Pulsatile Bioreactor for Conditioning Tissue Engineered Heart Valves

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Statement of Purpose: Valvular heart disease results in approximately 275,000 valve replacement procedures performed annually worldwide.[1] Structural deterioration, calcification, tissue overgrowth, and thromboembolism frequently lead to complications that contribute to the failure of native heart valves.

Existing heart valve replacements provide major improvements in cardiac function and life expectancy, but have significant limitations and eventually require surgical replacement within 15-20 years. These risks are particularly prominent in pediatric patients, who naturally outgrow their mechanical implants and where tissue derived non-living valves degenerate and calcify rapidly. Thus, tissue engineered implants capable of self-repair and growth in parallel with the developing child would be highly desired.

Tissue engineered heart valves (TEHVs) developed from decellularized and chemically stabilized porcine aortic heart valves seeded with adipose tissue-derived stem cells (ATSCs) may have the capability of self-remodeling and growing. ATSCs show potential for multiple differentiation in vitro and in vivo[2], and with the proper mechanical and environmental signals, may be directed to differentiate into a phenotype that resembles and functions as a valve interstitial cell.

To address this problem, we have developed a conditioning system that will provide physiological pressure and flow profiles to developing aortic valvular constructs, as well as the biological factors needed for cell proliferation and differentiation.

Methods: Our valve conditioning system (Figure 1a), contained in a cell culture incubator, is driven by an external respirator. Inside the incubator, there is a three-chambered bioreactor (2), that holds the heart valve (white), a pressurized compliance tank (3), a reservoir tank (4), one-way valves (5), pressure retaining valves(6), and pressure transducers.

The bioreactor (2) is six inches in diameter, made of acrylic plastic, and is completely transparent. The three chambers of the bioreactor are held together by stainless steel screws. A special removable holder is able to adapt to valves ranging in sizes from 20 to 30 mm. This bioreactor will produce pulsatile flows ranging from 50 to 2000 mL/min and systemic pressures from 10 to 240 mmHg. Other features of the sterile bioreactor include an unobstructed observation area with a wireless camera, a modular design allowing easy replacement of cell culture media, and multiple ports for media sampling or measurement.

Porcine aortic heart valves were decellularized with detergents and enzymes, and lightly cross-linked with penta-galloyl glucose[3], for controlled biodegradation. ATSC were isolated from rat inguinal fat and seeded on the valve scaffold.

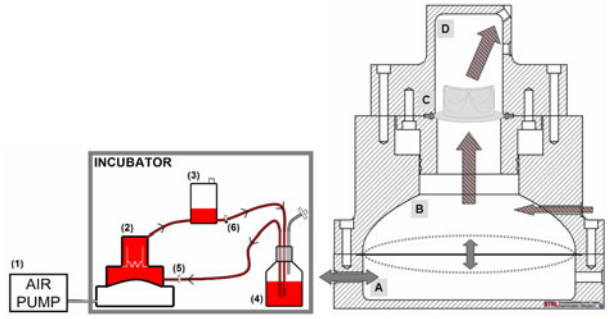

Figure 1a (left): *In vitro* heart valve conditioning system Figure 1b (right): Bioreactor design; Arrows - direction of flow

Results: The bioreactor (Figure 1b) functions as follows: The air chamber (A) is connected to the external pump and is the only chamber not filled with culture medium. It is separated from the pumping chamber (B) by a clear silicone rubber membrane. Through the power of the external air pump, this membrane will bulge into the pumping chamber and push the residing media through the heart valve (C) into the aortic chamber (D). Once completed, the pump will release pressure in the air chamber, allowing the membrane to fall down and draw culture medium in through the one way valves from the reservoir tank to fill the pumping chamber in preparation for the next cycle. Once through the valve, the medium will enter the aortic chamber and shortly thereafter flow into the compliance chamber for circulation.

The valve will be mounted onto a holder which will be then be mounted to the inferior side of the aortic chamber with stainless steel screws. With the addition of a sealing cover, the aortic chamber will have the additional benefit of creating a sterile chamber in which the aortic valve could be transported. TEHV scaffolds seeded with ATSCs and mounted into the bioreactor for 4 weeks would be analyzed for cell differentiation markers and tissue remodeling.

Conclusions: The conditioning system represents a dynamic three-dimensional cell culture setting designed to provide long-term optimal physiological conditions for TEHV development.

References:

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