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The independent role of cyclic flexure in the early in vitro development of an engineered heart valve tissue

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Abstract

Tissue engineered heart valves (TEHV) are being investigated as an alternative to current non-viable prosthetic valves and valved conduits. Studies suggest that pulse duplicator bioreactors can stimulate TEHV development. In the current study, a model system was used to determine if cyclic flexure, a major mode of heart valve deformation, has independent effects on TEHV cell and extracellular matrix (ECM) development. Ovine vascular smooth muscle cells (SMC) were seeded for 30 h onto strips of non-woven 50:50 polyglycolic acid (PGA) and poly-L-lactic acid (PLLA) scaffold. After 4 days of incubation, SMC-seeded and unseeded scaffolds were either maintained under static conditions (static group), or subjected to unidirectional cyclic three-point flexure at a physiological frequency and amplitude in a bioreactor (flex group) for 3 weeks. After seeding or incubation, the effective stiffness (E) was measured, with SMC-seeded scaffolds further characterized by DNA, collagen, sulfated glycosaminoglycan (S-GAG), and elastin content, as well as by histology. The seeding period was over 90% efficient, with a significant accumulation of S-GAG, no significant change in E, and no collagen detected. Following 3 weeks of incubation, unseeded scaffolds exhibited no significant change in E in the flex or static groups. In contrast, E of SMC-seeded scaffolds increased 429% in the flex group ($p<0.01$) and 351% in the static group ($p<0.01$), with a trend of increased E, a 63% increase in collagen ($p<0.05$), increased vimentin expression, and a more homogenous transmural cell distribution in the flex versus static group. Moreover, a positive linear relationship ($r^2=0.996$) was found between the mean E and mean collagen concentration. These results show that cyclic flexure can have independent effects on TEHV cell and ECM development, and may be useful in predicting the mechanical properties of TEHV constructed using novel scaffold materials.

Keywords: Tissue engineering; Heart valve; Smooth muscle cell; Bioreactor; Mechanical properties; Collagen

1. Introduction

Prosthetic heart valves are implanted in approximately 275,000 patients each year; however, even the most advanced designs are far from ideal [1]. Mechanical valves require life-long anticoagulation, with concomitant quality of life concerns [2,3], while bioprosthetic heart valves (BHV) incur structural degeneration in the long-term, thus restricting their use to older patients. Tissue engineered heart valves (TEHV) may circumvent these problems, potentially allowing for growth and remodeling, self-repair, and resistance to infection [4]. TEHV are conceptually appealing for use in the repair of congenital or acquired lesions in pediatric patients, as their potential to grow with the patient may mitigate the need for reoperations [5–7].

With notable exceptions [8–10], TEHV are generally constructed by seeding autologous cells onto anatomically shaped, porous scaffolds. While clinically feasible cell sources continue to be investigated [11–14], two distinct paradigms have emerged, distinguished by the choice of scaffold: synthetic versus natural. Natural materials (e.g., decellularized valve tissue) may possess proper anatomical structure, innate bioactivity, and tissue-like mechanical properties [15,16], however their

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inherent variability and potential immunogenicity represent significant hurdles. This is especially a concern with commercially available xenogenic tissues, in which clinical failures have been reported [10].

Synthetic biodegradable polyesters (e.g., polyglycolic acid (PGA), poly-L-lactic acid (PLLA)) represent the most broadly applied class of scaffold materials. They are non-immunogenic, do not elicit a significant chronic inflammatory response, and degrade by hydrolysis into naturally occurring metabolites [17]. From an engineering standpoint, synthetic scaffolds offer the further advantage of design flexibility; material and structural properties can be reproducibly controlled to meet functional requirements [18].

