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Electroconductive Scaffolds to Mature Induced Pluripotent Stem Cell-Derived Cardiomyocytes for Cardiac Tissue Engineering Applications

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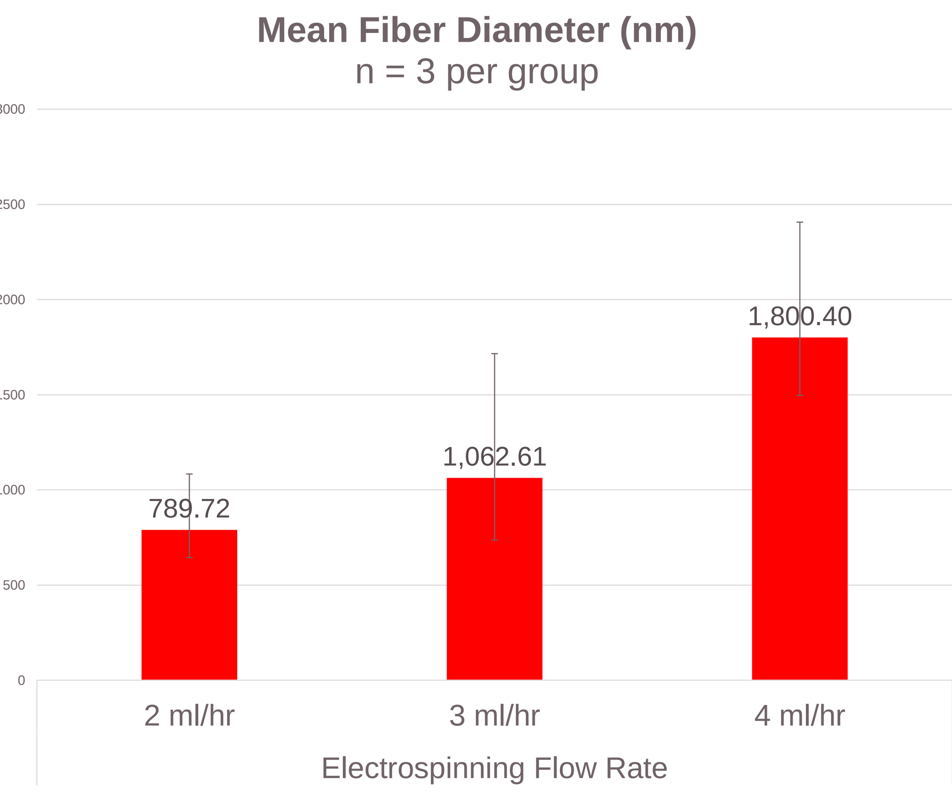
INTRODUCTION

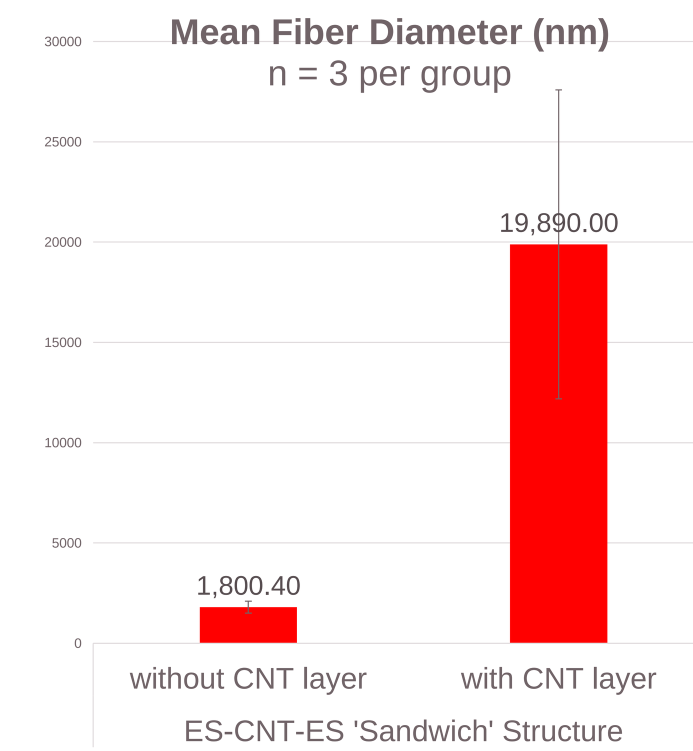
Heart disease is the leading cause of death in the U.S. [1]. Cardiac tissue engineering (CTE) aims to repair and replace cardiac tissue, offering a solution. Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) theoretically offer unlimited numbers of patient-specific CMs for CTE. However, iPSC-CMs are electrophysiologically immature compared to functional adult CMs, and therefore incapable of facilitating the heart's beating [2]. Hence, iPSC-CMs cannot be reliably used for CTE until there is a method developed to electrophysiologically mature them. **My research aims to incorporate carbon nanotubes (CNTs) into electrospun scaffolds to increase their electroconductivity, thus facilitating electrophysiological maturation of iPSC-CMs seeded onto them, and paving the way forward for the use of iPSC-CMs in CTE.**

