

5-5-2020

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Recommended Citation

VanDerWolde, Brooklyn K. and Hillson, Katelyn (2020) "Design of a 3D Printed Bioreactor for Bone Cancer Research," *The Journal of Undergraduate Research*: Vol. 17 , Article 6.

Available at: <https://openprairie.sdstate.edu/jur/vol17/iss1/6>

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Cover Page Footnote

Brooklyn Kay VanDerWolde is the recipient of a fellowship from the NASA South Dakota Space Grant Consortium.

Design of a 3D Printed Bioreactor for Bone Cancer Research

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INTRODUCTION

Bone cancers, both primary and metastatic, are aggressive with devastating consequences on patients' quality of life [1]. Bone cancer is most prominent during growth (10 to 19 years), aging (70+ years), and in survivors of other kinds of cancers such as breast and prostate cancers [1–4]. Astronauts who undergo microgravity and space radiation during long-term space missions also have an increased risk of bone tissue regeneration, including bone cancer. There is no cure for bone cancer. Patients who do survive suffer from severe pain and frequent fractures [5]. New targeted therapies require a fundamental understanding of tumor localization to the bone and the role of bone environment in cancer growth [3,4]. This, in turn, requires the development of an effective tissue model to replicate the 3D bone environment for bone cancer research.

A limited number of in vitro models capable of reproducing the complex microenvironment of bone tissue to support multi-cellular activity in a three-dimensional structure currently exist. The lack of appropriate models, in turn, stalls one's ability to understand disease progression and develop new treatments and therapies. Therefore, developing a bone tissue model and an associated bioreactor is critical to understand the risks associated with cancer progression and improve treatment and preventions related to those risks. This research focused on developing an optimized, automated 3D printed bioreactor to be used in a bone cancer microenvironment.

A bioreactor is a device used to house and support biological activity. Incorporating mechanical loading is necessary for bone tissue viability. We aim to create a new bioreactor design that can not only support long-term tissue viability for bone cancer but can also be easily assembled for efficient application in future research studies. These studies will include the development of an osteosarcoma, a type of bone cancer, microenvironment. The bioreactor design was based off a prior graduate student of Dr. Prasad [6], where the student successfully developed a compact bioreactor capable of supporting bone tissue ex vivo long-term. The developed system was demonstrated to maintain cancellous bone tissue viability (10 mm ϕ x 10 mm) viability for up to 35 days. The goal of this project was to create a more reliable, sustainable design while still maintaining a cost-effective and reproducible bioreactor.

MATERIALS AND METHODS

A compact tissue bioreactor was recently developed to support long-term bone tissue survival ex-vivo [6]. Several modifications were made to the original in-house developed setup. In particular, the preexisting bioreactor, which was made from nylon material, would warp, leak and become a tinted yellow shade with continued use. Therefore, a material change was necessary. A flexible silicone top cover needed to be redesigned to seal in the nutrient media without leaking. Finally, the loading in the bioreactor required manual placement over each of the wells and therefore required modification for loading multiple tissues at once. When developing the new bioreactor, it was essential to ensure that the design would allow fluid to flow past the bone on all sides.

Additionally, the bone and cancer cells respond to mechanical loading and fluid flow [7-8]. Therefore, there was a need to apply regular fluid perfusion and intermittent mechanical loading to the tissue. The section below describes the various bioreactor components.

The designed bioreactor assembly consists of these unique components, namely: (1) a tissue well to house the tissue, (2) a

camshaft assembly for mechanical loading, (3) a motor to control the cam-shaft loading, (4) a pump for fluid perfusion, and (5) a base platform to house the components. The base platform

incorporates areas for pipes, pump, motor, and cam-follower

assembly. These components and the base platform are shown in Figure 1.

All of the components that come in contact with the fluid and the tissue will be printed using ULTEM filament. This filament is known to be a biocompatible, water-resistant, high-temperature-withstanding material. The choice of material will ensure that the design will not be damaged when the bioreactor is autoclaved or exposed to the liquid nutrient media.

