

Perfusion Bioreactor Apparatus for Studying Osteoblast Proliferation

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Abstract—Research-aided development of synthetic tissue constructs resembling natural tissue shows promise for expanding the potential of regenerative medicine. Advancements in this field are enabled using devices that support a biologically active environment. Specifically, there exists a demand within academia for a low-cost, high output bioreactor to facilitate study on osteoblasts proliferation and differentiation. Our goal was to design and manufacture a perfusion bioreactor capable of testing six individual samples. Our perfusion bioreactor was engineered to achieve a specific range of shearing stress and pressure at a sample’s surface; while sustaining optimal conditions for cell culturing. SolidWorks 2019 and Ansys CFD 2019 were used for prototype development. The simulation testing generated data that was analogous to analytical calculations. This perfusion bioreactor comprises of four components and is readily assembled. The device allows for experimentation on numerous samples and enables repeated use. Potential for modification of bioreactor tubing exists to allow for variation of cell media properties in future cell studies.

I. INTRODUCTION

According to the United Nation, the World Health Organization, governments, and professional and patients' organisations have declared 2000-10 the “bone and joint decade” with the aim of improving the health-related quality of life of people with musculoskeletal conditions [1]. Recently, there has been significant investment in tissue engineering as researchers seek to enhance treatment for these conditions. The development of bioreactor devices provides scientists with the means to perform targeted research at the microscopic level.

This paper presents a perfusion bioreactor apparatus which enables study on the effects of osteoblast proliferation and differentiation when subjected to fluid shearing stress ranging from 0.8 to 3.0 Pa and relative pressure on the order of 7 kPa. The device is composed of nontoxic, autoclavable, 3D printable polycarbonate filament whose multichambered design capable of simultaneously experimentation on six individual samples. The device is inexpensive, compact, durable, and simple to assemble. The perfusion bioreactor works in conjunction with a peristaltic pump, cell media reservoir, and incubator. The goal of this apparatus is to simulate a biologically stable environment, while keeping cell media fluid properties stable.

II. MATERIALS AND METHODS

A. Prototype Design

The symmetrical, split-flow bioreactor design efficiently separates incoming cell media, directing it to individual samples. Consisting of four components, the bioreactor can be disassembled (Fig. 1) for cleaning and inspection. The first component, the inlet cap, connects the bioreactor to the peristaltic pump tubing. The second component, the fluid distribution module, gathers and directs incoming cell media to a sample. The next component is used to load the samples and position them in place. Finally, a reservoir collects cell media that has passed over the samples.

B. Analytics of Cell Media Properties

The bioreactor is designed to be compatible with a base medium of 1:1 mixture of Ham’s F12 Medium Dulbecco’s Modified Eagle’s Medium whose fluid properties are comparable to those of water. The mass flow rate (used in CFD simulations) output from the pump was verified experimentally. Basic governing equations for fluid flow and the provided properties of the cell medium were used to determine inlet boundary conditions and shear stress at the surface of a sample.

$$\dot{m} = \rho AV \quad (1)$$

$$\tau = \rho \frac{d\mu}{dy} \quad (2)$$

ANSYS CFD was used for simulating the flow of cell media within the apparatus. This was performed with the assumption of steady, incompressible, and fully-developed fluid flow.

C. Manufacturing Process

The perfusion bioreactor is designed to be 3D printed using hygroscopic, medical grade polycarbonate. Polycarbonate is autoclavable and can withstand temperatures up to 140°C while maintaining the strength and form. Printing may be done with Fortus Classic by Stratasys using PC-ISO (translucent) #310 20400 filament and PC BASS support material. This process has a printing tolerance of ± 0.127 mm.

