MINIATURIZATION OF AN OSTEOCHONDRAL BIOREACTOR SYSTEM

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INTRODUCTION

Osteoarthritis (OA) affects millions of people in the US and is primarily characterized by the progressive degeneration of articular cartilage in joints, such as the knee [1]. However, cartilage is not the only tissue affected by OA, the underlying bone is also impacted by the disease. In fact, damage to the bone may be deleterious for the above cartilage and may even initiate the disease [2]. Hence, it is important to study cartilage and bone as a single unit. However, most research has so far been focused on either cartilage or bone, studying them as separate tissues [2]. Creating a new method in which to study OA, putting cartilage and bone together as a single osteochondral unit, is paramount to advance the field of OA research and identify potential treatments. Currently at the Center for Cellular and Molecular Engineering, a bioreactor has been developed that uses mesenchymal stem cells (MSCs) to form a tissue that is part bone and part cartilage, which can be used to model the osteochondral unit as it is found in the knee [3]. The stem cells are loaded into a gel that is placed in the bioreactor and then exposed to separate medium streams that cause the stem cells on one side of the gel to differentiate into chondrocytes (cartilage cells), and the stem cells on the other side of the gel to differentiate into osteoblasts (bone cells). The final application of this bioreactor is to screen drug candidates that could alleviate or even stop OA [3]. Currently, the screening of drugs is a lengthy and cumbersome process, and the effects of many candidate drugs are fully elucidated only when they are tested in animals or in humans, the most costly and risky phase of drug development. With a new bioreactor that effectively mimics human tissues and simulates an in-vivo environment, the pharmacological testing of OA drugs would become safer for the patients and more effective, allowing for the identification of potentially harmful compounds much earlier.

OBJECTIVE

The current bioreactor, unfortunately, does not permit viewing of the cells during growth. This is inefficient because the cells cannot be imaged during differentiation. Viewing of the cells is essential in fluorescent testing, which will eventually be used to verify chondrogenesis in real time. Furthermore, the current bioreactor design requires a very high number of MSCs due to its relatively large volume. The scope of my project is to design an original osteochondral bioreactor for the Center for Cellular and Molecular Engineering for the study of OA. To improve upon the current design, I have developed the following design requirements: 1) to allow optical access to visualize cells in the process of differentiation and their response to a candidate drug and 2) to reduce the total amount of MSCs used in the reactor. The success criteria for the design is 1) efficient sealing, 2) allows for two separate medium channels that allow for diffusion through gel, and 3) differentiation of MSCs to create the engineered osteochondral tissue. My hypothesis is to see if a model can be designed that successfully meets all criteria.

METHODS

I have developed two preliminary models of the new bioreactor design via the computer aided design (CAD) software, SolidWorks (Figure 1 and Figure 2).

Figure 1. SolidWorks model of preliminary bioreactor model. This particular model has an attached base and two channels for the respective medium streams.

Figure 2. SolidWorks model of bioreactor that contains a removable base. Channels for medium streams run in parallel through the reactor.

The first model (Figure 1) is a single unit that is simple and can be easily used in preliminary testing. The attached base allowed for easy assembly for the initial testing. The second model (Figure 2) has a removable base that would offer a more efficient way to remove the tissue for examination after testing. Similar to the current bioreactor, there are two channels for the chondrogenic and osteogenic media, as well as a well for an o-ring to make the reactor water tight. These models have proceeded through rapid prototyping via 3D printing, and continued through a preliminary round of tests. The first test was to verify that the bioreactor was water tight.
and could be successfully sealed. This was done by filling the reactor with a liquid dye and then visually monitoring for leaks. The next test was to verify if the bioreactor allowed for an input solution to diffuse through a blank gel. For this test, the tissue chamber was filled with a gel and the diffusion of a dye colored fluid from the fluid channels was observed.

RESULTS

The results for the sealing and diffusion tests were both successful (Figure 3 and Figure 4).

Figure 3. The bioreactor filled with dye to show successful sealing.

Figure 4. Two dyes successfully diffused through the gelatin. The gelatin is shown at time =0 min, 20 min, and 24 hours.

The sealing test (Figure 3) was successful because the bioreactor did not leak when loaded with the liquid dye. The diffusion test (Figure 4) was successful because the dyes successfully diffused through the gel. At 20 minutes, the dye began diffusing and at 24 hours both dyes diffused through the gel. The open chamber allows for easy access to analyze the results of these tests. The chamber also has a much smaller volume and will require less MSCs.

DISCUSSION

In continuing the project, the next steps will be to (a) to finalize the bioreactor design, (b) to define a protocol to achieve well-defined differentiation of the cartilaginous and osseous components of the osteochondral unit, and (c) to test the bioreactor with human adult stem cells, assessing their differentiation and their response to a candidate disease modifying drug. To finalize the reactor, I will continue to decrease the volume of the required gelatin until the reactor creates a microtissue that most highly achieves the design requirements. In addition, I will use adult stem cells transfected with a reporter gene for green fluorescent proteins (GFP) that have been developed at the Center for Cellular and Molecular Engineering. These cells start producing GFP only when a gene of choice is activated. That is, if we decide to monitor the differentiation of cells, we can choose a gene like aggrecan, which is activated when stem cells differentiate into chondrocytes (cartilage cells). In this way it is possible to know that differentiation is taking place just by monitoring if the cells start to fluoresce green, i.e., if they start producing GFP. In this way, I will be able to determine the effectiveness of the bioreactor in close to real time, resulting in a quantitative analysis of its effectiveness. As aforementioned, this can only be done with a reactor that allows for direct viewing of the cells during differentiation. Ultimately, my goal is to design a bioreactor in which multiple osteochondral tissues can be developed and tested simultaneously.

Looking to the future, this bioreactor has the potential to be used for pharmacological screening. This means that the final bioreactor could be used in the development of pharmaceutical products to limit the number of compounds that will proceed through testing. This can potentially save billions of dollars, decreasing the cost of drug development and benefitting patients both in terms of savings and, most importantly, in terms of increased drug safety.

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REFERENCES