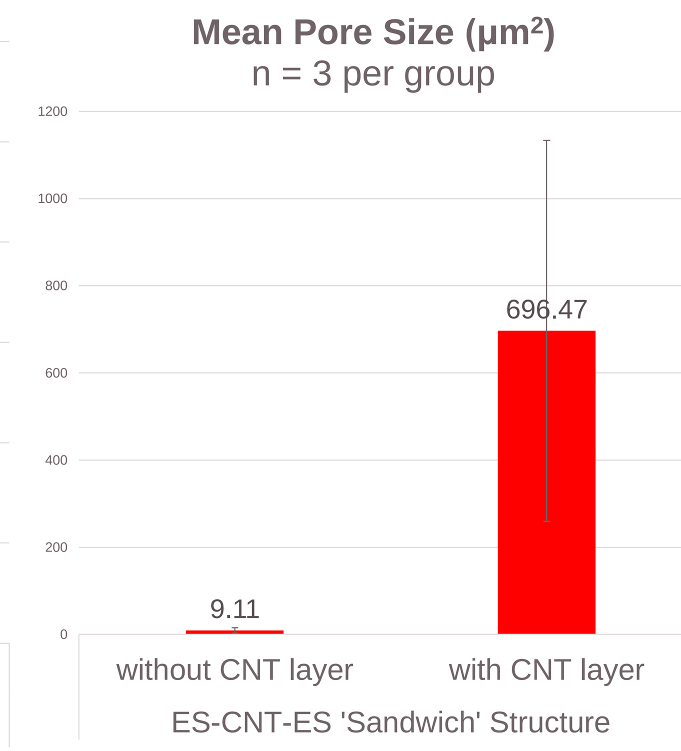
- SEM images analyzed from scaffolds electrospun at 2, 3, and 4 ml/hr flow rates using ImageJ to quantify fiber diameter
- Increasing electrospinning flow rate significantly increased fiber diameter

This demonstrates the ability to exert morphological control over electrospun fibers, which is crucial to achieve biomimicry of the cardiac extracellular matrix (ECM).



- SEM images analyzed from scaffolds without CNTs vs. with 'sandwich' CNTs using ImageJ to quantify fiber diameter and pore size
- Inclusion of CNTs into scaffolds significantly increased both fiber diameter and pore size

