# BIOMIMETIC BONE MARROW MICROENVIRONMENT FOR hMSC CULTURE

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## ABSTRACT

Degenerative musculoskeletal diseases, specifically osteoarthritis, affect approximately 1 out of 3 people aged 65 and over [1]. Noninvasive treatment approaches only alleviate symptoms and do not address the degeneration of joints [2,3]. As an alternative, our client is interested in employing a regenerative medicine approach with human mesenchymal stem cells (hMSCs). Thus, a new culture system is needed to maintain hMSCs in a quiescent state. Last semester, rheometer testing characterized bone marrow stiffness (E) within a range of 61.7- 312.8 kPa. However, we will proceed with literature values due to their consistency across studies. This semester, we fabricated RGD-conjugated PEG hydrogels with Young's moduli spanning the human bone marrow stiffness range. We also developed a bioreactor that maintains [$CO_2$] and [$O_2$] within ±1.5% of 1% and 5%, respectively. Future work will fabricate fibronectin-conjugated PEG and 2 additional bioreactor chambers, ultimately allowing for multivariate testing.

## SIGNIFICANCE

**Musculoskeletal Diseases:**

- Autoimmune, inflammatory, and degenerative diseases
  - ex: osteoarthritis affects 33.6% of individuals age 65+ [1]
- Potential treatment: regenerative medicine with human mesenchymal stem cells (hMSCs)

**Bone Marrow and hMSCs:**

- Niche of quiescent hMSCs; visco-elastic tissue [4]
- Complex environment allows maintenance of hMSCs in undifferentiated state
- Stiffness: 0.1-0.2 kPa [5] Oxygen tension: 1-5% [14]
- *In vitro* culture: loss of potency [5]

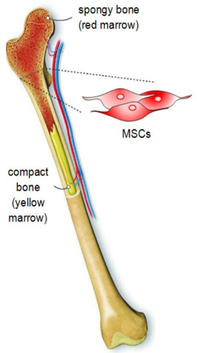



**Figure 1**. MSC source.

## PROBLEM STATEMENT

Current culture methods are variable in their ability to maintain hMSCs in a multipotent and quiescent state, and do not attempt to **recreate** the **physiological conditions** that **prevent differentiation**. For this reason, there is a need for a culture system that sustains cell quiescence by mimicking the bone marrow niche through **substrate stiffness** and **oxygen tension**.

## INNOVATION

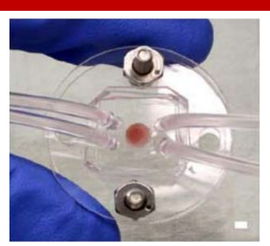


**Figure 2**. Bone marrow-on-a-chip [6].

- Cultures living bone marrow
- Retains hematopoietic stem cells and progenitor cells for one week
- Drawbacks:
  - Bone marrow generation in mouse
  - No consideration of oxygen tension or mechanical properties

## BIOREACTOR

**Aim:** Develop bioreactor that maintains user-set oxygen tension.
**Design:**

- Hypoxia chamber housed inside cell culture incubator, which provides temperature regulation

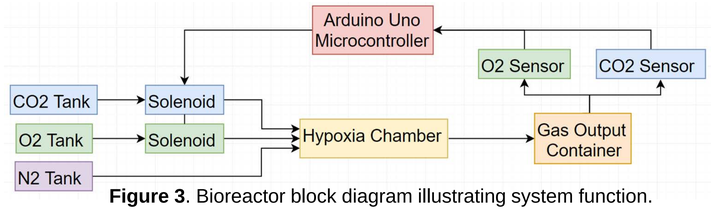



**Figure 3**. Bioreactor block diagram illustrating system function.

- Solenoid valves open for 2 seconds when $CO_2$ or $O_2$ concentrations drop below user-set values, close for 7 seconds.

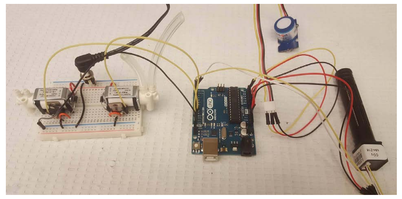
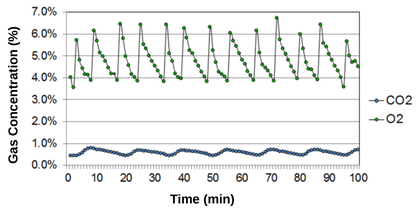


**Figure 4**. Bioreactor prototype, including circuit with solenoid valves, Arduino, and gas sensors.

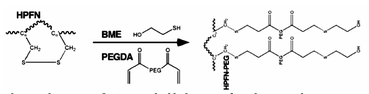
**Validation:**

- Monitor [$CO_2$] and [$O_2$] for 10 minutes once stable
- Variation within ±1.5% met for both gases, fine-tune flows to improve



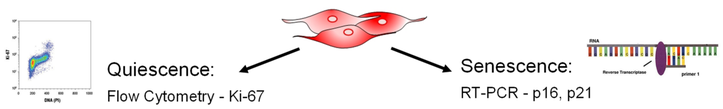

## FUTURE WORK

**Scaffold:** Fabricate fibronectin-conjugated PEG gel that allows a chemically-defined environment.




**Bioreactor:** Fabrication of 2 additional chambers to allow higher throughput evaluation of hMSC behavior at various oxygen tensions.

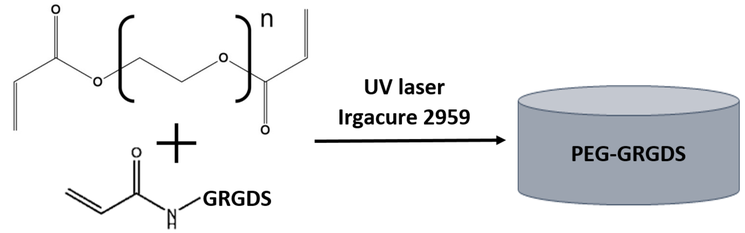
**Cell Culture Evaluation:** Culture hMSCs on scaffolds of varying stiffnesses, and on tissue culture plastic in varying oxygen tensions.




**Multivariate Testing:** Cell culture evaluation over a range of biomaterial stiffnesses and oxygen tensions could reveal the ideal combination of factors for maintaining hMSC quiescence *in vitro*.

## SCAFFOLD

**Aim:** Fabricate RGD-conjugated PEG hydrogels with Young's moduli spanning relevant human bone marrow stiffness range.
**Design:**




| | Primary Troubleshooting Issues | | | |
|---|---|---|---|---|
| **Gel Attempted** | **Hydrogel Formation** | **Peptide Lyophilization** | **Peptide Dialysis** | **Adhesion** |
| **3D Porous PEG** | ✗ | | | |
| **2D PEG-RGD** | | ✗ | ✗ | ✗ |
| **2D PEG-GRGDS** | | | ✗ | ✗ |
| **2D PEG-gelatin** | ✗ | | | ✗ |

**Validation:**

- Cell adhesion test with hMSCs
- Mass-spectrometry of peptide aminolysis products (future)
- Rheometry for Young's Moduli of 2.5 - 7.5 w/v% PEG gels (future)
- Experiment for effect of stiffness on quiescence (future)

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