

***In vitro* Growth and Differentiation of Neuronal Synaptic Network on PEDOT: Tosylate Substrate**

Krishnendu Chatterjee^{a,b,c}Ph.D Student, Department of Engineering and System Science, National Tsing Hua University, Hsinchu 300, Taiwan^a

Nano Science and Technology Program, Taiwan International Graduate Program, Academia Sinica, Taipei 115,

Taiwan and National Tsing Hua University, Hsinchu 300, Taiwan^bResearch Center for Applied Sciences, Academia Sinica, Taipei-115, Taiwan, R.O.C.^c

ABSTRACT: Establishing primary neural cultures on conducting polymers is a powerful technique for in-vitro analysis of primary structural networks and functional complexities of electro-chemical neural signaling. Several doped polymers like PEDOT materials have been developed and applied in the field of neuro-cultural studies, especially for electro-stimulation studies. In this work, tosylate doped PEDOT materials have been studied to grow primary cultured hippocampal neurons directly from neonatal rat brain, obtained via newborn litters of SD rats maintained in our lab. Immunofluorescence assay was performed to view structural integrity for individual neurons showing healthy growth, maturation and viability of cells grown onto such polymer. The functional potency of these neurons with respect to acute synaptic behavior was visualized using Ca^{+} ion modulated stimulus-response imaging. Experiments conducted over long growth periods showed PEDOT: tosylate capable of sustaining healthy primary neuron cultures for at least 15 days and potentially applicable for further in-vitro/in-vivo studies.

KEYWORDS: PEDOT: Tosylate, hippocampal neuron, conductive polymer, neural interface, calcium imaging

I. INTRODUCTION

Neurological disorders affect and debilitate the normal brain function in about 6% of the total population globally[1]. The possibilities for brain electrodes implants are endless but would need comprehensive study upon the effects of stimulation in various regions of the brain, the development of neural interfaces capable of high recording sensitivity, low impedance, specific charge injection capacity and long-term stability. The adverse brain tissue reactions upon electrodes implantation[2] along with poor electrical charge transfer, low sensitivity and high impedance of the electrodes[3] are the most common causes for botched brain electrodes implants. Brain neural network and cellular interactions are an extremely complex structure, but until recently most research groups failed to go beyond 2D cell culturing methodology; a barrier Mario Romero-Ortega et al.,[4] were able to overcome. Recreating a 3D complex, layered brain like microstructure through printing technique using a unique bio-ink consisting of peptide-modified biopolymer, gellan gum-RGD (RGD-GG) in conjugation with primary cortical neurons, they were able to study the formation of discrete layers of neuronal cells.

Several *in vitro* methods have been employed for studying neuron culture which includes earlier methods like hanging drop[5, 6], carrel flask[7, 8], roller tube[9], campanot chamber [10] to more modern microfluidic devices[11, 12] and patterned substrate[13, 14]. The assimilation of neurons on specialized substrates for controlled neural development in response to an artificial stimulus is in progress. By manipulating the microenvironment via mechanical or biochemical stimulus, modulation of neurons has been achieved[15, 16] to some extent focusing on their impulse-signaling. Conducting polymers (CPs) are well suited for inducing on demand neuronal response to electro-

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

stimulation[17, 18]. The mechanical and electrical properties for CPs can be fine-tuned[19] for mimicking the microenvironment to maintain and regulate neural stem cell differentiation.

Several conducting polymers have been employed for enhancing neuronal differentiation and promotion of neurite outgrowth like polypyrrole (PPy)[20], polyaniline (PANI)[21], polythiophene derivatives like Poly (3,4-ethylenedioxythiophene) PEDOT[22], etc. Amongst the CPs, the flexibility[23], biocompatibility and inherent electron transport properties make PEDOT a viable candidate for interfacing between neurons and organic bioelectronic interfaces. By having a dioxyalkylene bridging group across the 3- and 4-positions of its heterocyclic ring, the electrical conductivity of PEDOT is vastly improved due to the lowering of its band gap, reduction and oxidation potential, in comparison with other polythiophene derivatives[24]; which makes PEDOT have a significant edge over other conducting polymers in terms of chemical, environmental and thermal stability.

Surface topography is also an interesting way to influence and control neurite growth and differentiation [25, 26]. PEDOT has been utilized in conjugation with various materials like tosylate[27], polyethylene glycol (PEG)[28], poly (2-hydroxyethyl methacrylate) (pHEMA)[29], poly(styrene sulfonate) PSS[30], poly(L-lysine-graft-ethylene glycol) (PLL-g-PEG)[31],[32], polyurethane (PU)[33] and graphene oxide [34] for neurite outgrowth. The thickness and roughness of the PEDOT films directly influence the biological performance of the substrate, with the thicker and rougher coatings leading to better conductivity with proportionally reduced biological activity[35]. The in-vivo biocompatibility of PEDOT material has also been tested by seeding NIH3T3 fibroblasts on PEDOT films followed by its implantation subcutaneously[36], making it a viable candidate for the development of implantable neural prosthetics. PEDOT: PSS has been able to support the growth and differentiation of primary embryonic neurons[37],[38]; pre-layering it with poly-L-lysine further improves the long-term adhesion of neurons[39]. PEDOT: PSS-based microelectrodes have found applications in vivo, like recording brain activity[40], including superficial cortical activity[41] and electrocorticography (ECoG)[42] in rats amongst others. But although PEDOT: tosylate is better suited for biological studies in comparison with other PEDOT-based composites[27],[35] (PEDOT/ PSS and PEDOT/ CIO₄), there is a dearth of study focusing on growth, differentiation and long-term maintenance of neuron culture on it.