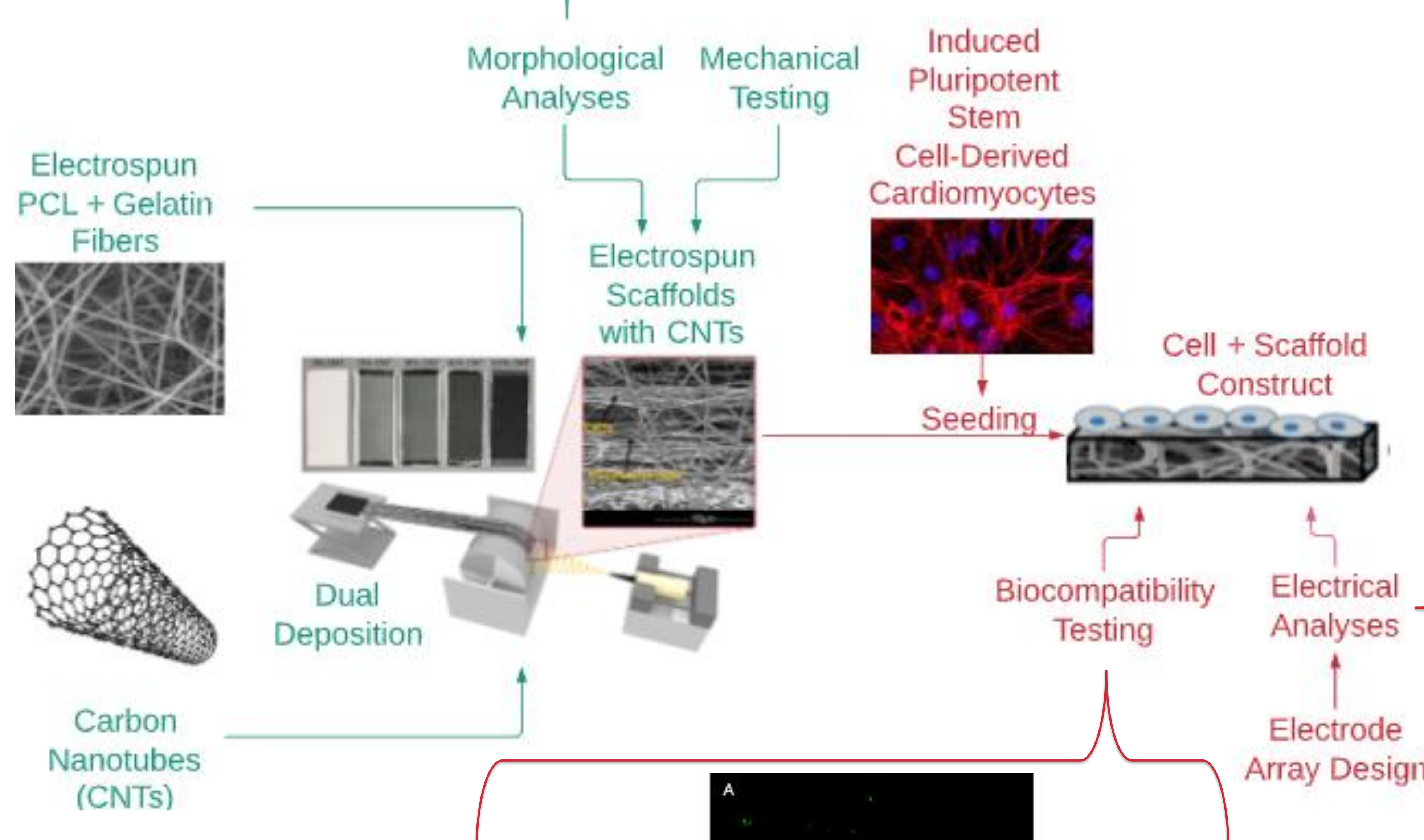


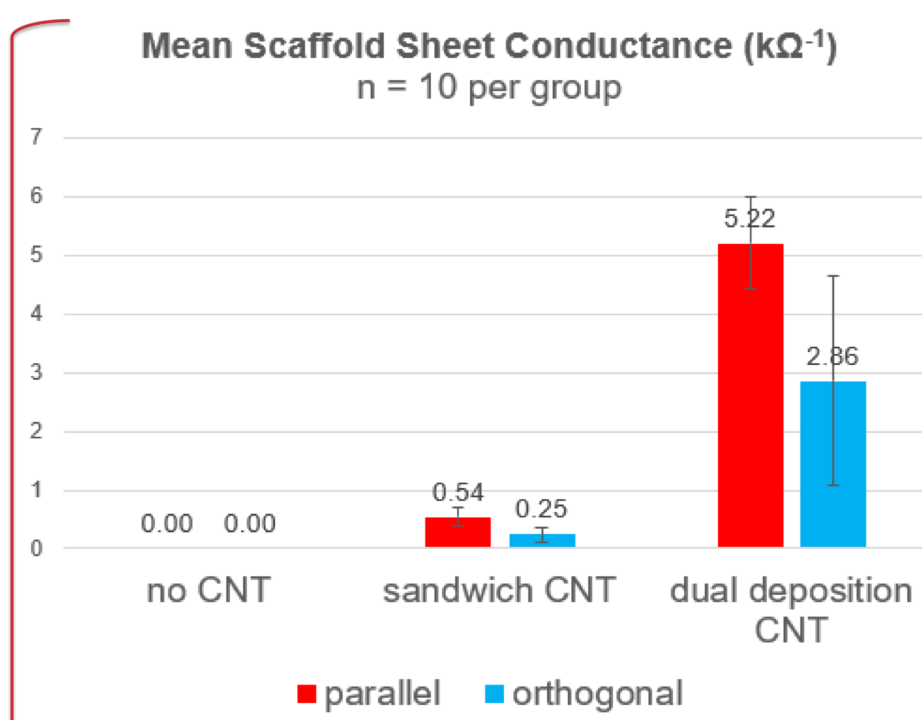


- Polycaprolactone (PCL) and gelatin dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol at 20% w/v
