# DIFFERENTIATION OF MESENCHYMAL STEM CELLS (MSCs) TO FUNCTIONAL NEURON ON GRAPHENE-POLYCAPROLACTONE NANOSCAFFOLDS

**Debika Debnath**[1], Manisha Singh[3], Sonali Rawat[3], Mahak Tiwari[3], Ankarao Kalluri[1], Deepika Gupta[5], Bhushan Dharmadhikari[3],Prabir Patra[1,2], and Sujata Mohanty[3]

•[1]Department of Biomedical Engineering, University of Bridgeport, Bridgeport, Connecticut 06604, United States

•[2]Department of Mechanical Engineering, University of Bridgeport, Bridgeport, Connecticut 06604, United States

•[3]Department of Electrical Engineering, University of Bridgeport, Bridgeport, Connecticut 06604,United STtates

•[4]Stem Cell Facility (DBT- Centre of Excellence for Stem Cell Research), All India Institute of Medical Sciences, New Delhi 110029, India.

•[5]Smita Lab, Department of Textile Technology, Indian Institute of Technology, New Delhi, India

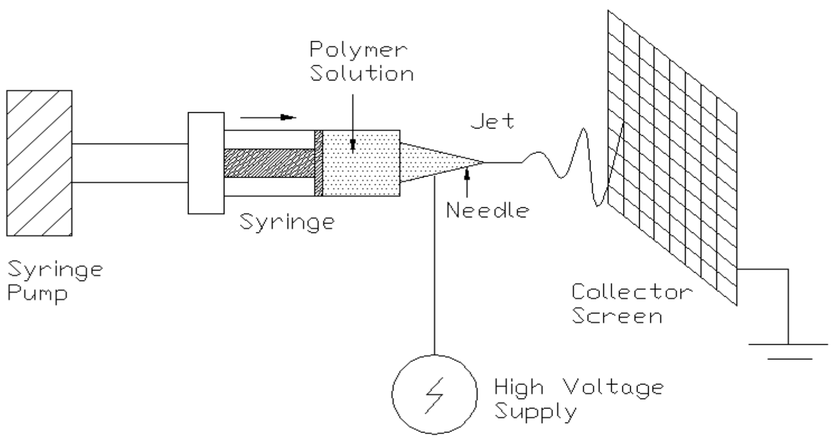
## ABSTRACT

Spinal cord is an important part of the central nervous system that controls all activities of the body. It is a tubular bundle of nerve fibers and tissues connecting brain to nearly all parts of the body. Nerve cells in an adult human body do not divide and make copies of themselves. Therefore, in case of an injury or damage to any part of spinal cord causes permanent changes to strength, sensation and other body functions. The field of tissue engineering and regenerative medicine which aims to replace and repair damaged tissues, organs or cells entails for effective methods for fabricating biological scaffolds.

Here we present synthesis of fibrous scaffolds by a process called electrospinning that can provide a microenvironment in-vitro for differentiation and proliferation of functional neurons from mesenchymal stem cells. These nanofibrous PCL scaffolds with graphene as filler materials are engineered in such a way so as to provide topological, biochemical as well as electrical cues that can enhance neurite extension and penetration. Poly(ε-caprolactone) (PCL) is a FDA approved synthetic biodegradable polyester extensively used in biomedical applications. Graphene, a single layer carbon crystal, based nanomaterials have recently gained considerable interest for tissue engineering applications including osteogenic, neural and differentiation in other lineages due to their favorable chemical, electrical and mechanical properties. Our final aim is that the the functional tissues or organs developed in vitro shall be implanted inside body to rehabilitate the biological function that was lost due to injury, abnormality or loss.

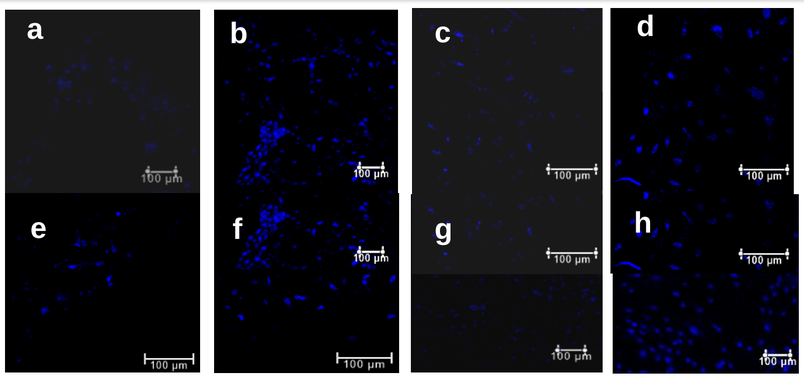
## WORK FLOW

- Fabrication of PCL and PCL-G scaffolds: Nanofibrous scaffold was formed from stretching graphene dispersed viscoelastic polymer solution uniaxially under an applied voltage.