In this study, we synthesized PEDOT: tosylate substrate for the investigation of its biocompatibility when seeded with post-natal rat hippocampal neurons *in vitro*. The PEDOT: tosylate material was surface coated with poly-L-lysine for improving the growth and differentiation of primary neuron cells. The poly-L-lysine PEDOT: tosylate were tested for its viability for the growth and differentiation of neurons *in vitro* for hippocampal cultures. Calcium imaging and immunofluorescence study were undertaken to study the synaptic activity, physiological and morphological analysis of the neuronal cells when interfaced with the PEDOT-based substrate. Fig 1 depicts the schematic for the preparation, coating and experimental design.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

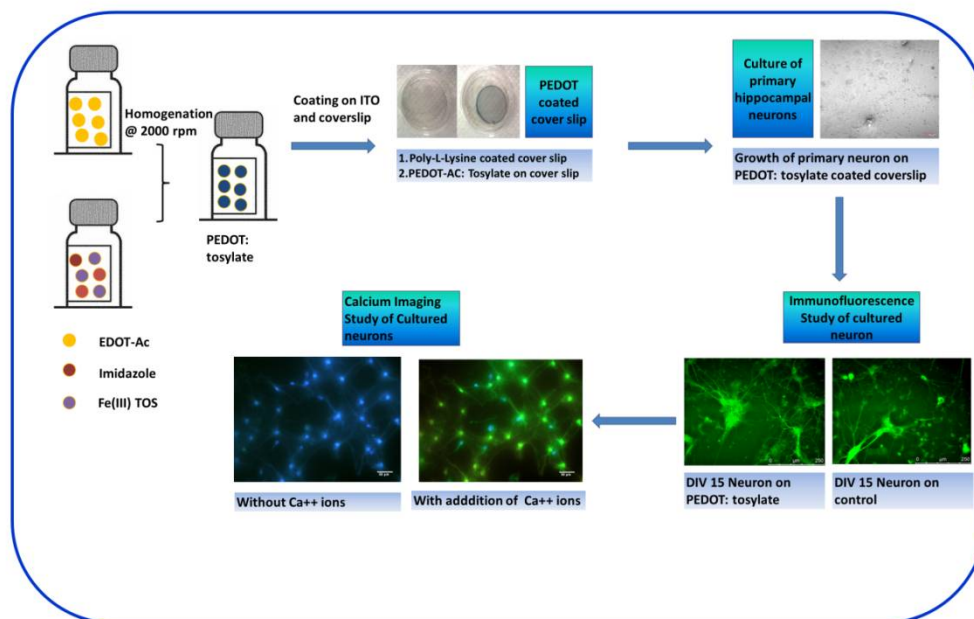


Fig 1: Schematic overview of preparation, coating and experimental design of the study.

II. MATERIALS AND METHODS

II.1 PREPARATION OF PEDOT:TOSYLATE FILMS.

Preparation of PEDOT: Tosylate was conducted following the protocol of Hsiao et al.,[43] with some minor modifications. For obtaining PEDOT:tosylate film, solutions of imidazole (IM) (TCI Chemicals) (1.47 M; inhibitor) and Fe (III)TOS (Sigma-Aldrich) (2.56 M; oxidizing agent) was prepared separately in MeOH at 60°C. The preparation of PEDOT-Ac precursor involved the mixing of EDOT derivative (67 mg of EDOTAc) with 1.8 mL each of as prepared IM and Fe(III)TOS solution followed by spinning at 2000 rpm (10 s) for homogenization. It was then coated on ITO glass (RiDisplay Corporation) ($<10 \Omega \text{ sq}^{-1}$) and the coated film changed from yellow to dark green/blue upon polymerization (10 min) on a hotplate (105 °C). The film was then cooled to room temperature followed by washing (thrice) with MeOH to remove the unutilized EDOTAc monomers and excess Fe(III)TOS.

II.2 ANIMAL MAINTAINENCE AND ETHICAL APPROVAL

Two weeks pregnant SD rats were procured from BioLASCO Taiwan Co., Ltd for the purpose of hippocampal neuron extraction. Subsequent to procurement rats were housed under a pathogen-free condition with controlled light (12 hour light/dark schedule; lights on 6am-6pm) and humidity. Autoclaved water and food were given to the rat *ad libitum*. Bedding consisted of wood shavings (Aspen Shavings NEPCO). Cages were cleaned twice a week and sterilized every fortnight. The rat handling was done using its tail for all practical purposes. The protocol of the animal experiment was approved by and all experiments were performed according to the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee. They were handled according to the established guidelines for animal care at the center under procedures in compliance with Taiwanese Law.

II.3 ISOLATION AND CULTURE OF PRIMARY HIPPOCAMPAL NEURON FROM BRAIN

Prior to hippocampal neuron isolation, all things were sterilized by autoclaving and UV exposure in the hood. Newborn rat pups were sacrificed within 16 h and brain tissue obtained using standard dissection techniques. Hippocampal

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

neurons were isolated from post-natal rat[44] pups for neuron cultures., with minor adjustments. Instead of trypsin, papain (0.6 mg/mL) was used in HBSS for digestion purpose and horse serum was utilized for the purpose of enzyme inactivation after digestion step. All other steps remaining same as reported[44].

The neurons were plated on glass coverslips (control), PEDOT: PSS and PEDOT: tosylate, coated with poly-L-lysine (1 mg/mL in 0.1 M borate buffered saline. Each coverslip or the PEDOT-coated surface was incubated with 1 ml of poly-L-lysine than it was removed after overnight incubation and washed with DI water. The plates can be stored up to a week at 4° C filled with Hank's Balance Salt Solution (HBSS) (Thermo Fisher Scientific).

