Assembly of a Pulsatile Flow Bioreactor System to Facilitate Oscillatory-flow Conditions to Optimize *In Vitro* Engineered Valve Tissue Growth

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Introduction: Tissue engineering and regenerative medicine show promise for application in various diseases ranging from structural tissues such as cartilage, skin, and bone, to organs such as liver, kidney, and myocardium [1]. In particular, critical heart valve diseases in children present one of the most challenging problems with patients having extremely limited treatment options. However, the probability of success for treating such diseases leveraging a tissue engineering approach appears promising. Previous work in our lab has shown direct improvement in gene expression supporting the valve phenotype when stem-cells were conditioned under native, human aortic flow pulsatile flow conditions [2]. Moreover, a specific magnitude of flow oscillatory flow patterns yielded the most desirable expression; however, these studies were conducted in monolayer culture. To extend this work to 3-dimensional tissue growth, the objective of the current investigation was to assemble an *in vitro* system which could deliver physiologically-relevant pulsatile flows to growing engineered valve tissue constructs.

Materials and Methods: The bioreactor system consisted of two U-shaped tubular conditioning chambers that house the tissue samples in a sterile, physiologically relevant pulsatile flow environment. Components of the system include a pulsatile pump, two conditioning chambers, two conical adaptors that connect the chambers to the pump inflow and outflow, a flowmeter, and three pressure transducers. Each chamber was built with commercially available CNC equipment. The surface of the chamber also includes a viewing port for sample imaging. The pump, flowmeter, and pressure transducers were components of a pulse duplicator system from Vivitro Labs, Victoria, BC, Canada. The pump was controlled by Vivitest software and fluid pressures were measured at three specific sites: at pump exit (P1), in the bioreactor chamber (P2), and at the chamber exit (P3). The software's S35 waveform at 70 beats per minute and a stroke volume of 40mL was used. The flowmeter was also positioned at the pump exit to obtain upstream volumetric flow rate. Measurements were captured for ten cycles, and the averages were used for data analysis. The volumetric flow rate inside the bioreactor chamber was subsequently calculated using the Bernoulli's equation and basic conservation of mass principles.

Results and Discussion: Our findings revealed a consistent flow profile shape within the bioreactor chamber as compared to the upstream waveform at the pump exit (Figure 1). Flow dissipation through tubing connections between the pump and the bioreactor led to a roughly 22% reduction in flow rate magnitudes within the bioreactor, which however could be easily scaled-up by augmenting the magnitude of the upstream (Q1) profile. Previous valve tissue engineering studies have used bioreactor systems to run steady flow experiments in combination with cyclic flexure [3]. However, native circulatory flow conditions are highly pulsatile with specific waveforms. Moreover, our recent findings suggest that fluid oscillations induced from pulsatile flow are important in enhancing valvular matrix generation and gene expression [3]. Our current updates to the bioreactor system will permit physiological components of pulsatile flow-induced oscillations to be used for conditioning developing, 3-dimensional engineered valve tissue constructs.

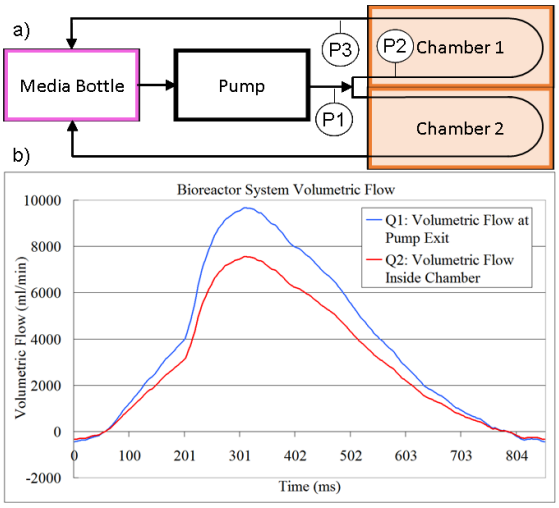

Figure 1: a) Schematic of system's flow loop. b) Q1 and Q2 waveforms showed close correlation of flow profile shape between output at the pump exit and inside the bioreactor chamber. Q1 could be scaled-up by ~22% to induce its current magnitude within the bioreactor chamber.

Conclusions: We have confirmed that the newly designed bioreactor system, with a simple scale-up of the upstream flow profile magnitudes, is capable of subjecting samples to pulsatile flow-induced oscillations. The integrity of the waveform shape is concomitantly maintained, thereby allowing the user to customize a flow profile at the user-interface for delivery to engineered valve tissue specimens within the chamber of a flow conditioning device.

References:

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