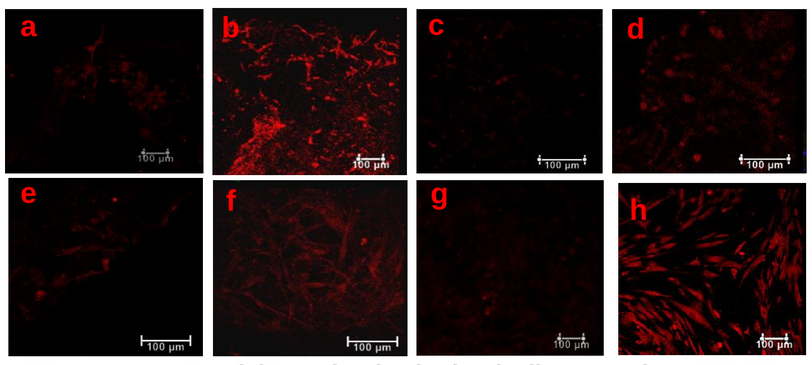



- Seeding of MSCs and their differentiation on scaffold: The MSCs were induced to differentiate into neurons due to topological and electrical effect of scaffold, and biochemical effect of EGF, FGF2 and Oxysterol.
- SEM studies: Ultrastructural analysis of scaffolds with or without cells were performed.
- Contact angle measurement: To determine hydrophobicity and hydrophilicity of scaffold.
- Confocal Studies: Functional neuronal markers were studied in uninduced and induced MSCs for analysis of cytoskeleton arrangement

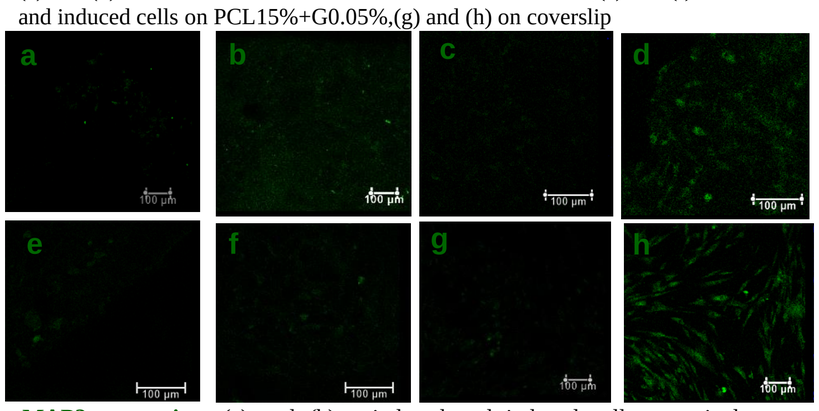
## RESULT



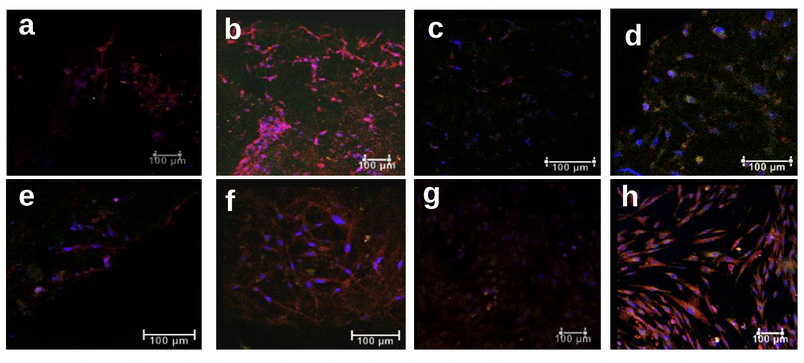

**DAPI**. (a) and (b) uninduced and induced cell respectively on PCL15%, (c) and (d)uninduced and induced cells on PCL15%+G0.01%,(e) and (f) uninduced and induced cells on PCL15%+G0.05%,(g) and (h) on coverslip