II.4 IMMUNOFLUORESCENCE STUDY

Specific immunofluorescence study was undertaken to differentiate between the growths of neuron morphology on poly-L-lysine coated glass coverslips (control) and PEDOT-based material. The cultured neurons (DIV 15) were washed with phosphate buffer saline (PBS) (Thermo Fisher Scientific) thrice and then fixed with 4% paraformaldehyde (Sigma-Aldrich) in PBS overnight. The slides were subsequently blocked with blocking buffer (3% bovine serum albumin (Sigma-Aldrich), 0.3% Triton X 100 (Sigma-Aldrich) in PBS) at room temperature for 30 mins and incubated with mouse monoclonal anti-MAP2 antibody (1:1000 dilution; Sigma-Aldrich #M4403) at room temperature for 1 h. The slides were then washed with washing buffer (PBST) (0.5% Tween 20 (Sigma-Aldrich) in PBS) thrice before incubating with goat anti-mouse IgG-TRITC antibody (1:200 dilution, Sigma-Aldrich #T5393). The samples were then visualized with an Olympus 1X81 microscope.

II.5 CALCIUM IMAGING

Calcium imaging; a powerful physiological tool for studying synaptic function and plasticity of neurons was performed. Calcium indicators can be categorized primarily into either ratiometric dyes (Fura 2 AM) and single wavelength dyes (Fluo 4 AM). Zeiss AxioVert 200 system (Lambda 10-2 Optical Filter Change) was used to record changes in calcium ion concentration in randomly identified neurons (DIV 14). Fura 2 AM (Abcam) is a ratiometric calcium probe capable of measurement of basal calcium levels and a small rise in calcium ion concentration. For Ca imaging purpose, the coverslip was washed with HBSS thrice and incubated with Fura 2 AM (2 μ M final conc) for 45 mins at 37 °C (in incubator). Then cells were again washed with HBSS thrice and incubated in HBSS for 30 mins at 37 °C (in incubator). The cell culture coverslips incubated with Fura 2 AM were then placed in a perfusion chamber. After that, the perfusion apparatus so installed was adjusted to aspirate liquids at 50 μ L/ sec. The internal and platform dual temperature controller was maintained at 37° C. The focal plane was subsequently adjusted to clearly focus on the hippocampal neurons under the microscope. The samples are then excited rapidly with 340 nm (high calcium) and 380 nm (low calcium) while the emission is collected at around 510 nm. The image analysis was performed using MetaFluor. The ratiometric measurement of the signal which is collected at 510 nm after excitation at 340/ 380 nm gives a measure of the instantaneous calcium ion change. Fluo 4 AM calcium probe (same methodology as Fura 2 AM for dye incubation) was also utilized to study the accumulation of calcium ions (5 mM) in neurons (DIV 14) with an Olympus 1X81 microscope.

II.6 DATA ANALYSIS

The calcium imaging data captured employing Fluo 4 AM were processed by MetaMorph software. For the purpose of measuring fluorescence intensity, 10 randomly chosen neurons were analyzed on the control and PEDOT: tosylate before and after the addition of calcium ions. All experiments were performed in triplicates. Two-tailed Student t-test was performed for studying the statistical significance of the data; with $p < 0.05$, considered to be statistically significant. The values are shown by mean \pm standard deviation.

III. EXPERIMENTAL RESULTS

III.1 PEDOT: TOSYLATE SUPPORTING THE GROWTH OF HIPPOCAMPAL NEURONS.

The rats procured were in good shape and well cared for. The rat pups were born healthy and within 16h, the obtained hippocampal neurons were healthy with good anchorage and dendritic network.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

The rats procured were in good shape and well cared for. The rat pups were born healthy and within 16h, the obtained hippocampal neurons were healthy with good anchorage and dendritic network.

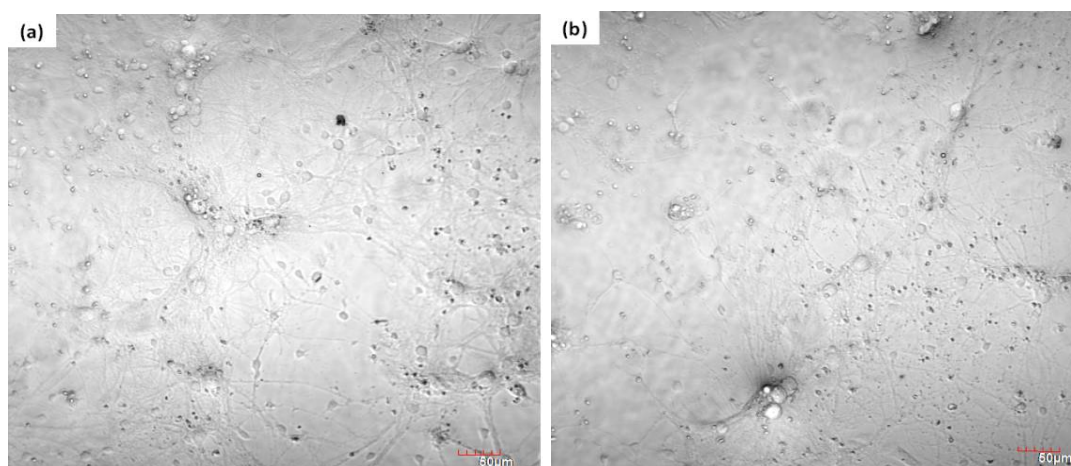


Fig 2: DIC image of rat hippocampal neurons. Neuron cultured on poly-L-lysine coated glass cover slip (control) and PEDOT: tosylate on DIV 15.

