Optimizing ATDC5 Seeding of Graphene Foam for Cartilage Tissue Engineering

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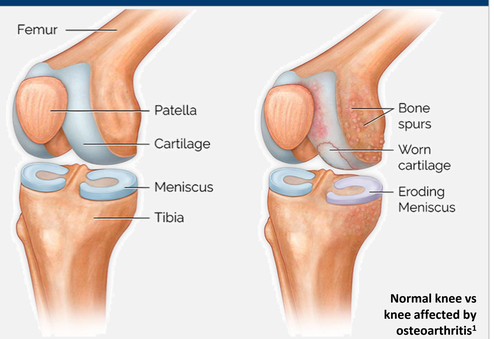
I. Introduction

Osteoarthritis (OA):

- 11th leading cause of disability worldwide
- Impacts 50% US population over 65
- Cartilage has limited regenerative capacity
- Current treatments are inadequate and expensive

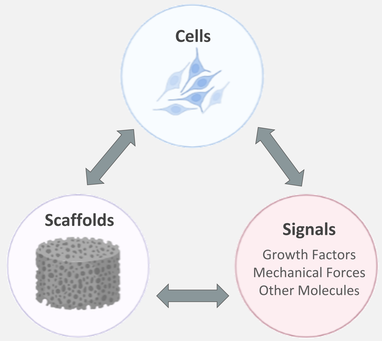


Normal knee vs knee affected by osteoarthritis[1]

Prospective Treatment → Tissue Engineering (TE)

Advantages of Tissue Engineering:

- Patient specific (individualized stem cell treatment)
- Regenerative approach
- Ability to utilize bioscaffolds to match mechanical properties of target tissue



Role of Bioscaffolds

People need a sturdy, reliable place to live

Cells need a framework to grow and thrive

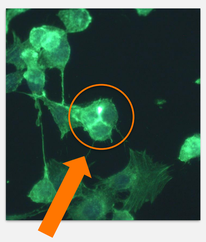
Graphene Foam → Prospective Bioscaffold:

- Superior mechanical strength which matches target tissue
- High electron mobility
- Thermal conductivity
- Biocompatible

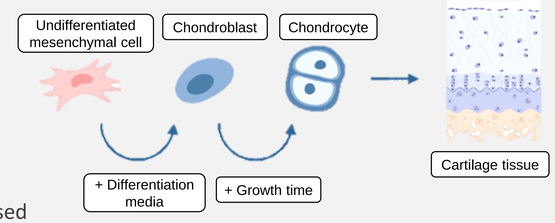
Challenge → Controlling cell differentiation:

- Must optimize cell adherence
- Must optimize cell characterization

Purpose: The goal of this work is to optimize the ATDC5 seeding and characterization protocols during 3D cell culture on graphene foam bioscaffolds for cartilage tissue engineering.

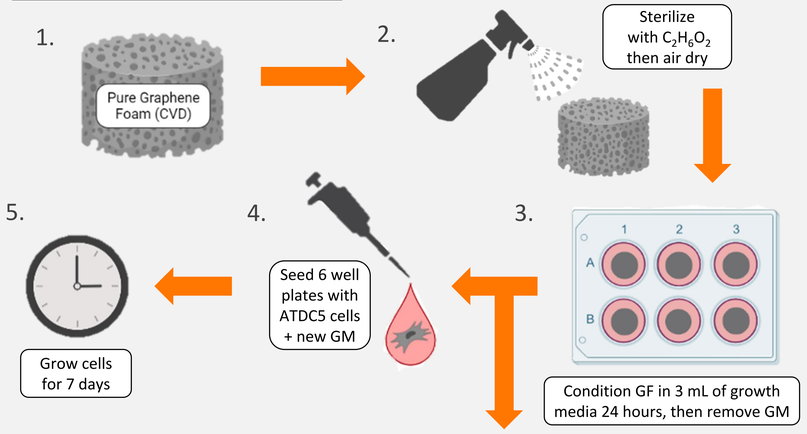


Three chondrocytes enclosed by one membrane



II. Materials and Methods

Cell Culture Workflow:



