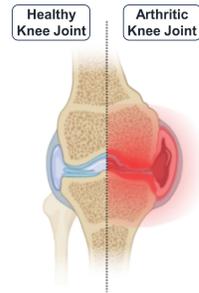


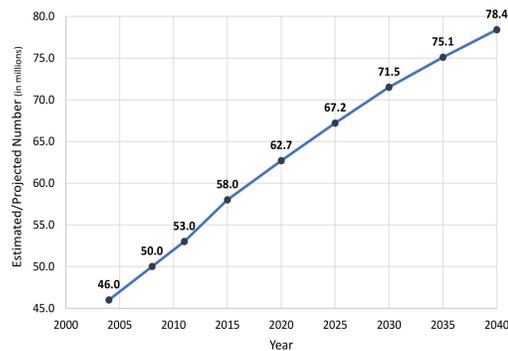
INTRODUCTION

Osteoarthritis (OA):

- Chronic joint disease, leading to the degradation of protective cartilage over time
- 250 million people all over the world suffer from OA^[1]
- Limited capacity for self-repair
- Current treatment is insufficient for long-term health



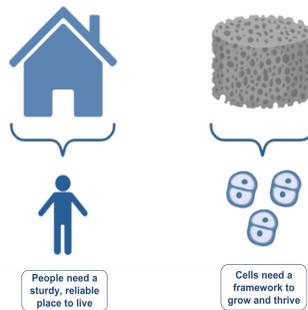
Estimated and Projected Number of Adults with Diagnosed Arthritis^[2]



TISSUE ENGINEERING – PROSPECTIVE TREATMENT

Advantages:

- Patient-specific regenerative approach to healing
- Utilization of bio-scaffolds to match the mechanical properties of native tissue
- Graphene Foam (GF) is a biocompatible, highly conductive material
- Other helpful characteristics: thermal conductivity, mechanical strength



PROBLEM:

External stimuli direct cell lineage in ways that need to be optimized to drive cell behavior in 3D environments

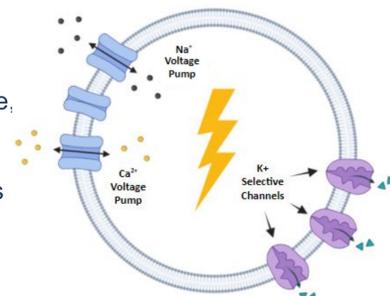
APPROACH:

Applying direct electrical stimulus to control the resting membrane potential of ATDC5 cells on GF bio-scaffolds and encourage chondrogenic differentiation

MEMBRANE POTENTIAL

What is it?

- The voltage difference across the cell membrane, which influences volume and regulates ion exchange. MP modulates responses to physiochemical signals and determines cell interaction
- We will optimize the ion transport mechanism for K⁺ and Ca⁺ channels, which impact chondrocyte differentiation



ELECTRICAL STIMULATION

- Application of an outside voltage will force cells to open ion pumps/channels. These channels cause an influx of ions, thus regulating chondrogenesis
- Previous data suggests need for improved voltage, waveform, and frequency

Initial Experimental Parameters

# of Technical Replicates	Applied Voltage (Vpp)	Applied Frequency (kHz)
3	0.5	1

MATERIALS AND METHODS

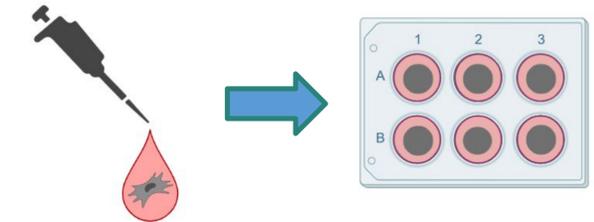
1

Preliminary electrical testing on three samples of GF in cell media. Measured vs. input voltage and current values (AC):

Well #	Average reading from input (mV): 0 Vpp 1 kHz	Average reading from input (mV): 0.5 Vpp 1 kHz	Average current reading (μA)
1	0.029	0.052	0.050
2	0.034	0.055	0.052
3	0.036	0.062	0.053

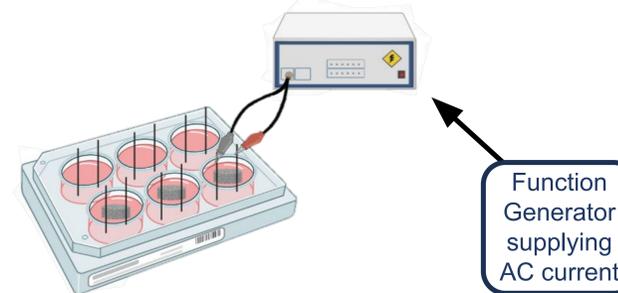
2

Sterilization of electrical stimulation apparatus, then seeded with 1.5 x 10⁵ ATDC5 cells on day 0.