Tissue Well

The tissue housing area (Figure 1c) consists of four identical, individually 3D printed tissue wells. Each well has a base platform at the center to house a bone sample and has an inflow-outflow channel for the nutrient media. Figure 2a shows the CAD model, and Figure 2b shows its printed form. The base platform has a porous structure that allows fluid contact of the bone sample at the

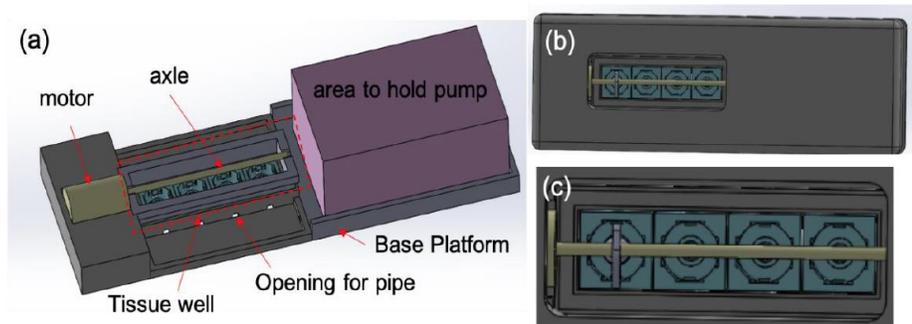


Figure 1: The components of the bioreactor (a) isometric view of the bioreactor setup where the cylindrical motor is attached to the axle, which will contain four cams evenly spaced over the four tissue wells. The "area to hold pump" houses a low flow peristaltic pump inside. (b) Base view of the bioreactor. (c) Top view of bioreactor zoomed in to show tissue wells where bones will be housed.

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base as well as for continuous contact. The edges of the platform are raised 1mm to allow a tight hold on the bone once placed and loaded. Each side was designed to have a hook. A follower will be held in place by rubber bands stretching across these hooks, as shown in Figure

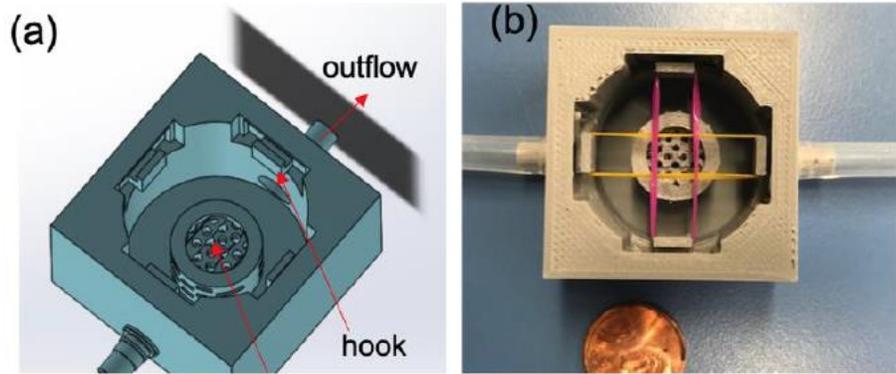


Figure 2: Tissue wells to house bone samples (a) CAD model of the well, and (b) 3D printed component

2b. Hollow extrusions protrude

from two opposite sides to allow plastic hoses to fit snugly over them. This creates a flow channel to enter and exit the tissue well. A total of four of these wells are made and all fit together in a linear row on the base platform, as shown in Figure 1c.

Base Platform

The base platform consists of a multi-component frame assembled to hold the tissue wells, motor, cam axle and pump. The main element is the base framework which contains an area to hold the tissue wells in a line at the center (Figure 3a). Each of the tissue wells in the base platform contains two holes on the side to fit the extrusion of the tissue well, which connects with the inflow and outflow pipe. An empty tray on both the left and right side holds hoses when not in use. An indentation around the framework allows the top cover to snap onto the framework when the tissue well is in use. The top frame (Figure 3b) fastens to the base frame. When in use, a transparent plastic layer will cover the tissue well to seal the nutrient media in the tissue well. On top of this component there are two gaps on opposite ends for the shaft to rest. A motor holder was designed to secure the motor while the bioreactor is in use (Figure 3c). It consists of a block with an indentation to hold the motor at the predetermined height necessary for the camshaft to rest in the nested cutouts on the top.

