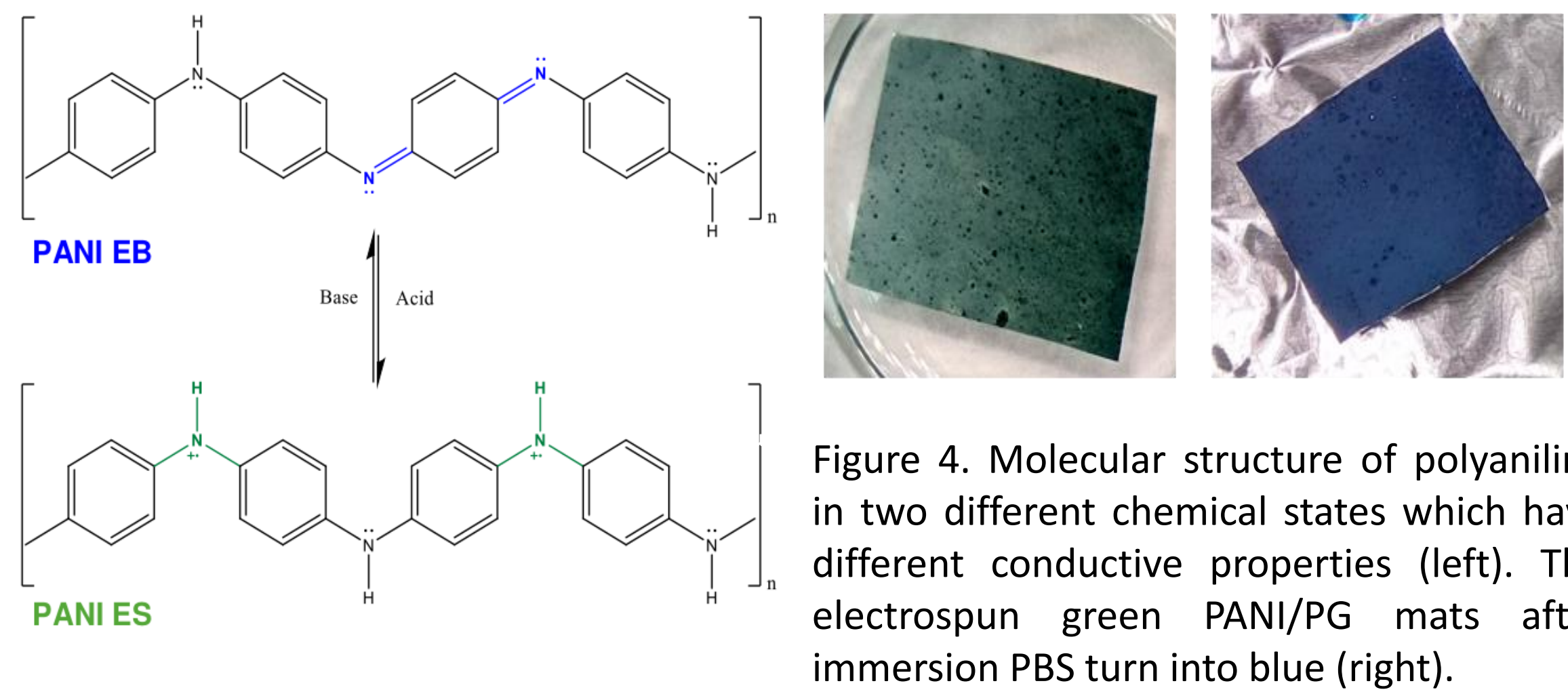


Figure 5. Energy-dispersive X-ray spectroscopy(EDX) analysis and comparison of composition of the fiber region (yellow) and spot region (red)