The neuronal cells were able to attach to the PEDOT substrates after seeding and begin neurite extension within 1 day. Fig 2 clearly demonstrates that the growth and differentiation of the cultured neurons on the PEDOT-based substrate are morphologically similar to that of the control substrate. TOS doped PEDOT substrates showed more neuronal aggregation compared to control but overall proved to be compatible with neural primary cultures.

III.2 IMMUNOFLUORESCENCE STUDY INDICATING BIOCOMPATIBILITY OF PEDOT: TOSYLATE

The hippocampal neuron cultures (control, PEDOT: PSS & PEDOT: tosylate) were immune-labeled following the previously mentioned protocol for quantifying the growth and development of neurons.

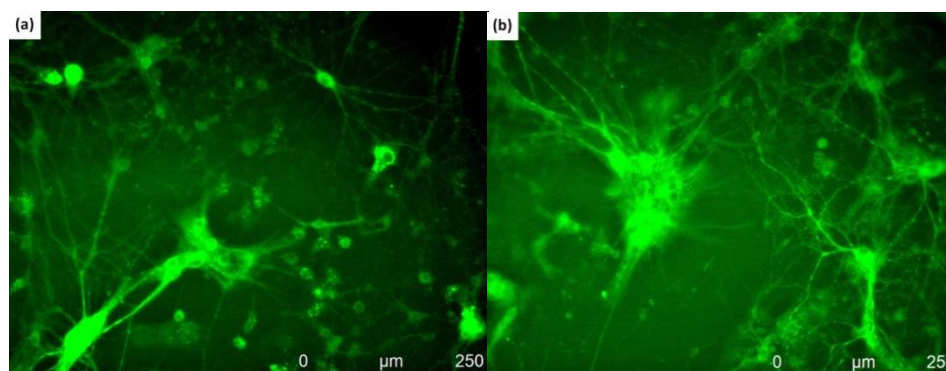


Fig 3: Immunofluorescence imaging of neuron culture. (a) Neurons (DIV 15) cultured on poly-L-lysine-coated glass (Control) (b) PEDOT: PSS and PEDOT: tosylate. Cells were stained with antibodies anti MAP2 (green).

Anti-MAP2 antibody stained neuron culture (DIV 15) images is shown in Fig 3. Fig 3 visibly illustrates similar growth and differentiation of neuronal cells, comparable to both the control (Fig 3a) and PEDOT: tosylate (Fig 3b). We can conclude that PEDOT: tosylate layers exhibit healthy morphological features including neurite

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

development and formation of arborizations along with comparable network size and cell density when matched to neuronal culture maintained on control substrate.

III.3 CALCIUM IMAGING INDICATING HEALTHY NEURONAL GROWTH AND SYNAPTIC RESPONSE ON PEDOT: TOSYLATE.

Polystyrene sulfonate doped PEDOT (PEDOT: PSS) have been able to support growth and differentiation of hippocampal neurons in comparison to control substrate, showing similar neuronal passive properties including membrane capacitance, input resistance and resting membrane potential, evaluated by single cell patch clamp recording[22]. Patch-clamp recordings can differentiate between neuronal subtypes but fail to investigate the neuronal plasticity and synaptic response, which can be obtained from calcium imaging studies. Fig 4, shows the calcium imaging experiments conducted employing ratiometric dye - Fura 2 AM, on postnatal rat hippocampal neurons (DIV 14) cultured on control substrates to evaluate the responses of external chemical stimulus (EGTA- Ca^{2+} chelator & Ca^{2+} ions).

