Development of a bioreactor aimed at designing spatial and temporal drug delivery profiles for bone regeneration protocols

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Introduction

The Clinical Problem

Bone disease and injury affect millions of people around the world, resulting in weakened, degraded, and broken bones**. Mobility and limb function are often limited** even after treatment or surgery, primarily due to lost bone. Approximately **1.5 million non-union fractures occur each year** in the United States alone; this type of bone injury almost never heals properly [1].

Left: Non-union fracture of the femur [2].

Casts are often used to keep a bone in place after injury [3].

Potential Solution: Creating a Bioreactor to Further Develop Bone Scaffold Drug Delivery Protocols

Regeneration of lost bone can be achieved through implantation of a bone scaffold followed **by targeted delivery of angiogenic and osteogenic growth factors**. The spatial and temporal delivery profiles of these growth factors play a key role in the extent of vascularized bone formation.

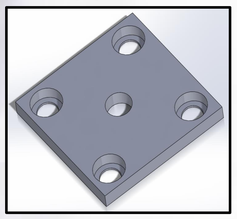
Our **newly developed bioreactor facilitates large scale testing** of many **different delivery profiles**. Further exploration of delivery profiles, scaffold materials, and biological factors can lead to discovery of **more successful bone regeneration protocols**.

Bioreactor Mechanical Design

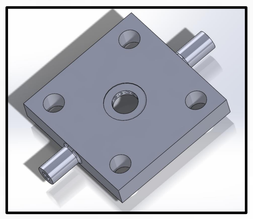
The purpose of the bioreactor is to provide a closed environment enabling 3-D bone/blood vessel growth to study the outcomes of different growth factor profiles.

3 Component System

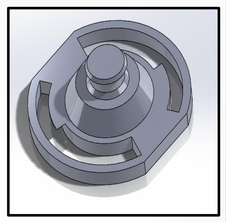
(1) Lid



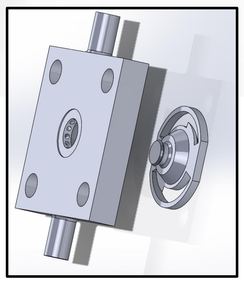
(2) Central Reactor



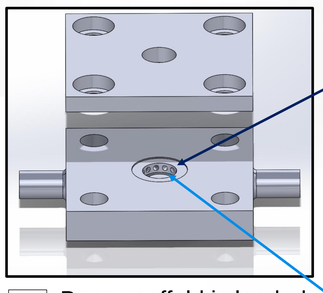
(3) Base



Bone scaffold loading & assembly of bioreactor

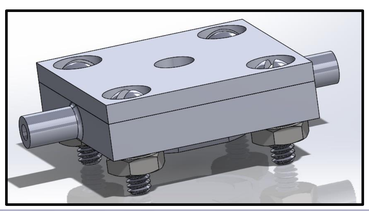


1 Base is inserted into bottom of reactor.



2 Bone scaffold is loaded into the central well

3 Glass cover slip is placed after loading



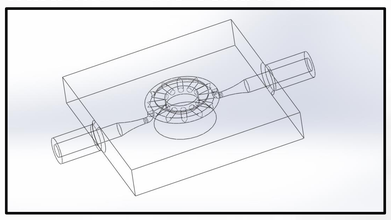
4 Lid is locked in place with screws and nuts

Bioreactor Fluidic Design & Verification

Step Design and Ring Design Flow Simulations

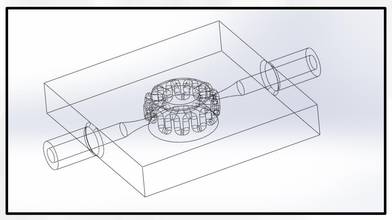
Two different flow pathway designs were proposed. The primary deciding factor for choosing between the two was:

Which design allows fluid to **penetrate the central well most quickly**, while showing the **most uniform, even distribution of fluid** within the central well?