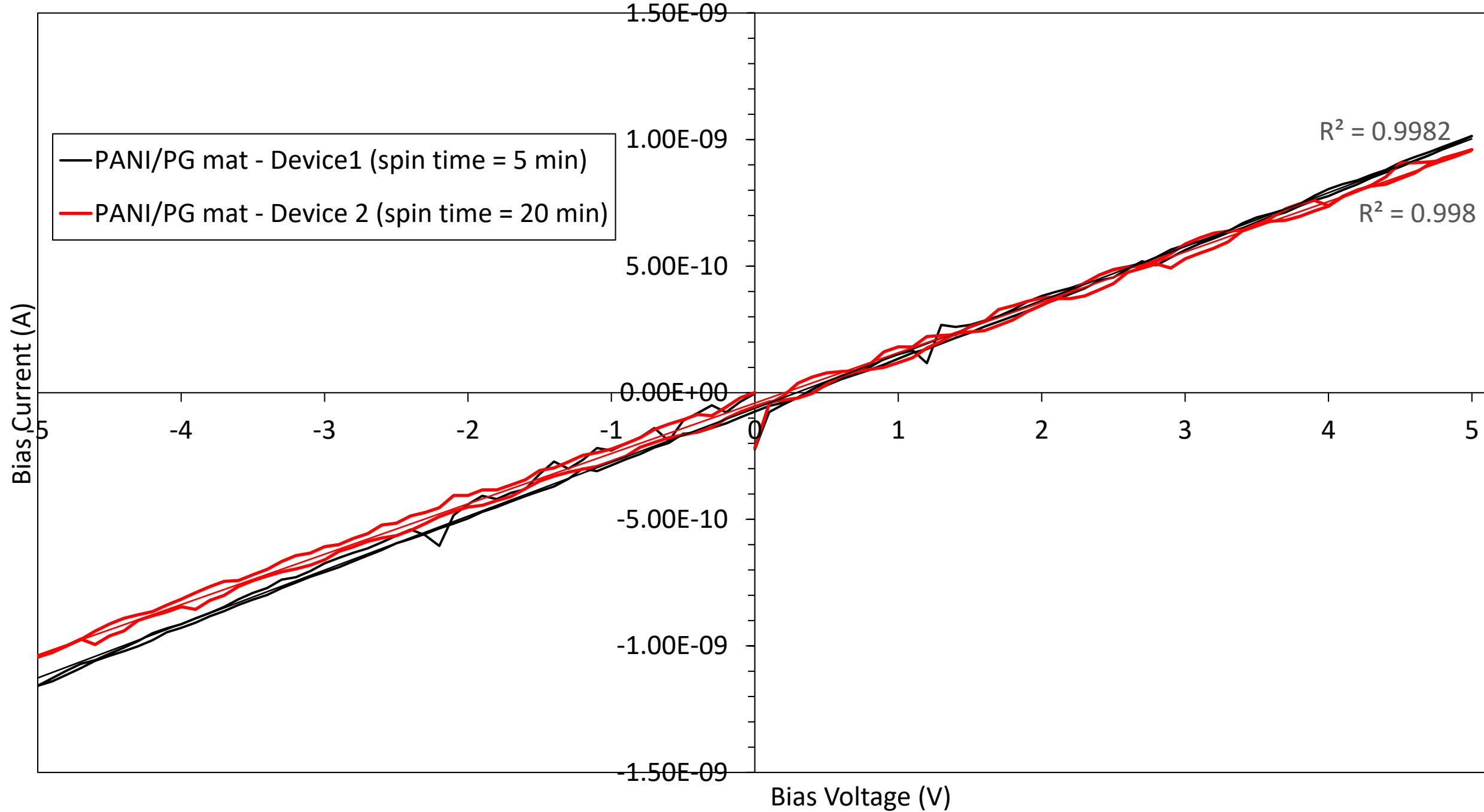


Figure 6. Current versus voltage (I-V) curve at a voltage range of -5 V to +5 V for two devices with fiber mats with different thickness.