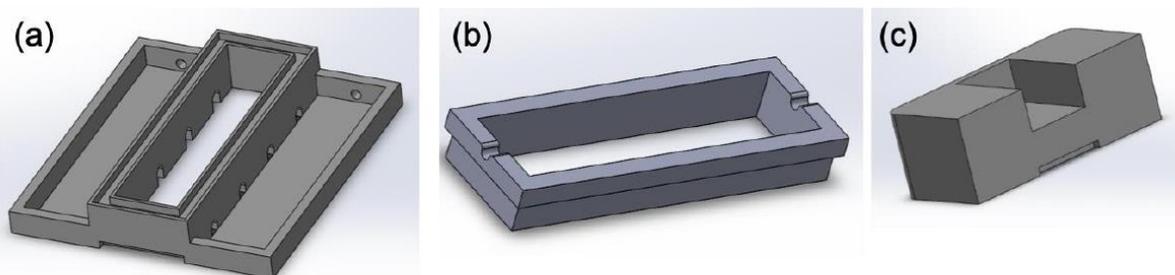


Figure 3: Components of the base platform (a) Base framework which houses the tissue wells, (b) top cover of the tissue well area

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The pump holder was designed to secure a peristaltic pump (Figure 4). It is made of two pieces that can either be assembled using a robust and biocompatible glue or potentially 3D printed holder, depending on the size of the printer. On the right side of the pump holder there is a lip that fits into the base to appear as if it is one single piece when assembled (Figure 4a). The bottom of the pump holder, where the pump sits, is a simple design that snaps into the rest of the bioreactor to secure the pump in place while the bioreactor is in use (Figure 4b).

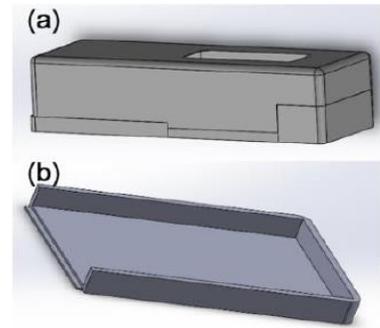


Figure 4: Components to hold pump of the bioreactor

Motor and Cam Shaft

The bioreactor design also includes a motor and camshaft assembly (Figure 5) for mechanical stimulation of the bone tissue in the bioreactor. The cam was designed both as a rounded teardrop shape (Figure 5a) as well as a circle with offset diameter. A hole passing through the cam will hold the axle of the motor during its rotation to apply the desired force. The follower was designed as a conic shape that is flat on both ends and has a hole passing through the configuration in four directions, depicted in Figure 5b. These holes allow the rubber bands to pass through the follower and secure it above the bone sample. The cam-follower assembly was designed to include a camshaft with four evenly spaced cams that exert a force onto the followers resting above each bone sample to simultaneously load the bones. The camshaft is connected to a motor that rests in the notches of the top frame described above. Each cam is offset by 90 degrees and is designed to load each cam with the same force and duration mechanically. This ensures that the camshaft will apply the maximum predetermined load to each bone sample. Motion analysis studies were performed in SolidWorks to determine the ideal shape and size of the cam-follower geometry.