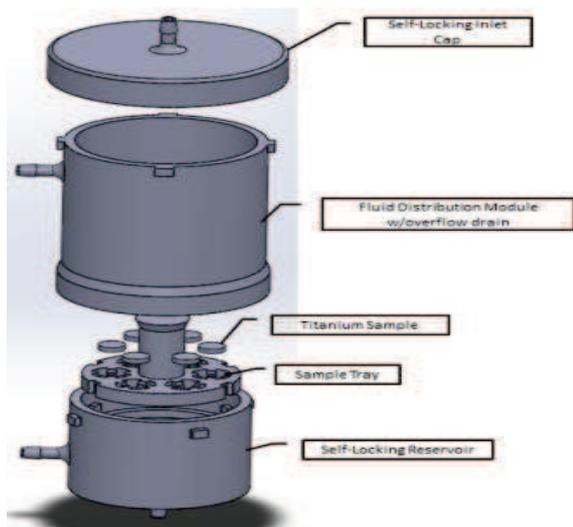


Figure 1: Bioreactor Assembly (SolidWorks)

III. RESULTS

Variation of the tubing diameter and length of the bioreactor fluid distribution module affected the mechanical fluid properties of the cell media. Various tube geometries were tested to attain the required shear stress and pressure ranges required for research. The effects of varying tube geometry are presented in Table 1. Using the simulation data, it was decided that the fluid distribution module produces the necessary range of shear stress and pressure at the sample face using 8 mm diameter tubing.

Table 1

	Tube Length			Tube Diameter		
	40 mm	400 mm	1 m	6mm	8mm	10mm
Shear Stress at center (Pa)	0.833097	0.83126	0.82857	4.27851	1.78162	1.13044
Shear Stress near edge (Pa)	0.970442	1.04298	1.13795	3.67917	1.65526	1.09331
Pressure at center (Pa)	2451.028	3937.616	9780.570	8170.904	7901.351	6749.539
Pressure near edge (Pa)	2447.310	3934.508	9763.280	76383.950	7144.23	6288.048

The distribution of shear stress and pressure on the face of the sample varied independently of the tubing diameter. The manner in which the cells are seeded onto a sample disc results in the majority of cells gathering near the center of the disc. Given this, shear stress and pressure values were measured with respect to the radial distance from the center of the sample. These results are presented in Figures 2 and 3. As expected, shear stress and pressure are higher at locations further from the center of the sample. Varying the length of distribution module tubing did not show significant changes on the shear stress at the sample surface, however, data shows that pressure increases proportionally when tubes are elongated. The pressure variation with respect to radial distance from the sample center is attributed to the difference in diameter of the tubing and the sample disc.

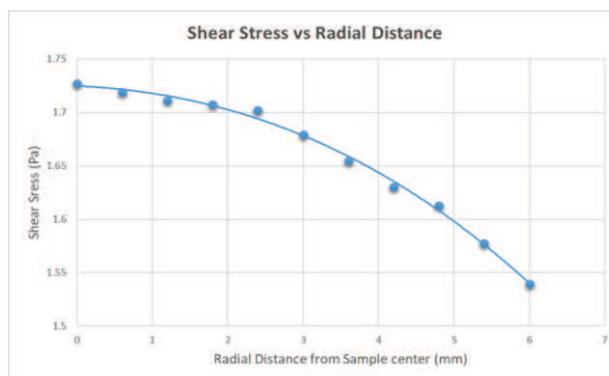


Figure 2: Variation of Shear Stress at Sample Surface

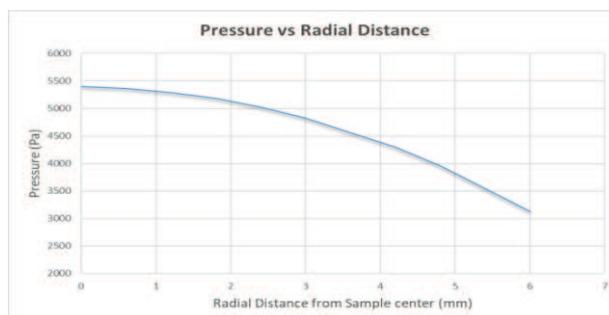


Figure 3: Variation of Pressure at Sample Surface

This work marks an important step in the development of appropriate tube geometries. Moreover, the use of inexpensive materials and simple geometry modifications makes this device easily adaptable for inducing a variety of shear stress and pressure ranges in future research.

IV. CONCLUSIONS

A perfusion bioreactor apparatus was designed for studying the effects of shearing stress and pressure exposure on osteoblast proliferation and differentiation. Various bioreactor tube geometries were designed and tested through simulation. Additionally, the 3D printed manufacturing process enables the possibility of modifying bioreactor tubing geometry for future study.

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