FUNCTIONAL TISSUE ENGINEERING OF THE AORTIC HEART VALVE

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INTRODUCTION

Aortic heart valve dysfunction is commonly treated with replacement of the valve. To overcome the shortcomings of currently used mechanical valves, xenografts and homografts, efforts are being made to create fully autologous heart valve replacements using the concepts of tissue engineering. To this end autologous cells are seeded onto pre-shaped biodegradable scaffolds and cultured under conditions that mimic the physiological valve environment. This includes mechanical and/or biochemical conditioning in bioreactors to enhance tissue formation and organization. The scaffold provides initial anchorage and support for the cells, until they have produced and reorganized their own extra cellular matrix (ECM) to form a functional tissue. Ideally, the rate of tissue formation is proportional to the rate of scaffold degradation. Moreover, the engineered tissue should meet and maintain the specific mechanical behavior and load bearing properties of the native aortic heart valve.

To date, tissue engineered heart valves show promising results when used to replace the pulmonary valve in animal studies [1,2]. The mechanical properties of these valves, however, are insufficient to withstand the functional demands on the high-pressure, aortic side of the circulation. To satisfy these demands, and hence to develop a functional, load-bearing aortic heart valve, our studies aim at improving ECM architecture and properties via optimal mechanical conditioning protocols.

The present study concentrates on relationships between mechanical conditioning, tissue formation and ECM remodeling using an in-vitro model system of engineered heart valve material.

MATERIALS AND METHODS

Engineered heart valve tissue

Human saphenous vein cells were harvested and expanded in culture. Subsequently, 4-5 $\times$ 10^6 cells (passage 5-6) were seeded per cm^2 onto 4 $\times$ 1 $\times$ 0.1 cm strips of biodegradable polymer scaffold material in three seeding steps and grown under static conditions for 7 days in a specially designed straining device [Fig. 1].

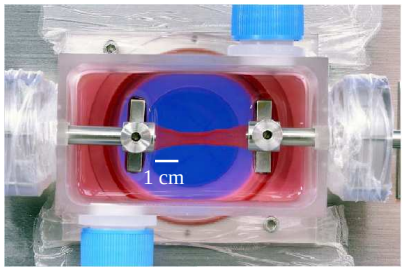

Figure 1. Culture chamber of straining device with engineered tissue construct

Mechanical conditioning

The straining device was used to subject tissue constructs to cyclic, physiologically relevant strains from day 8 to 21 in culture. The device consists of a stainless steel frame that can be mounted to the stage of an inverted confocal laser scanning microscope (CLSM) to monitor tissue formation via a circular cover slip. The frame holds a vented polycarbonate culture chamber with stainless steel clamps for the tissue construct. One of the clamps is connected to a computer-driven linear actuator allowing cyclic axial straining of the construct at predetermined frequencies. The whole set-up can be kept sterile for over 3 weeks and is placed inside an incubator during conditioning experiments.

Five constructs were strained with increasing strains (per 3 days) up to 7-10% maximum strain at a frequency of 1 Hz, whereas four constructs served as unstrained controls.

Evaluation of tissue properties

Qualitatively, tissue constructs were evaluated with histology (HE staining and M. Trichrome staining), SEM, TEM, and CLSM. The latter technique was also used to visualize cellular orientation and

viability with a combination of the viable fluorescent probes Cell Tracker Green and Propidium Iodide (Molecular Probes) [3].

Biochemical measurements of DNA, GAG, and hydroxyproline content after 21 days of culture gave further information about tissue proliferation and ECM deposition. Mechanical properties of the constructs were evaluated using uniaxial tensile testing.

RESULTS

Throughout the culture period constructs showed high viability. In strained constructs cells were observed to orient predominantly parallel to the direction of applied strain, whereas unstrained controls showed more random cell orientations [Fig 2]. It is expected that this cellular alignment will favor collagen deposition and fiber orientation in the direction of applied strains.

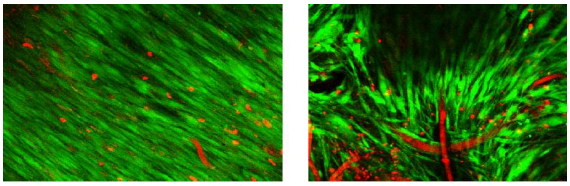

Figure 2. Cell orientations visualized with CLSM of strained (left) and unstrained constructs (right), day 21.

Further qualitative analyses demonstrated more pronounced and organized tissue formation in strained constructs as compared to unstrained constructs [Fig. 3]. Moreover, a smoother and denser surface layer was produced with increasing maximum strain levels [Fig. 4].

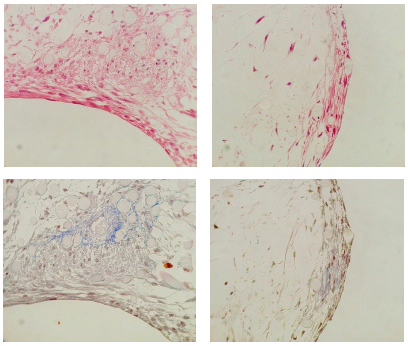

Figure 3. Top: HE (× 40), bottom: M. Trichrome (× 40) of 10% max. strained constructs (left) and unstrained constructs (right), day 21.

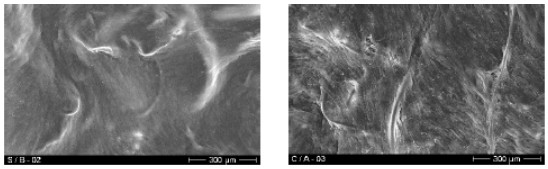

Figure 4. Scanning electron micrograph of surfaces of strained (left) versus unstrained (right) constructs, day 21.

DNA, GAG and collagen content, as well as mechanical properties [Fig. 5] were significantly higher in strained versus unstrained constructs at maximum strain levels exceeding 9%.

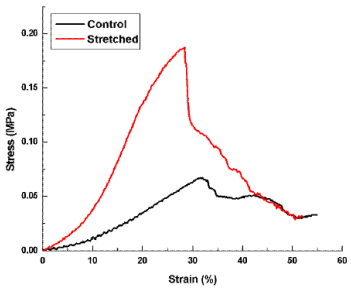

Figure 5. Typical stress-strain relationships of 10% max. strained constructs versus unstrained constructs

CONCLUSION

Mechanical conditioning using cyclic straining is a promising method to improve tissue formation and properties of engineered heart valve material. Contrary to what is currently done when mechanically conditioning tissue engineered heart valves [2] and what has been hypothesized so far [4], we conclude that large strains are beneficial in early tissue development and should be applied at an early stage of the culture protocol to improve the load bearing properties of the tissue. As the load bearing properties are primarily dependent on collagen formation and architecture, which in turn are mainly determined by cyclic straining, the application of fluid shear stresses seems less critical for conditioning heart valve tissue at an early stage. Currently, straining devices for applying optimal straining levels and directions in complex 3D heart valve conduits are being developed.

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