- Electrospun at varying (2, 3, 4 ml/hr) flow rates with 14 kV applied voltage and 15 cm die-to-collector distance (DCD)

CNTs deposited using two distinct methods:

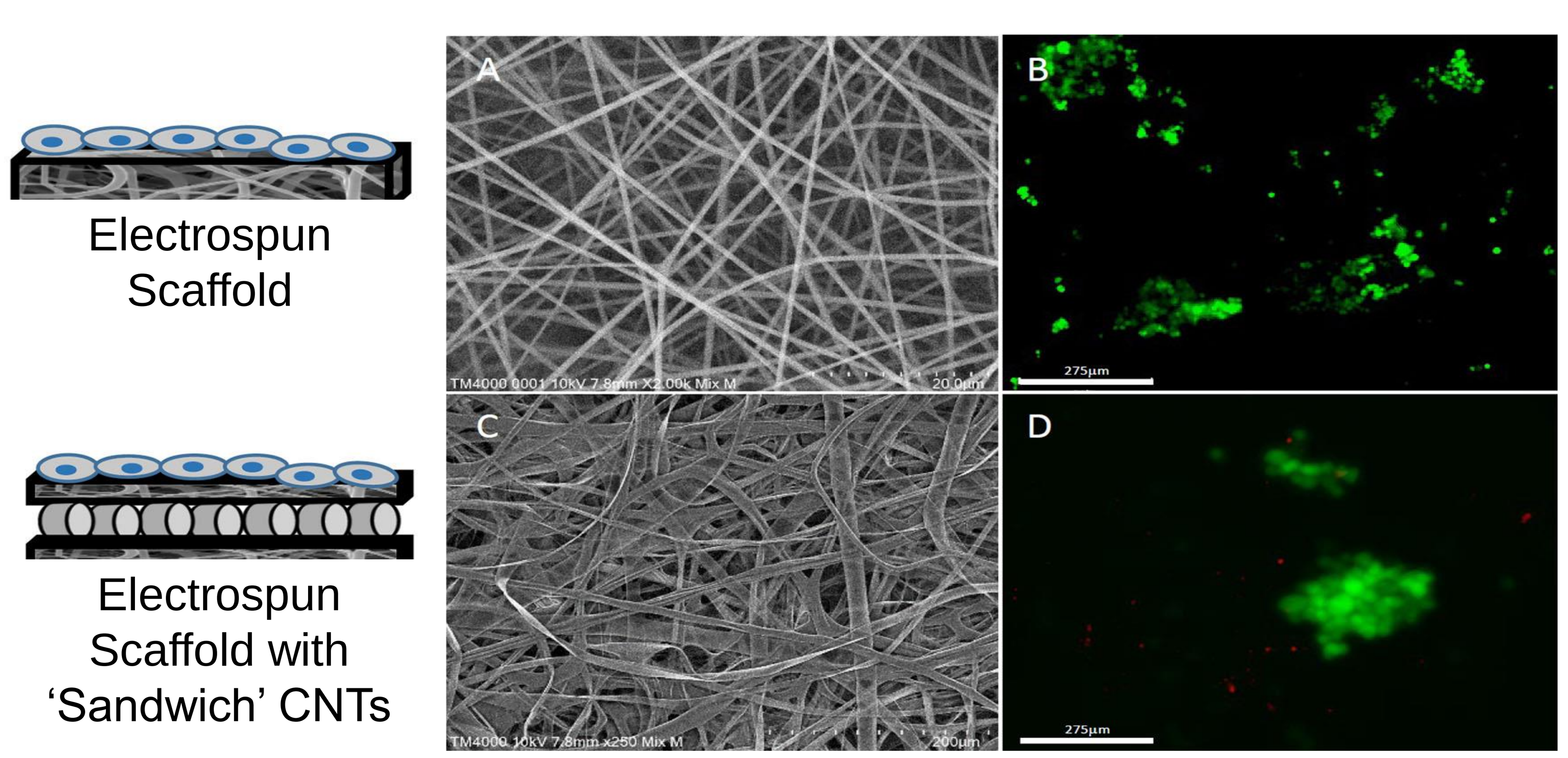
1. Sandwich: Fibers electrospun, CNT array stretched across them, another layer electrospun on top
2. Dual Deposition: Fibers electrospun onto same rotating collector that 2.5 layers of CNTs deposited onto

