Design of a High-Throughput Flow Perfusion Bioreactor System for Tissue Engineering

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Flow perfusion culture is used in many areas of tissue engineering and offers several key advantages. However, one challenge to these cultures is the relatively low-throughput nature of perfusion bioreactors. Here, a flow perfusion bioreactor with increased throughput was designed and built for tissue engineering. This design uses an integrated medium reservoir and flow chamber in order to increase the throughput, limit the volume of medium required to operate the system, and simplify the assembly and operation.

Overview of Flow Perfusion Bioreactors

FLOW PERFUSION BIOREACTORS are commonly used in many areas of tissue engineering.^{1–5} Such systems are designed to perfuse culture medium through the interconnected pores of a tissue culture scaffold. This method of culture has two primary advantages. Perfusion culture can be used to increase the rates of mass transport through the interior of the scaffold compared with the rate of diffusion alone⁶ and can be used to apply mechanical stimulation, in the form of shear stress, to cells in the scaffold, in order to achieve a desired cellular response.^{6,7}

There are ubiquitous challenges encountered with perfusion bioreactors, and their use with specific tissues, such as cartilage, often presents several additional unique challenges. One of the primary limitations to perfusion cultures is the relatively low-throughput nature of these systems. This is primarily due to space limitations, as perfusion bioreactors require relatively large amounts of space to operate in a controlled environment. However, the time intensive assembly and operation can further limit output. Chondrogenic cultures often necessitate the use of growth factors, and with the large volumes of culture medium that are often necessary to operate perfusion bioreactors, incorporating growth factors into the culture medium can be costly. Additionally, the rabbit osteochondral defect model is one of the most widely used animal models in articular cartilage engineering.⁸ Here, the critical-sized defect is 3 mm in diameter.⁸ Thus, the ideal perfusion bioreactor for cartilage engineering would be capable of culturing scaffolds of this size but be easily adaptable to other scaffold sizes.

The objective of this work was to design a flow perfusion bioreactor for tissue engineering, while addressing some of the challenges encountered with perfusion bioreactors, primarily the low-throughput nature. Additionally, due to our

interest in cartilage engineering, the design considered the earlier-mentioned challenges specific to this field. The primary design requirement of the system was the ability to provide a consistent and controllable media flow rate through the scaffolds. Additionally, the design was intended to allow for high-throughput cultures, while minimizing the volume of medium required to operate the system. The bioreactor was designed to initially culture scaffolds of 3 mm in diameter but to be easily adaptable to additional scaffold sizes. Additionally, the system was designed to eliminate air bubbles before reaching the scaffolds in order to not disrupt the cultures. Finally, all parts were designed to be autoclavable and easily handled and assembled under sterile conditions.

Design Philosophy

In many perfusion bioreactor designs, the medium is contained within reservoirs, separate from the flow chamber where the scaffolds are perfused.⁶ Here, by integrating the medium reservoir and flow chamber into one unit, the amount of medium and tubing and the number of parts required to run the system are significantly reduced. In combination with perfusing the scaffolds from top to bottom, the air bubbles are prevented from accumulating below the scaffolds and are eliminated before reaching the scaffolds and disturbing the culture. Finally, culturing multiple scaffolds per flow channel provides significant flexibility when selecting a volumetric flow rate, as the culture medium is divided between multiple samples as the scaffolds are perfused.

Design

The bioreactor design, shown in Figure 1, consists of four main components. The scaffold holder, medium reservoir, and peristaltic pump and tubing.

FIG. 1. (A) Schematic of assembled bioreactor unit with the integrated medium reservoir and flow chamber. (B) Image of assembled bioreactor unit.

Scaffold holder

Scaffolds are contained within custom-made, autoclavable, polycarbonate scaffold holders, shown in Figure 2. Each scaffold holder is designed to contain 10 scaffolds 3 mm in diameter, distributed axisymmetrically in order to ensure an equal division of flow through the scaffolds. This scaffold holder can be easily adapted to fit other scaffold sizes, as shown in Figure 3. Samples are pressfit from the bottom of the holder and supported from below by a stainless steel 316 mesh (McMaster-Carr, Elmhurst, IL). The support mesh slides into place and is held by two tabs on the bottom of the

FIG. 2. Scaffold holder and stainless steel 316 support mesh. Ten scaffolds, 3 mm in diameter, are distributed axisymmetrically and pressfit into position. A support mesh slides into place to prevent the movement of scaffolds during culture.

scaffold holder. The thickness of the scaffold holder was chosen so that each individual flow channel in the holder is able to contain an appropriate volume of cell solution for seeding scaffolds. This dimension (11.5 mm) is significantly more than the entrance length required for fully developed flow at the scaffold surface,⁹ which is necessary to minimize entrance effects.

Medium reservoir lid and base

The medium reservoir consists of two pieces machined from stainless steel 316. The two pieces fit together to form a base and lid. The scaffold holder is placed in between the two pieces with nitrile rubber o-rings (McMaster-Carr) on top and bottom. The medium reservoir is compressed together and held with stainless steel bolts and wingnuts. Medium is contained both above and below the scaffolds, with ~25 mL contained below the scaffolds. The system can operate with as little as 10 mL in the reservoir above the scaffolds, but can store as much as 120 mL, bringing the total medium volume range in the system to 35 to 145 mL.

Medium is changed through a port of the lid that can be sealed by a silicone rubber stopper (McMaster-Carr). Two additional ports lead to two polypropylene, barbed, male threaded adapters (Cole Parmer, Vernon Hills, IL). One adapter leads to a syringe filter that facilitates gas exchange, and the other adapter connects to the inlet tubing. The reservoir base contains an adapter that leads to the outlet tubing.