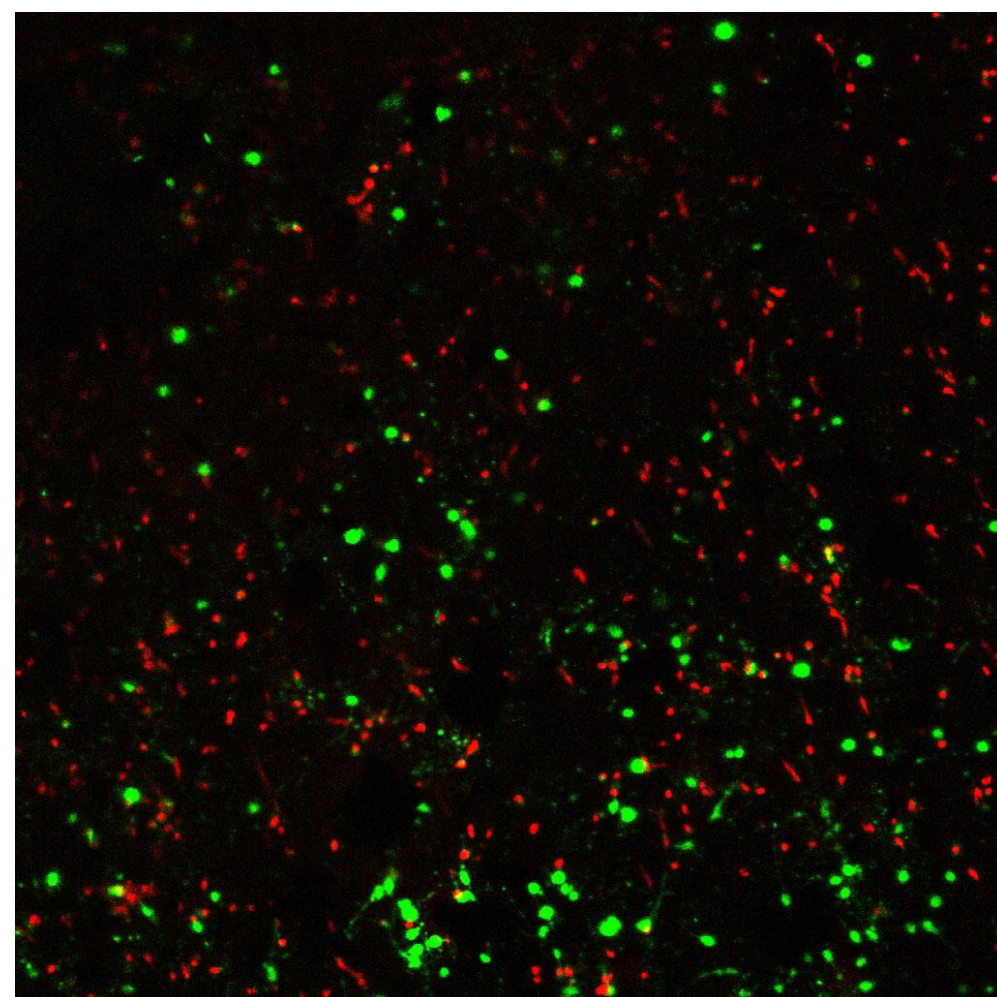
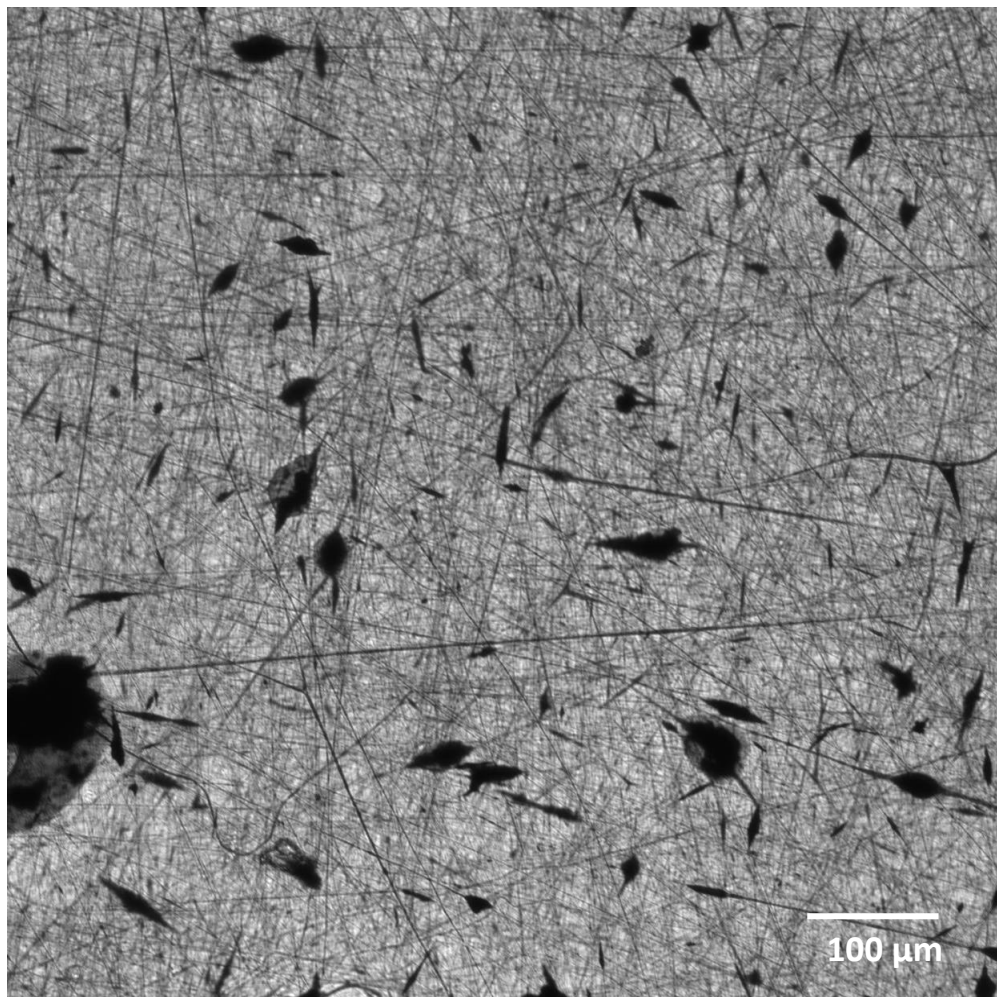