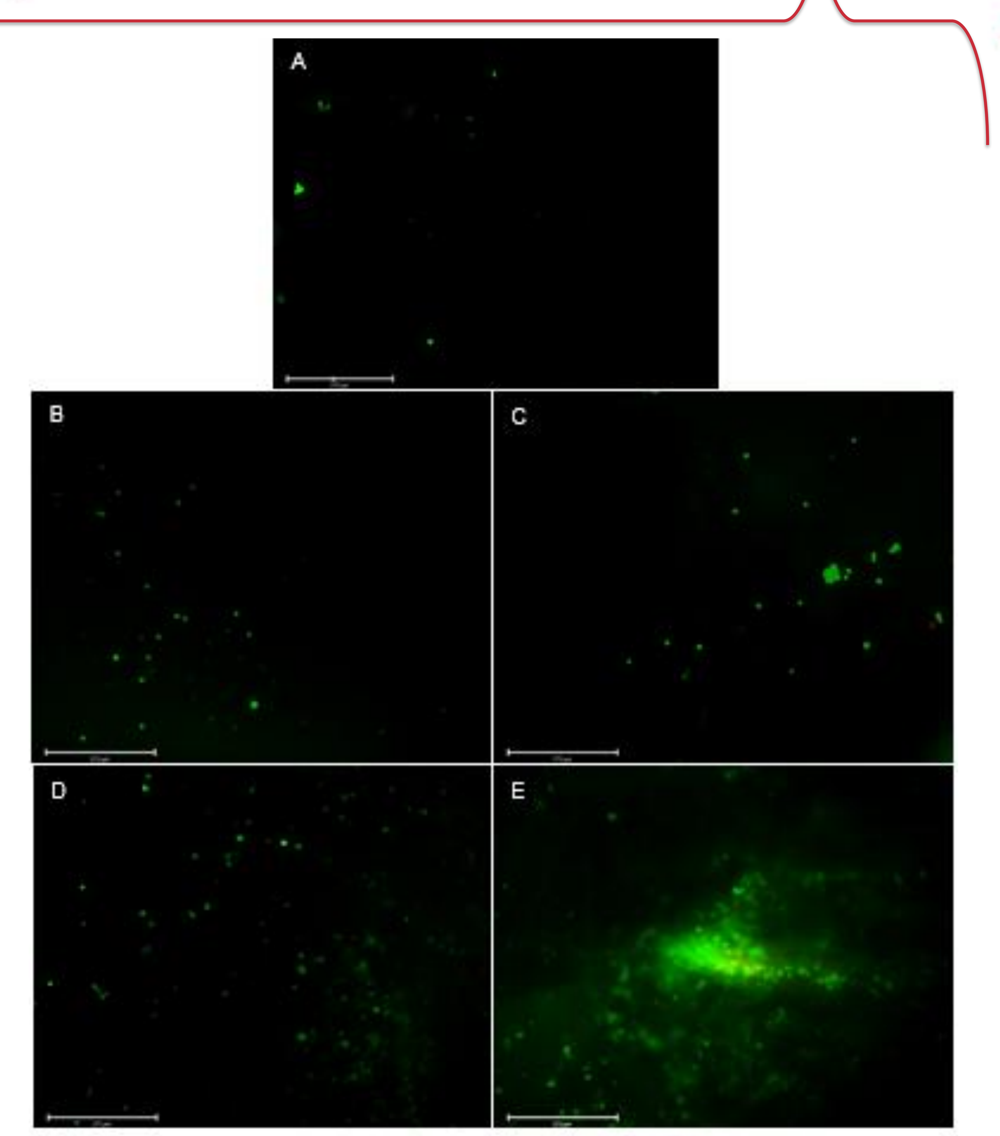




- Multimeter quantified scaffold resistance, from which conductance was calculated
- **Inclusion of CNTs significantly increased scaffold conductance**
- **Dual deposition method increased conductance significantly more than 'sandwich' method**
- Mean conductances for all scaffolds containing CNTs classify them as **highly conductive**
- Conductance measured higher parallel to CNT arrays than orthogonally, suggesting anisotropic effect

NIH 3T3 murine fibroblasts seeded at 10^6 cells/cm² onto 1x1 cm² scaffolds with and without CNTs. Live/dead assays performed at day 2 revealed **good cellular attachment with no cytotoxicity** for scaffolds both with and without CNTs.





- Live/dead assays performed using NIH 3T3 cells over a month-long period
- Live/dead assays shown from days (A) 2, (B) 7, (C) 14, (D) 21, and (E) 28 of scaffold containing CNTs via 'sandwich' method
- **Scaffolds both with and without CNTs demonstrated no cytotoxicity and high biocompatibility over a month-long period**
- NIH 3T3 fibroblasts **proliferated well** on the scaffolds and **migrated throughout the fibers**

CONCLUSIONS

The preliminary data presented here demonstrates the feasibility of an electroconductive CTE scaffold to mature iPSC-CMs. **The morphological control, cellular attachment and proliferation, biocompatibility, and increased conductance due to CNT presence provide a promising foundation for the development of a scaffold with electroconductive properties capable of electrophysiologically maturing iPSC-CMs, opening the door for their use in a myriad of life-saving CTE applications.**

NEXT STEPS

- Mechanical testing of scaffolds
- Immunostaining and live/dead assays over a month-long period to compare NIH 3T3 response to all scaffold types (varying flow rate, PCL:gelatin ratio, CNT presence and deposition method)
- qPCR analysis of cellular response
- Electrical analysis of scaffolds using four-probe method
- Use of iPSC-CMs to assess biocompatibility and cellular response
- Assessment of electroconductive properties of iPSC-CM + scaffold constructs
- Use of voltage-sensitive dye to confirm iPSC-CM response to physiological signals
- Application of electrical pulsing to pace beating activity of iPSC-CM + scaffold constructs

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[2] Roshanbinfar K, et al. (2018): Electroconductive Biohybrid Hydrogel for Enhanced Maturation and Beating Properties of Engineered Cardiac Tissues. *Advanced Functional Materials,* 28(42), 1803951.