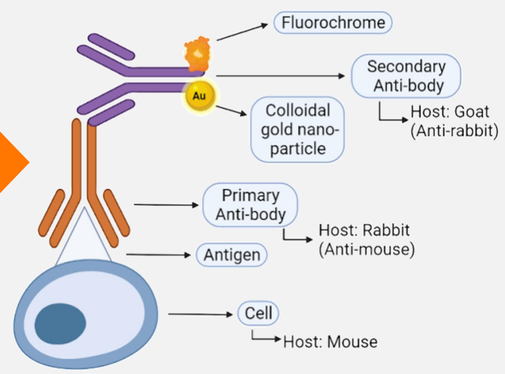
	Trial 1	Trial 2
Plate Treatment	None	Anti-Adherence Rinse
Cell Density (approx.)	$5.5 * 10^3$ cells	$5.5 * 10^3$ cells

Characterization Techniques:

Structural properties	Fluorescence Imaging Microscopy	Scanning Electron Microscopy (SEM)	Microcomputed Tomography
Porosity			✓
Pore size		✓	✓
Surface Roughness		✓	✓
Pore Interconnectivity			✓
Live cell observation	✓		
Surface to volume ratio			✓
Composition GF		✓	
Topography GF		✓	

Using these techniques individually is inadequate for cell characterization grown in 3D on GF. Our lab formulated a technique for imaging cells using primary and secondary antibody staining to bind the actin of the cell with colloidal gold, making cells detectable and discernible on GF using MicroCT.

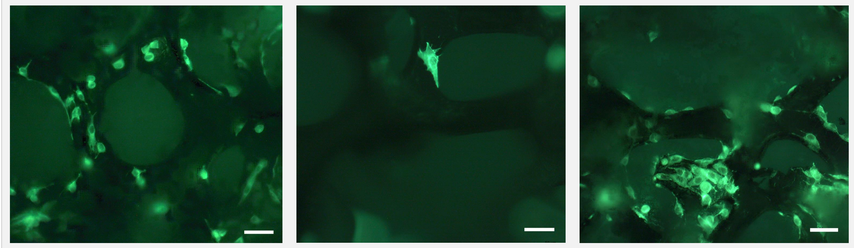
Antibody staining schematic:



III. Results/Discussion: Quantifiable data

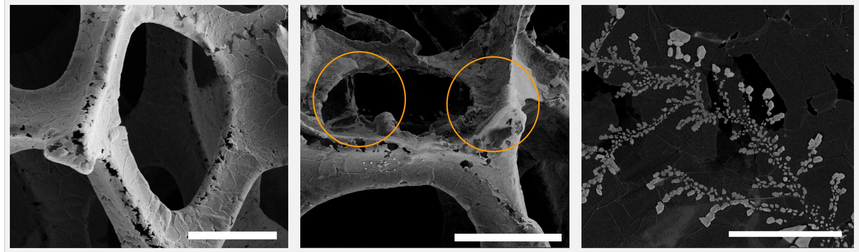
Fluorescence Imaging Microscopy:

Scale bars = 50 microns



Scanning Electron Microscopy:

[2] SEM Images

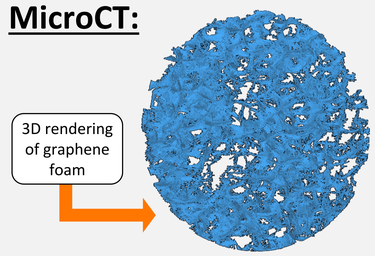


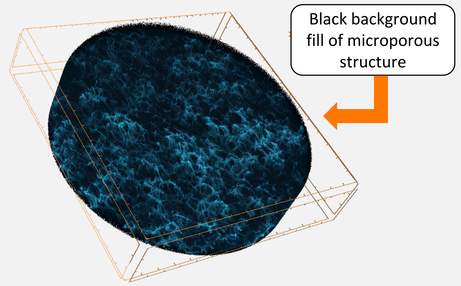
Bare GF (Scale bar = 100 µm)

GF with ATDC5 cells (Scale bar = 100 µm)

GF with PBS deposits (Scale bar = 10 µm)

MicroCT:





IV. Conclusion/ Future Work

- The anti-adherence rinse plate treatment resulted in minimal cell adherence to glass chamber slides forcing cells to adhere to the GF.
- No plate treatment resulted in most of the cells adhering to the bottom of the culture dish and few to GF, indicating that using anti-adherence rinse is the best method for seeding GF.
- Storing fixed cells on GF in PBS before staining resulted in salt crystals.
- A methanol rinse will be implemented as an alternative solution in the future to mitigate noise and nonspecific imaging during MicroCT. This work will lead to using graphene foam bioscaffolds as an active scaffold for electrical stimulus during 3D cell culture.

V. Acknowledgements and References

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[1] https://www.mbortho.com/patients/education/Knee-Arthritis.htm.
[2] Jacob Manzi for SEM images