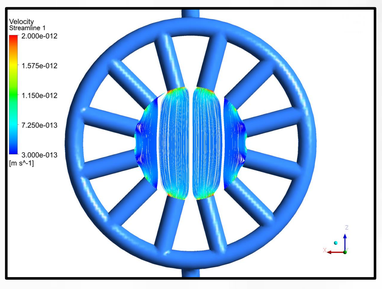
Ring Model

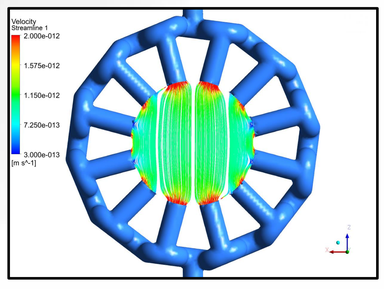
Consists of an **outer ring with evenly spaced pores** leading to the central well.



Step Model

Exhibits a **winding, sinusoidal path** along the outside with pores placed at the peaks of each wave.

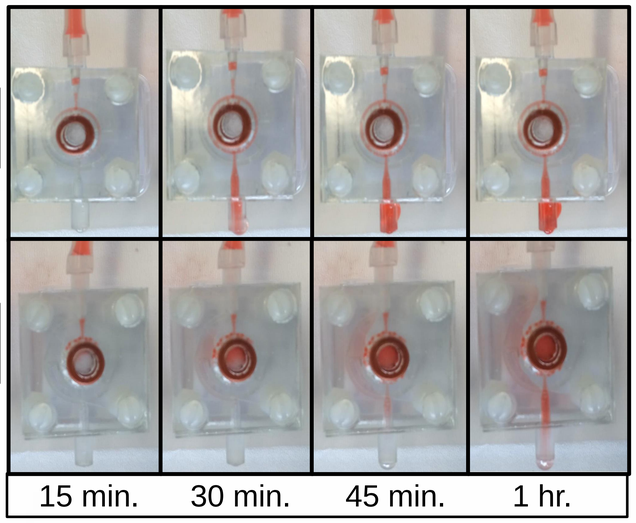




- Fluid flow simulation results for the **ring design show very little flow into the central well**. Most fluid seems to continue flowing through the ring until it exits through the outlet.
- **The step design shows significantly more flow velocity through the central well** and also exhibits flow through almost all pores, **hinting at even distribution of the fluid** throughout the well.

Confirmation of Simulation Results Through Flow Tests

A 1 hour time-lapse recording of fluid flow through each reactor model showed **strong agreement with the simulation results**:

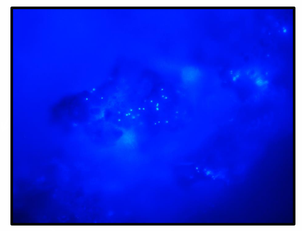


Based on these results, the **decision was made to proceed with the step model.** Uniform distribution of the water and dye solution used in this study can translate to **uniform and even distribution of growth factors onto the scaffold.**
This subsequently can lead to the **design of optimal release profiles** to generate vascularized bone.

Imaging Contents of Bioreactor

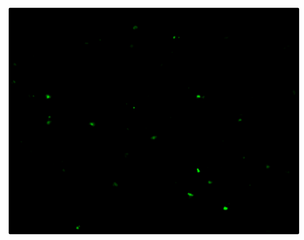
- Fluorescent beads (blue) were added to bone cement and loaded into the bioreactor**. Images of the beads and bone cement** were captured through an inverted microscope.

Bright-field (10x)

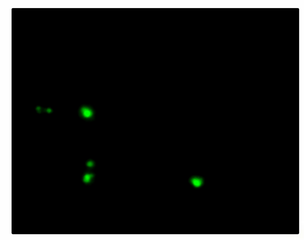


UV (10x)

- Fluorescent (green) human umbilical vein endothelial cells **(HUVECs) were seeded onto the bone scaffold** inside the reactor and **imaged successfully**.



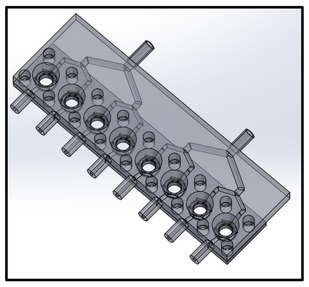
Blue (4x)



Blue (10x)

Future Work

- Continuation of the project involves seeding MSCs inside the reactor and **pumping growth factors through the bioreactor at varying concentrations and rates**.
- We seek to further understand **interplay between the effects of osteogenic and angiogenic growth factors**.
- We also aim at testing of an **"array" design consisting of many reactors** arranged in parallel.



- Such a design will allow for automated, **large sample size growth factor studies**.
- Can **increase throughput and decrease experiment** preparation time significantly.

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References

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