Peristaltic pump and tubing

The inlet and outlet tubing is made of platinum-cured silicone (Masterflex tubing; Cole Parmer). Platinum-cured tubing is used to reduce the amount of leachable chemicals and is relatively low-protein binding. Further, the tubing is gas permeable to oxygen and carbon dioxide. A short segment of mechanically stronger, neoprene, nongas-permeable tubing is used within the pump (Pharmed tubing; Cole Parmer). With the tubing size used in this system (Cole

FIG. 3. The scaffold holder is easily adaptable to other scaffold sizes and numbers. Shown here from left to right are scaffold holders designed to support twenty 2-mm scaffolds, ten 3-mm scaffolds, ten 5-mm scaffolds, and four 8-mm scaffolds.

Parmer L/S 13) the pump is capable of accurately and consistently pumping flow rates from 0.06 to 6.0 mL/min through each flow chamber. This flow rate is divided equally between all scaffolds in one flow chamber. Each pump is capable of driving 12 flow chambers, given that the pump is a 12-channel peristaltic pump (Cole Parmer).

Representative Results

Scaffolds were fabricated by electrospinning poly(ϵ -caprolactone) meshes with an average fiber diameter of 10 μ m using previously established methods.¹⁰ Scaffolds 3 mm in diameter and 1.0–1.1-mm thick were die-punched using a 3-mm dermal biopsy punch. Bovine chondrocytes were isolated and pooled from the femoral condyle of three 7–10-day-old calves (Research 87, Boylston, MA) using established methods.^{11,12} Primary chondrocytes were seeded at a density of 50,000 cells per scaffold and incubated overnight. Cultures were then continued either in the flow perfusion bioreactor or under static conditions in a 24-well culture plate. Perfusion cultures were performed with a flow rate of 0.03 mL/min through each scaffold. All constructs were cultured in chondrocyte proliferation medium,¹¹ which consisted of Dulbecco's modified Eagle's medium, 10% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA), 1% nonessential amino acids, 0.4 mM proline, 10 mM HEPES buffer, and 50 mg/L ascorbic acid, and penicillin, strepto-

mycin, and fungizone. After 14 days, samples were removed from culture, rinsed in phosphate-buffered saline, and fixed with 10% neutral-buffered formalin (Fisher Scientific, Pittsburgh, PA). Samples were then soaked in 70% ethanol, embedded in HistoPrep freezing medium (Fisher Scientific), frozen at -20°C , and sectioned using a cryotome (Leica Biosystems, Richmond, IL). Sections from each scaffold ($n=2$ scaffolds per culture condition) were cut to a thickness of 5 μ m, mounted onto Superfrost Excell glass slides (Fisher Scientific), and stained using Safranin O histological stain to visualize the distribution of extracellular matrix. Images were obtained using a light microscope (Zeiss Axio Imager 2; Carl Zeiss, Oberkochen, Germany) with a video camera attachment (Zeiss Axio Cam MRc5; Carl Zeiss). Representative images are shown in Figure 4. In all scaffolds evaluated there was a significant increase in chondrocyte extracellular matrix in perfused scaffolds, compared with static. These results demonstrate the significant enhancement that can be seen with flow perfusion culture, consistent with previous studies.⁹

Design Limitations

As with any bioreactor design, there are several limitations to this system. To begin with, while culturing multiple scaffolds per flow channel allows for high-throughput cultures, all of the scaffolds within one flow chamber share a

FIG. 4. Representative histological sections of electrospun poly(ϵ -caprolactone) scaffolds with bovine chondrocytes cultured for 14 days under (A) static and (B) flow perfusion conditions. Images show histological sections stained with Safranin O to visualize cartilaginous matrix. Extracellular matrix proteoglycans are stained red, and representative examples are indicated with yellow arrows. Representative examples of poly(ϵ -caprolactone) fibers are marked with blue arrows. Scale bar represents 100 μ m in both images. These images demonstrate the significant increase in cartilage-like matrix as a result of perfusion culture.

common medium reservoir and thus should be within the same experimental group. Similarly, all of the samples in a single scaffold holder must be harvested at the same time point. However, multiple bioreactor units can be used to incorporate additional groups and time points in one experiment. Finally, the medium flow is divided between all scaffolds in a flow chamber, so in order for the flow rate to be evenly distributed between all scaffolds, the resistance of each scaffold to fluid flow must be equal. Thus, each scaffold must have relatively uniform interior geometries and approximately equal scaffold thicknesses. Further, if one or more scaffolds were damaged or shifted during culture, the flow rate through all scaffolds in the flow chamber would be affected. However, scaffolds are both pressfit into the scaffold holder and supported by the stainless steel mesh to prevent such problems. Finally, with the integrated medium reservoir and flow channel, portions of the culture medium are contained both above and below the scaffolds. It is intended that medium changes be performed from the port on the top of the reservoir; however, ~25 mL of medium is contained below the scaffold holder and cannot be changed using this method, which could allow for the undesired accumulation of waste products. However, in some cases, partial medium replacements could be advantageous as they allow endogenous cytokines to remain in low levels.¹³ Further, this limitation can be overcome by increasing the frequency of medium changes or increasing the volume of medium contained in the top region of the medium reservoir (capable of holding 10 to 120 mL) in order to reduce the significance of the unchangeable 25 mL.

Conclusion

In conclusion, a flow perfusion bioreactor system has been designed and built for high-throughput cultures. This system contains an integrated medium reservoir and flow chamber in order to reduce the number of parts and volume of medium required. Additionally, multiple samples per flow chamber allow for increased production capacity. Further, this system is able to operate with a relatively low volume of medium and has simplified assembly and operation.

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Disclosure Statement

No competing financial interests exist.

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