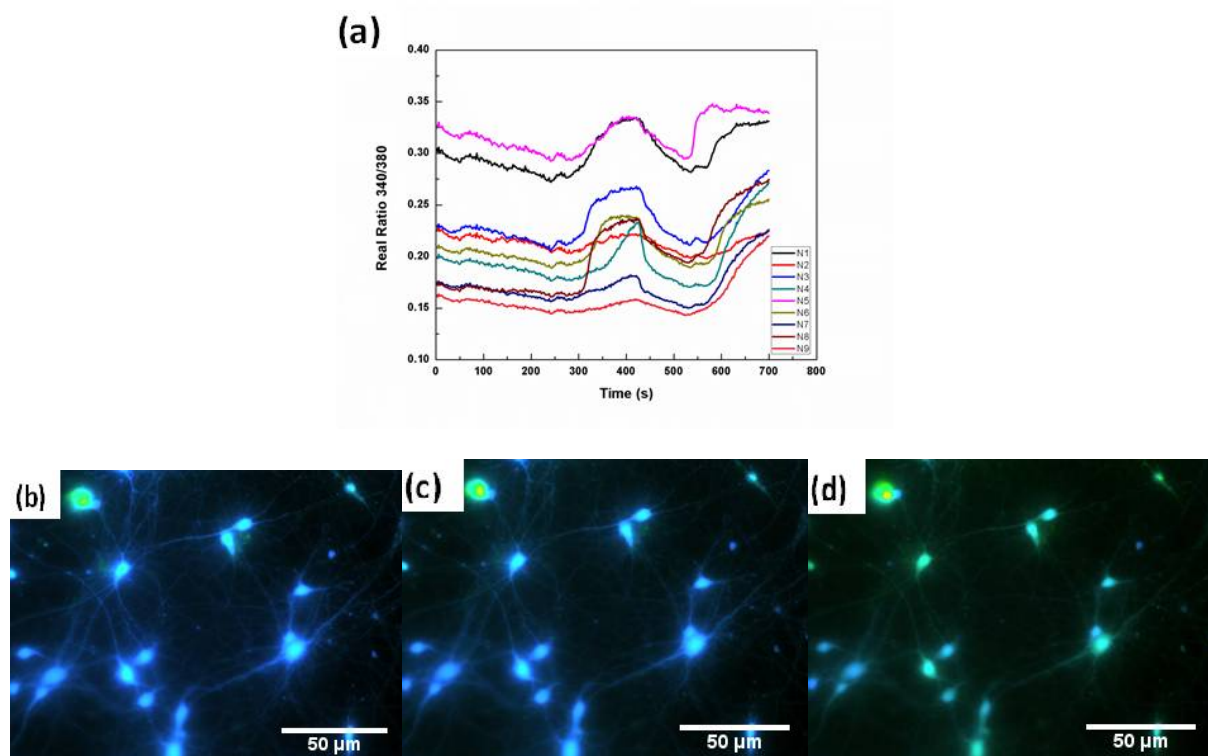


Fig 4: Calcium imaging studies employing Fura 2 AM (a) Real fluorescent intensity (background subtracted) of Fura 2 AM inside cultured rat neuron on control (b) HBSS addition for 50 cycles (c) EGTA (10mM) added for 100 cycles (d) 5mM CaCl_2 in HBSS was added for 50 cycles.

The steps involved in Ca imaging using Fura 2 AM (Fig 4a) are as follows-

1. HBSS (with Ca^{++} ions) was added for 50 cycles (each cycle = 2 sec). There is no increase or decrease of Ca^{++} ions conc both inside and outside the cell. (340 and 380 steady) Ratio steady (Fig 4b).
2. EGTA (10mM) was added to the system for further 100 cycles. This lead to decreasing Ca^{++} ions conc. (340 decreasing and 380 increasing). Ratio decreases (Fig 4c).
3. Next 5mM CaCl_2 in HBSS was added for 50 cycles leading to increase of Ca ions inside cell. (340 increasing and 380 decreasing). Ratio increases (Fig 4d).

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

4. EGTA (10mM) was added to the system for further 75 cycles. This lead to decreasing Ca^{++} ions conc. (340 decreasing and 380 increasing). Ratio decreases.
5. Next 5mM CaCl_2 in HBSS was added for 75 cycles leading to increase of Ca ions inside cell. (340 increasing and 380 decreasing). Ratio increases.

The working distance of the 20x objective lens (Objective Fluar 20x/0.75, Carl Zeiss) was around 0.60 mm, which was suitable for cell studies on coverslips (0.17 mm) but unsuitable for thicker ITO glasses (1.1 mm). So, for studying the synaptic activity of neurons cultured on PEDOT: tosylate, calcium imaging was performed employing Fluo 4 AM dye on a 1X81 microscope (20x/0.5 objective lens, working distance 2.1 mm).

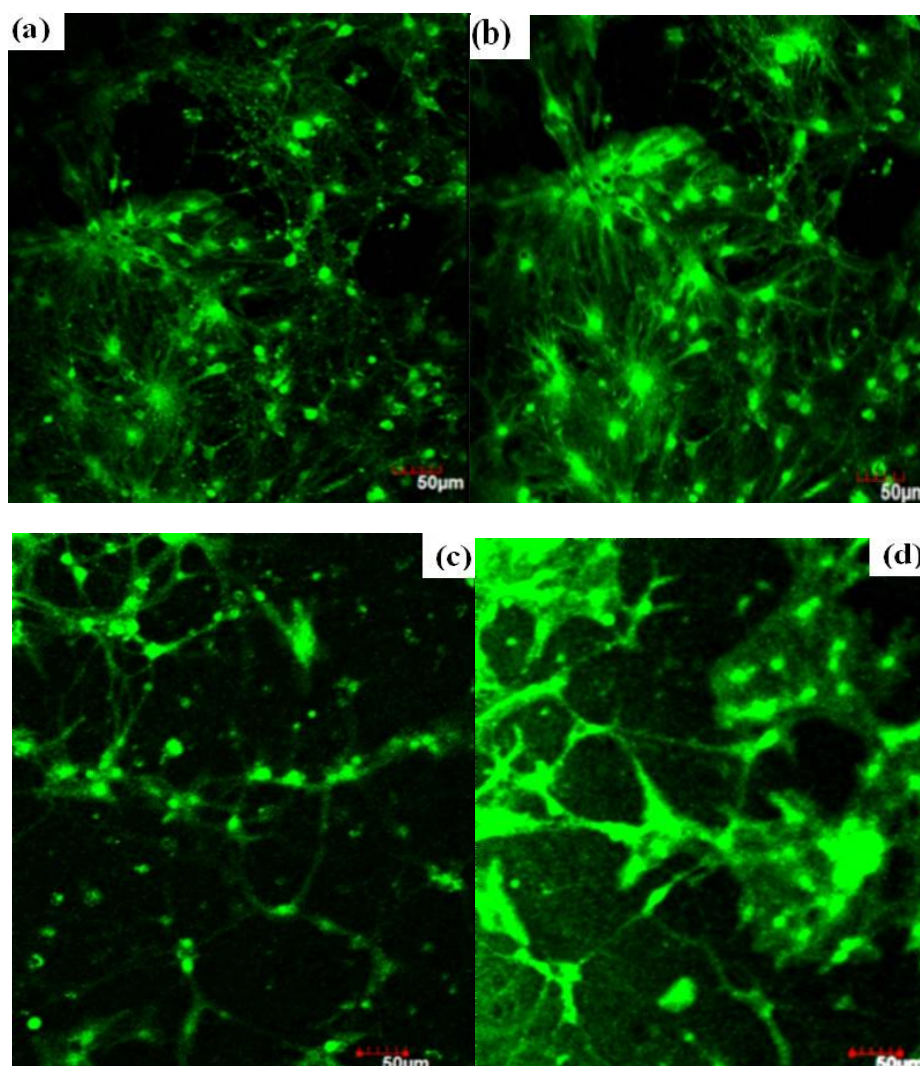


Fig 5: Calcium imaging studies employing Fluo 4 AM. Neuron culture (DIV 14) on (a) Control – before addition of Ca^{2+} ions (b) Control – 10 mins after addition of Ca^{2+} ions (5 mM in HBSS) (c) PEDOT: tosylate before addition of Ca^{2+} ions (d) PEDOT: tosylate before addition of Ca^{2+} ions (5 mM in HBSS).