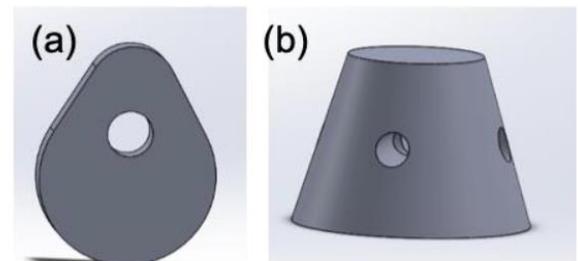


Figure 5: Geometry of (a) Cams and (b) follower geometry

RESULTS

A preliminary experimental and theoretical analysis was performed to calibrate the cam-follower assembly

Experimental Calibration of Cam Shaft

The original mechanical loading design consisted of a single cam attached to a motor that was manually slid along the bioreactor. It was to be run over each bone sample for an hour each day [6]. The new design proposed here consists of a new camshaft, where each cam is evenly spaced to fit over its follower existing above the bone samples. This allows one to mechanically stimulate all of the wells without having to move the load every hour. Preliminary experiments were run to test the newly

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proposed multi-cam design for many different combinations of cam offsets and cam geometries. Figure 6a below depicts a test run with 45-degree offsets and circular cams. A re-calibrated force sensor hooked up to an Arduino was used for this step. The process of sensor and cam calibration as proposed earlier [6] was used for this work and hence not described here. Figures 6b and 6c show the results of loading six cams, where each cam's center is offset by a different amount, and therefore possesses a different radius from the center of the shaft to the distance where the cam applies its maximum force to the calibration sensor. As one can see, the larger the radius, or offset, of the cam, the greater the force exerted by the cam is.

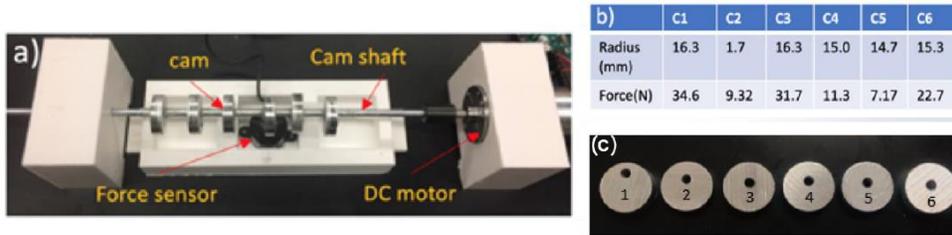


Figure 6: (a) cam follower calibration setup; (b) Table containing forces exerted by circular cams of varying offsets; (c) Images of the six cams of various offsets

SolidWorks Simulation Results

SolidWorks simulations were also performed to calculate the force applied by the cam assembly. The material, along with the speed and rotation of the motor, was specified for the simulations. Results from the simulations showed that there were approximately 5 newtons of force being applied to the bone every 2.5 seconds (Figure 7).

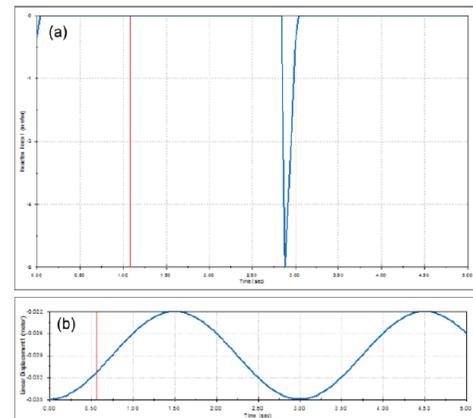


Figure 7: SolidWorks Simulation (a); Applied force in the Z direction: 5 newtons every 2.75 seconds; (b) Displacement in the Z direction

CONCLUSION/ FUTURE WORK

By modifying the recently developed bioreactor, we have developed and proposed a new design to make the bioreactor affordable, compact and automated. The use of ULTEM filament in the new design will ensure a sustainable design capable of long-term use in future cancer studies. The tissue bioreactor can be easily assembled using readily available materials and off-the-shelf components; hence, this bioreactor has broader implications in clinical and lab setups worldwide. Future work with this bioreactor includes (1) 3D printing the proposed design and testing its biocompatibility post sterilization, (2) fixing a combination of cam-follower assembly to provide ideal loading environment, (3) applying the design to create an osteosarcoma microenvironment and (4) applying the setup for bone cancer research. All of these works are currently ongoing or will be

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proposed shortly in Dr. Prasad's lab. These tasks will provide a standard protocol for multicellular feasibility using bone and cancer cells for applications in cancer research and drug development.

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