Figure 7. Phase contrast (above) and fluorescence microscopy (below) images (using live/dead staining) of OPCs seeded on electrospun PANI/PG membrane after 24 h in proliferation medium.

CONCLUSIONS

- PANI/PG based nanofiber mats have been fabricated by electrospinning (Fig. 3) by improving the ability to process PANI through addition of polycaprolactone (PCL) and gelatin; PANI – intrinsically conductive polymer, PCL – provides mechanical strength and gelatin – enhances cell adhesion and proliferation (Fig. 7).
- FE-SEM shows the morphology and a fiber diameter distribution dependent on the collector and drum speed. The spot regions on fiber mats have similar composition to the fiber region, as seen in the EDX analysis (Fig. 5).
- Challenges in measuring the conductance of the PANI/PG mats and the observed mat resistance is of the order of 10⁹ Ω (Fig. 6), which needs to be lowered to achieve current flow at biologically relevant voltages during stimulation.
- Initial cell viability study on the PANI/PG mats suggests no cytotoxicity for cell culture of OPCs.
- Customized device fabrication for seeding OPCs on PANI/PG mats by incorporating Pt electrodes for electrical stimulation
- The next step is to improve the conductance of the electrospun mats and their stimulation with cell relevant potential difference to observe its effect on myelination, based on the expression of differentiation markers by OPCs.

References

1. Kolodziejczyk, K., Saab, A. S., Nave, K.-A., & Attwell, D, 2010, F1000 Biology Reports, 2, 57
2. Cognato et al., 2004, J Cell Biol 167:365-375; Jagielska et al., 2012, Stem Cells Dev 21:2905-2914; Relucio et al., 2012, Glia 60:1451-1467