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

As can be clearly seen from Fig 5, the neurons were grown on PEDOT: tosylate (Fig 5 c-d) displayed similar synaptic activity with regard to control substrate (Fig 5 a-b), indicating its ability to support functionally active mature neurons. Fluo 4 AM fluorescence intensity was found to be similar before and after addition of calcium ions (5 mM), amongst the control (99.48 ± 24.47 arbitrary units (Fig 5a); 197.71 ± 26.39 arbitrary units (Fig 5b)) and PEDOT: tosylate (95.56 ± 30.18 arbitrary units (Fig 5c); 185.25 ± 38.8 arbitrary units (Fig 5d)) respectively; $p < 0.05$, *T*-Test (Fig 5). These results indicate the similarity in size and activity of neuronal network cultured in control and PEDOT: tosylate, after under a week of *in vitro* culture. The fluorescent intensity was found to almost double for neuronal cultures for both the control and PEDOT: tosylate samples upon addition of calcium ions (5 mM). Also, it is quite evident from Fig 5, that neurons have a tendency to aggregate on PEDOT: tosylate during growth and development.

IV. CONCLUSION

In this study, we have successfully utilized tosylate doped PEDOT materials as PEDOT: tosylate conducting polymeric substrate for establishing primary neural cultures. By studying hippocampal neuronal growth on the substrate, we found such material capable of harvesting healthy primary neural networks as compared to PSS-doped counterparts as well. Hence this material is well suited for exploitation and industrial applications in future neural prosthetics and implantable electronic devices. The as-synthesized PEDOT: tosylate substrate was coated on ITO glass, followed by poly-L-lysine coating to improve the neuronal viability before seeding of the primary hippocampal neuron. The immunofluorescence study performed on mature (DIV 15) neuron culture on PEDOT: tosylate, showed good biocompatibility, morphological integrity and maturation phases compared to control. In a further study, we characterized the neurons via calcium response imaging for probing the functional characteristics. The synaptic activity measured in terms of fluorescent intensity employing calcium indicators, pointed towards comparable response times, stimuli- receptor sensitivity and usual structural patterns and signaling cascades generated in response to external electrical pulse stimuli from both control and PEDOT: tosylate. The calcium imaging experiments conducted on mature (DIV 14) neuron culture exhibited similar synaptic behavior. Though direct electro-simulation studies are yet to be performed, this paper reports the initial growth characteristics of structurally and functionally potent, viable neural cells, that conclusively launches tosylate doped PEDOT as a feasible for neural culture. Also, considering the biocompatibility and other advantages PEDOT: tosylate possess over other PEDOT composites[35], this material could provide an option exploited for developing neural prosthetics and implantable devices for *in vivo* disease model studies.

ACKNOWLEDGEMENTS

This study was supported by the Academia Sinica Research Project on Nano Science and Technology. The author would like to acknowledge the contributions of Dr. Peilin Chen for his supervisory role and timely suggestions from time to time, along with Dr. Yu-Sheng Hsiao for providing the coverslips and ITO glass coated with PEDOT: tosylate. The author would also like to thank Dr. Shun-Min Yang and Dr. Chin Yi Chen for their guidance in establishing neuron culture. Ms. Sreerupa Sarkar and Mr. Abhishek Pathak provided valuable inputs in improving the quality of the manuscript.

REFERENCES

1. M. L. Kringelbach, N. Jenkinson, S. L. F. Owen, and T. Z. Aziz, "Translational principles of deep brain stimulation," *Nat Rev Neurosci*, vol. 8, no. 8, pp. 623-635, 2007.
2. V. S. Polikov, P. A. Tresco, and W. M. Reichert, "Response of brain tissue to chronically implanted neural electrodes," *Journal of Neuroscience Methods*, vol. 148, no. 1, pp. 1-18, 2005.
3. S. F. Lempka, S. Miocinovic, M. D. Johnson, J. L. Vitek, and C. C. McIntyre, "In vivo impedance spectroscopy of deep brain stimulation electrodes," *Journal of neural engineering*, vol. 6, no. 4, pp. 046001-046001, 2009.
4. R. Lozano, L. Stevens, B. C. Thompson, K. J. Gilmore, R. Gorkin, 3rd, E. M. Stewart, M. in het Panhuis, M. Romero-Ortega, and G. G. Wallace, "3D printing of layered brain-like structures using peptide modified gellan gum substrates," *Biomaterials*, vol. 67, pp. 264-73, 2015.
5. R. G. Harrison, "The outgrowth of the nerve fiber as a mode of protoplasmic movement," *Journal of Experimental Zoology*, vol. 9, no. 4,