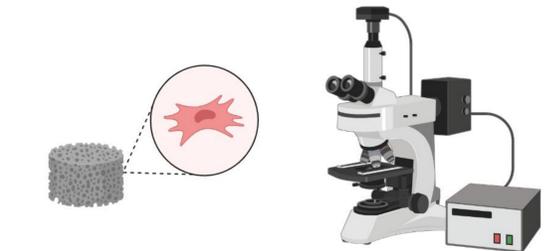
3

Apply electrical stimulus at 0.5 Vpp/1 kHz for 3 consecutive days. 10 minutes of stimulation was achieved each day per well

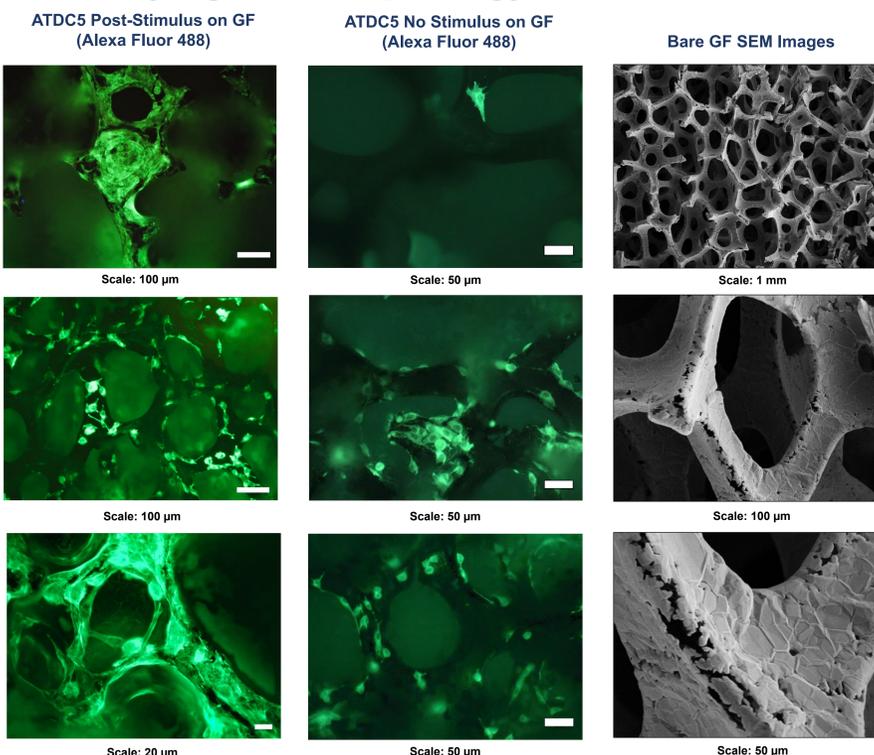


4

Grow cells to 7 days, then fix using paraformaldehyde and apply actin fluorescent stain. Compare stimulated cells to unstimulated cells on GF



Quantifying Cell Morphology and GF Structure



Results and Future Work:

- Fluorescent imaging indicates optimal cell growth after seven days
- Stimulus with these parameters doesn't kill ATDC5 cells under direct stimulation, and these parameters should be used for future experiments at variable applied voltages (0.3 V and 0.7 V)
- Under the applied stimulus, the morphology of ATDC5 cells changes, becoming more fibrous and elongated
- This experiment will be repeated precisely and characterized with the following: RAMAN spectroscopy, Microcomputed tomography (MicroCT), and gene expression (COL2A1, AGC, SOX-9)

Impact:

- The ability to quantify cell morphology on GF after direct electrical stimulus has never been done. As compared to applying an electric field, characterizing cells with further techniques will allow us to quantify the impact on differentiation and proliferation of mesenchymal stem cells in a 3D environment

Acknowledgments:

[1] Primorac D, Molnar V, Rod E, Jeleč Ž, Čukelj F, Matišić V, Vrdoljak T, Hudetz D, Hajsok H, Borčić I. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. *Genes (Basel)*. 2020 Jul 26;11(8):854. doi: 10.3390/genes11080854. PMID: 32722615; PMCID: PMC7464436

[2] Arthritis. BMUS: The Burden of Musculoskeletal Diseases in the United States. (n.d.). <https://www.boneandjointburden.org/fourth-edition/iii0/arthritis> (supplementary information credited to CDC)

This work was supported under the National Science Foundation CAREER Award No. 1848516 and NSF REU Site: Materials for Society Award No. 1950305. The authors acknowledge additional support from the Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408, P20GM109095, and 1C06RR020533.

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