While TEHV based on synthetic scaffolds have successfully functioned in the pulmonary circulation of sheep for at least 20 weeks [19,20], several issues need to be addressed to fully lay the foundation for clinical applications. For example, while explanted TEHV have revealed a tri-layered structure reminiscent of the native semilunar valve [11,20], it is unclear if the layers are functionally equivalent to the fibrosa, spongiosa, and ventricularis layers of the native valve [21,22]. Furthermore, the role of mechanical factors (e.g., pulsatile flow) in the development of a native or TEHV is largely unknown [23,24]. Thus, before TEHV designs for clinical applications can be developed, the fundamental mechanisms underlying TEHV development must be identified and quantified.

Pulse duplicator bioreactors have yielded insight into the factors influencing TEHV development [11,19,20,25,26]. These studies suggest that mechanical factors (e.g., cyclic flexure, fluid shear stress, tension and pressure) may act independently or synergistically to modulate extracellular matrix (ECM) development, cell phenotype, and organ-level morphology. Pulse duplicators, however, can only provide data on the combined effects of such factors, as the hemodynamic parameters are coupled (e.g., pressure-flow). To define the role of individual factors, bioreactors have been designed to investigate the independent effects of laminar flow (shear stress) [27], cyclic tensile strain [28–30], and in our laboratory, cyclic flexure [31].

Native heart valve leaflets withstand nearly 3×10^9 cycles during an average human lifespan. During the opening and closing phases of normal valve function, native and BHV valve leaflets are subjected to substantial amounts of flexural deformation [32,33]. Moreover, TEHV are subjected to cyclic flexure during in vitro conditioning in pulse duplicator bioreactors and in vivo following implantation [34]. While our laboratory has firmly implicated cyclic flexure in the progression of BHV failure [33], its potential role in maintaining native valve homeostasis or promoting TEHV development has yet to be established.

To investigate the independent effects of cyclic flexure on in vitro TEHV development, we designed a bioreactor with the capacity to provide cyclic three-point flexure to 12 rectangular samples of TEHV biomaterial [31]. We previously demonstrated that cyclic flexure can induce both quantitative and qualitative changes in the flexural mechanical properties of non-woven PGA/PLLA scaffolds dip-coated with poly 4-hydroxybutyrate (P4HB) (Tepha, Inc., Cambridge, MA). In the current study, we extended upon our previous work by evaluating the effects of cyclic flexure on cell and ECM development in mechanically stable scaffolds seeded with cells. This novel feature of our experimental study allowed for evaluation of ECM development *independent* of concurrent changes in scaffold mechanical properties.

2. Materials and methods

2.1. Bioreactor design and operation

The bioreactor used in this study has been described previously [31]. In brief, a linear actuator (UltraMotion, Mattitick, NY) controlled by Windows-based software (Si Programmer; Applied Motion Products, Watsonville, CA) is used to provide cyclic three-point flexure to 12 rectangular biomaterial samples (maximum dimensions $\sim 25 \times 7.5 \times 2$ mm). Sterility is maintained within two chambers flanking the actuator, each containing 6 individual polysulfone culture wells (25.4 mm diameter, 16 mm deep). Each sample is loaded into a culture well between 4 stainless steel stationary posts, and is subsequently bracketed from overhead by 2 centrally positioned, stainless steel flexure pins. The flexure pins are linked to the actuator, and thus samples can be subjected to either unidirectional or bi-directional three-point flexure, as depicted schematically in Fig. 1. The bioreactor can be cold gas sterilized by ethylene oxide, and was designed to be operated inside a standard cell culture incubator.

2.2. Scaffolds

Scaffolds were a non-woven 50:50 blend of PGA and PLLA fibers (Albany International Research, Mansfield, MA). The PGA/PLLA scaffolds had an approximate fiber diameter of 0.012–0.015 mm and density of 61.75 mg/ml. Rectangular scaffold samples were cut to size ($25 \times 7.5 \times 1$ mm) and the initial (prior to sterilization) effective stiffness (E) of each sample was measured using a three-point flexure test (see *Effective stiffness measurement*). Scaffolds were then cold gas sterilized with ethylene oxide prior to use.

2.3. Culture medium

The culture medium was Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and L-glutamine