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

- pp. 787-846, 1910.
6. R. G. Harrison, M. J. Greenman, F. P. Mall, and C. M. Jackson, "Observations of the living developing nerve fiber," *The Anatomical Record*, vol. 1, no. 5, pp. 116-128, 1907.
7. A. Carrel, and M. Burrows, "Cultivation of adult tissues and organs outside of the body," *Journal of the American Medical Association*, vol. 55, no. 16, pp. 1379-1381, 1910.
8. A. Carrel, "A METHOD FOR THE PHYSIOLOGICAL STUDY OF TISSUES IN VITRO," *The Journal of Experimental Medicine*, vol. 38, no. 4, pp. 407-418, 1923.
9. U. De Boni, A. A. Chong, and L. A. Hawthorn, "Organotypic development of neonate rabbit hippocampus in roller tube culture," *Acta Neuropathologica*, vol. 65, no. 1, pp. 53-61, 1984.
10. R. B. Campenot, "Local control of neurite development by nerve growth factor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 10, pp. 4516-4519, 1977.
11. L. J. Millet, and M. U. Gillette, "New perspectives on neuronal development via microfluidic environments," *Trends in neurosciences*, vol. 35, no. 12, pp. 752-761, 2012.
12. J. Wang, L. Ren, L. Li, W. Liu, J. Zhou, W. Yu, D. Tong, and S. Chen, "Microfluidics: A new cosset for neurobiology," *Lab on a Chip*, vol. 9, no. 5, pp. 644-652, 2009.
13. P. Massobrio, M. Tedesco, C. Giachello, M. Ghirardi, F. Fiumara, and S. Martinoia, "Helix neuronal ensembles with controlled cell type composition and placement develop functional polysynaptic circuits on Micro-Electrode Arrays," *Neuroscience Letters*, vol. 467, no. 2, pp. 121-126, 2009.
14. U. Frey, U. Egert, F. Heer, S. Hafizovic, and A. Hierlemann, "Microelectronic system for high-resolution mapping of extracellular electric fields applied to brain slices," *Biosensors and Bioelectronics*, vol. 24, no. 7, pp. 2191-2198, 2009.
15. K. Saha, A. J. Keung, E. F. Irwin, Y. Li, L. Little, D. V. Schaffer, and K. E. Healy, "Substrate Modulus Directs Neural Stem Cell Behavior," *Biophysical Journal*, vol. 95, no. 9, pp. 4426-4438, 2008.
16. Y. Xu, Y. Shi, and S. Ding, "A chemical approach to stem-cell biology and regenerative medicine," *Nature*, vol. 453, no. 7193, pp. 338-344, 2008.
17. R. A. Green, N. H. Lovell, G. G. Wallace, and L. A. Poole-Warren, "Conducting polymers for neural interfaces: Challenges in developing an effective long-term implant," *Biomaterials*, vol. 29, no. 24, pp. 3393-3399, 2008.
18. A. Williamson, J. Rivnay, L. Kergoat, A. Jonsson, S. Inal, I. Uguz, M. Ferro, A. Ivanov, T. A. Sjöström, D. T. Simon, M. Berggren, G. G. Malliaras, and C. Bernard, "Controlling Epileptiform Activity with Organic Electronic Ion Pumps," *Advanced Materials*, vol. 27, no. 20, pp. 3138-3144, 2015.
19. O. P. Dimitriev, D. A. Grinko, Y. V. Noskov, N. A. Ogurtsov, and A. A. Pud, "PEDOT:PSS films—Effect of organic solvent additives and annealing on the film conductivity," *Synthetic Metals*, vol. 159, no. 21–22, pp. 2237-2239, 2009.
20. L. Zhang, W. R. Stauffer, E. P. Jane, P. J. Sammak, and X. T. Cui, "Enhanced Differentiation of Embryonic and Neural Stem Cells to Neuronal Fates on Laminin Peptides Doped Polypyrrole," *Macromolecular Bioscience*, vol. 10, no. 12, pp. 1456-1464, 2010.
21. B. Massoumi, S. Davtalab, M. Jaymand, and A. A. Entezami, "AB(2) Y-shaped miktoarm star conductive polyaniline-modified poly(ethylene glycol) and its electrospun nanofiber blend with poly(epsilon-caprolactone)," *RSC Advances*, vol. 5, no. 46, pp. 36715-36726, 2015.
22. G. Cellot, P. Lagonegro, G. Tarabella, D. Scaini, F. Fabbri, S. Iannotta, M. Prato, G. Salviati, and L. Ballerini, "PEDOT:PSS Interfaces Support the Development of Neuronal Synaptic Networks with Reduced Neuroglia Response In vitro," *Frontiers in Neuroscience*, vol. 9, pp. 521, 2015.
23. E. J. S. K.E. Aasmundtveit, L.A.A. Pettersson, O. Inganas, T. Johansson and R. Feidenhans, "Structure of thin films of PEDOT," *Synthetic Material*, 1999.
24. C. A. Thomas, K. Zong, P. Schottland, and J. R. Reynolds, "Poly(3,4-alkylenedioxythiophene)s as Highly Stable Aqueous-Compatible Conducting Polymers with Biomedical Implications," *Adv Mater*, vol. 12, no. 3, pp. 222-225, 2000.
25. A. Ferrari, M. Cecchini, M. Serresi, P. Faraci, D. Pisignano, and F. Beltram, "Neuronal polarity selection by topography-induced focal adhesion control," *Biomaterials*, vol. 31, no. 17, pp. 4682-94, 2010.
26. A. Ferrari, P. Faraci, M. Cecchini, and F. Beltram, "The effect of alternative neuronal differentiation pathways on PC12 cell adhesion and neurite alignment to nanogratings," *Biomaterials*, vol. 31, no. 9, pp. 2565-73, 2010.
27. C. Salto, E. Saindon, M. Bolin, A. Kancierzewska, M. Fahlman, E. W. H. Jager, P. Tengvall, E. Arenas, and M. Berggren, "Control of Neural Stem Cell Adhesion and Density by an Electronic Polymer Surface Switch," *Langmuir*, vol. 24, no. 24, pp. 14133-14138, 2008.
28. E. A. Ostrakhovitch, J. C. Byers, K. D. O'Neil, and O. A. Semenikhin, "Directed differentiation of embryonic P19 cells and neural stem cells into neural lineage on conducting PEDOT-PEG and ITO glass substrates," *Archives of Biochemistry and Biophysics*, vol. 528, no. 1, pp. 21-31, 2012.
29. S. Baek, R. Green, A. Granville, P. Martens, and L. Poole-Warren, "Thin film hydrophilic electroactive polymer coatings for bioelectrodes," *Journal of Materials Chemistry B*, vol. 1, no. 31, pp. 3803-3810, 2013.
30. Y. Furukawa, A. Shimada, K. Kato, H. Iwata, and K. Torimitsu, "Monitoring neural stem cell differentiation using PEDOT-PSS based MEA," *Biochimica Et Biophysica Acta-General Subjects*, vol. 1830, no. 9, pp. 4329-4333, 2013.
31. C.-H. Lu, Y.-S. Hsiao, C.-W. Kuo, and P. Chen, "Electrically tunable organic bioelectronics for spatial and temporal manipulation of neuron-like pheochromocytoma (PC-12) cells," *Biochimica Et Biophysica Acta-General Subjects*, vol. 1830, no. 9, pp. 4321-4328, 2013.
32. Y.-S. Hsiao, C.-W. Kuo, and P. Chen, "Multifunctional Graphene-PEDOT Microelectrodes for On Chip Manipulation of Human Mesenchymal Stem Cells," *Advanced Functional Materials*, vol. 23, no. 37, pp. 4649-4656, 2013.
33. M. Sasaki, B. C. Karikkineth, K. Nagamine, H. Kaji, K. Torimitsu, and M. Nishizawa, "Highly Conductive Stretchable and Biocompatible Electrode-Hydrogel Hybrids for Advanced Tissue Engineering," *Advanced Healthcare Materials*, vol. 3, no. 11, pp. 1919-1927, 2014.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