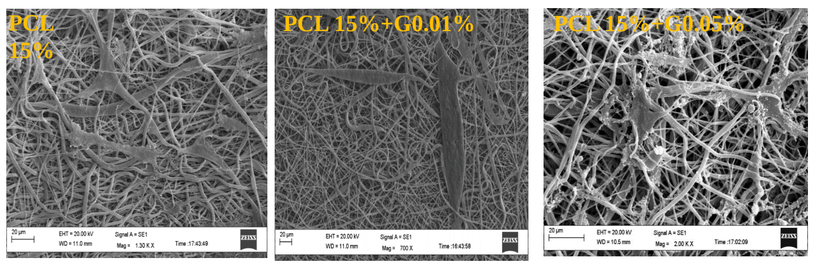

**TH expression**. (a) and (b) uninduced and induced cell respectively on PCL15%, (c) and (d)uninduced and induced cells on PCL15%+G0.01%,(e) and (f) uinduced and induced cells on PCL15%+G0.05%,(g) and (h) on coverslip



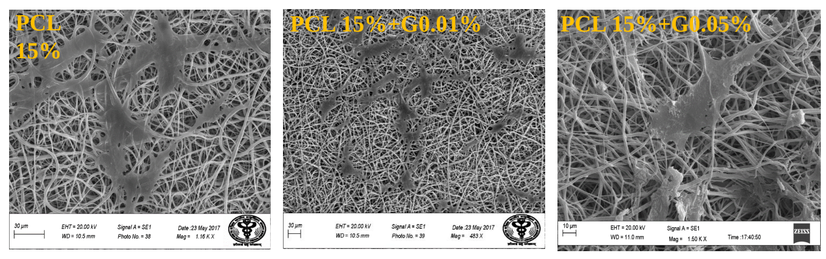

**MAP2 expression**. (a) and (b) uninduced and induced cell respectively on PCL15%, (c) and (d)uninduced and induced cells on PCL15%+G0.01%,(e) and (f) uinduced and induced cells on PCL15%+G0.05%,(g) and (h) on coverslip



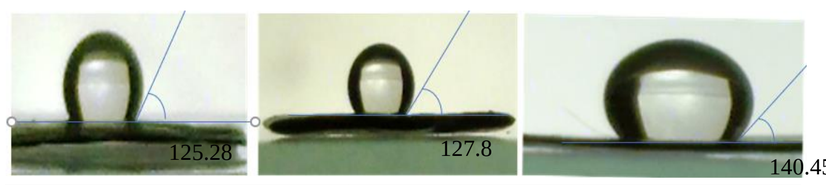

**MERGE.** (a) and (b) uninduced and induced cell respectively on PCL15%, (c) and (d)uninduced and induced cells on PCL15%+G0.01%,(e) and (f) uninduced and induced cells on PCL15%+G0.05%,(g) and (h) on coverslip




SEM images of uninduced cells seeded on PCL15%, PCL15%+G0.01%, PCL15%+G0.05% respectively




**SEM images of induced cells** seeded on PCL15%, PCL15%+G0.01%, PCL15%+G0.05% respectively




| | 15% PCL | 15% PCL+ 0.01% Gr | 15% PCL+ 0.05% Gr |
|---|---|---|---|
| Contact Angle | 125.28 | 127.8 | 140.45 |

## CONCLUSION

1. This work demonstrates the key role of graphene as filler in composites scaffolds which significantly provides permissive surfaces for protein and cell adhesion as well as electrically stimulate axonal growth which overall increases the biological responses.
2. Nano fibrous assembly of PCL-G facilitated/ promoted attachment and spreading of MSCs with typical neuronal morphology and strong cell matrix interaction, specifically inPCL15%+G0.01.
3. Differentiation of cultured MSCs into mature, functional neurons was further validated by IFstudies.
4. Thus, we envisaged that such a platform can serve as a powerful tool for developing future therapies for any diseases and injuries of the spinal cord.

## REFERENCES

1. Chen, M., Patra, P. K., Warner, S. B., & Bhowmick, S. (2007). Role of fiber diameter in adhesion and proliferation of NIH 3T3 fibroblast on electrospun polycaprolactone scaffolds. *Tissue Engineering*, *13*(3), 579-587.
2. Ghaderi, E., Shirazian, S. A., & Rahighi, R. (2016). Rolled graphene oxide foams as three-dimensional scaffolds for growth of neural fibers using electrical stimulation of stem cells. *Carbon*, *97*, 71-77.
3. Johnson, D. W., Dobson, B. P., & Coleman, K. S. (2015). A manufacturing perspective on graphene dispersions. *Current Opinion in Colloid & Interface Science*, *20*(5-6), 367-382.Akhavan, O., dispersions. *Current Opinion in Colloid & Interface Science*, *20*(5-6), 367-382.
4. Aphale, A. N., Mahakalkar, K., Macwan, I. G., Mukerji, I., Cox, P. J., Mahapatra, M., & Patra, P. K. (2016). Fabrication and Experimental Analysis of Axially Oriented Nanofibers. *Journal of nanoscience and nanotechnology*, *16*(3), 2668-2676.