34. X. Luo, C. L. Weaver, S. Tan, and X. T. Cui, "Pure graphene oxide doped conducting polymer nanocomposite for bio-interfacing," *Journal of Materials Chemistry B*, vol. 1, no. 9, pp. 1340-1348, 2013.
35. S. Baek, R. A. Green, and L. A. Poole-Warren, "The biological and electrical trade-offs related to the thickness of conducting polymers for neural applications," *Acta Biomater*, vol. 10, no. 7, pp. 3048-3058, 2014.
36. S.-C. Luo, E. Mohamed Ali, N. C. Tansil, H.-h. Yu, S. Gao, E. A. B. Kantchev, and J. Y. Ying, "Poly(3,4-ethylenedioxythiophene) (PEDOT) Nanobiointerfaces: Thin, Ultrasoother, and Functionalized PEDOT Films with in Vitro and in Vivo Biocompatibility," *Langmuir*, vol. 24, no. 15, pp. 8071-8077, 2008.
37. S. M. Richardson-Burns, J. L. Hendricks, B. Foster, L. K. Povlich, D.-H. Kim, and D. C. Martin, "Polymerization of the conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) around living neural cells," *Biomaterials*, vol. 28, no. 8, pp. 1539-1552, 2007.
38. Y. Furukawa, A. Shimada, K. Kato, H. Iwata, and K. Torimitsu, "Monitoring neural stem cell differentiation using PEDOT-PSS based MEA," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1830, no. 9, pp. 4329-4333, 2013.
39. J. E. Collazos-Castro, J. L. Polo, G. R. Hernández-Labrado, V. Padial-Cañete, and C. García-Rama, "Bioelectrochemical control of neural cell development on conducting polymers," *Biomaterials*, vol. 31, no. 35, pp. 9244-9255, 2010.
40. D. Khodagholy, J. Rivnay, M. Sessolo, M. Gurfinkel, P. Leleux, L. H. Jimison, E. Stavrinidou, T. Herve, S. Sanaur, R. M. Owens, and G. G. Malliaras, "High transconductance organic electrochemical transistors," vol. 4, pp. 2133, 2013.
41. D. Khodagholy, J. N. Gelinas, T. Thesen, W. Doyle, O. Devinsky, G. G. Malliaras, and G. Buzsaki, "NeuroGrid: recording action potentials from the surface of the brain," *Nat Neurosci*, vol. 18, no. 2, pp. 310-315, 2015.
42. D. Khodagholy, T. Doublet, M. Gurfinkel, P. Quilichini, E. Ismailova, P. Leleux, T. Herve, S. Sanaur, C. Bernard, and G. G. Malliaras, "Highly Conformable Conducting Polymer Electrodes for In Vivo Recordings," *Advanced Materials*, vol. 23, no. 36, pp. H268-H272, 2011.
43. Y. S. Hsiao, S. C. Luo, S. Hou, B. Zhu, J. Sekine, C. W. Kuo, D. Y. Chueh, H. H. Yu, H. R. Tseng, and P. Chen, "3D bioelectronic interface: capturing circulating tumor cells onto conducting polymer-based micro/nanorod arrays with chemical and topographical control," *Small*, vol. 10, no. 15, pp. 3012-7, 2014.
44. J. Nunez, "Primary Culture of Hippocampal Neurons from P0 Newborn Rats," *Journal of Visualized Experiments : JoVE*, no. 19, pp. 895, 2008.