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Heart valve scaffold fabrication: Bioinspired control of macro-scale morphology, mechanics and micro-structure

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ABSTRACT

Valvular heart disease is currently treated with mechanical valves, which benefit from longevity, but are burdened by chronic anticoagulation therapy, or with bioprosthetic valves, which have reduced thromboembolic risk, but limited durability. Tissue engineered heart valves have been proposed to resolve these issues by implanting a scaffold that is replaced by endogenous growth, leaving autologous, functional leaflets that would putatively eliminate the need for anticoagulation and avoid calcification. Despite the diversity in fabrication strategies and encouraging results in large animal models, control over engineered valve structure-function remains at best partial. This study aimed to overcome these limitations by introducing double component deposition (DCD), an electrodeposition technique that employs multi-phase electrodes to dictate valve macro and microstructure and resultant function. Results in this report demonstrate the capacity of the DCD method to simultaneously control scaffold macro-scale morphology, mechanics and microstructure while producing fully assembled stent-less multi-leaflet valves composed of microscopic fibers. DCD engineered valve characterization included: leaflet thickness, biaxial properties, bending properties, and quantitative structural analysis of multi-photon and scanning electron micrographs. Quasi-static *ex-vivo* valve coaptation testing and dynamic organ level functional assessment in a pressure pulse duplicating device demonstrated appropriate acute valve functionality.

1. Introduction

Heart valve disease represents a major cause of morbidity and mortality, with age and sex adjusted prevalence for the US population reported as 2.5% [1], with mitral and aortic regurgitation or stenosis being frequent syndromes. While the incidence of different etiologies varies based on age and geography [2], the major mechanisms initiating valvular maladies remain degenerative, rheumatic or inflammatory processes, ischemic cardiomyopathy and congenital malformations [3]. Globally, the valve replacement and repair market was estimated as $2.8 B with an expected compound annual growth rate of 9.1% [4].

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Two classes of heart valve prostheses respond to these clinical and market needs: mechanical and bioprosthetic. Mechanical valves benefit from longevity, but come with the burden of chronic anticoagulation therapy due to an elevated risk for thrombosis and thromboembolism [5]. Conversely, bioprosthetic valves do not require chronic anticoagulation therapy. However, their long term performance and durability remains plagued by failure modalities that include leaflet tear and calcific degeneration, with the latter risk elevated in younger patients [6]. Tissue engineered heart valves (TEHVs) have been proposed to resolve these issues by implanting a scaffold that is replaced or extensively augmented with endogenous tissue growth, leaving autologous, functional leaflets that would putatively eliminate the need for anticoagulation therapy and avoid tissue calcification. While this approach has never been fully demonstrated *in vivo* [7–9], a wide variety of efforts have been directed to overcome the substantial challenges associated with translating TEHV technology toward clinical practice. Multidisciplinary strategies have been explored in terms of cell source [10–12], engineered construct conditioning regimens [13,14], and transcatheter deployment strategies [15,16]. Processing technologies developed include non-woven meshes molded by heat welding [17], salt leaching [11], stereolithography [18], polymer dipping [19,20], fiber-reinforced composite molding [21], electrospinning [22], jet spraying [23] and acid etching of Nitinol sheets [24,25]. In developments based on natural materials, of promise are decellularized tissue sheets [26] and whole organ decellularization protocols for allografts [27,28] or xenografts [29,30] as well as collagen cross-linking strategies for pericardial tissue [31] and *in vivo* [32] or in vitro [14,33] conditioning that facilitates the structured growth of neo-tissue.

Despite the diversity in fabrication strategies reported and some of the encouraging results in large animal models, control over engineered construct structure-function remains at best partial. More specifically, the current technologies do not allow for the combined engineering of four major design factors: I) macroscopic morphology and size (e.g. aortic, mitral, tricuspid, pulmonary indications), II) in-plane mechanics (e.g. biaxial response), III) out of plane mechanics (e.g. bending rigidity), and IV) microstructure (e.g. fiber diameter, pore size). This study aims to overcome these limitations by introducing an electrodeposition technique that employs multi-phase electrodes to control valve macro and microstructure and resultant function.

The double component deposition (DCD) technique reported here utilizes electrodes composed of electrically conducting/insulating materials to selectively orient fiber deposition on anatomy inspired valve geometries (Figs. 1–3). The first component, made of electrically conducting metallic alloy, acts as an electrospun polymer collection surface, while the second component, made of materials with lower conductivity, reduces fiber deposition in regions which would otherwise receive excessive mass accumulation during the fabrication process. Results in this report demonstrate the capacity of the DCD method to simultaneously control scaffold macro-scale morphology, mechanics, and microstructure while producing stent-less multi-leaflet valves composed of microscopic fibers. DCD engineered valve characterization included: leaflet thickness, biaxial properties, bending properties, multi-photon and scanning electron microscopy quantitative structural analysis, quasi-static *ex-vivo* valve coaptation testing and dynamic organ level functional assessment in a pressure pulse duplicator apparatus.

2. Methods

2.1. Mandrel design for double component fibers deposition (DCD)

The DCD mandrel model was developed in Solidworks (Waltham, MA, USA) and consisted of two components. The heart valve shaped conductive component was obtained by machining of aluminum alloy whereas the non-conductive component was made of acrylonitrile butadiene styrene (ABS) processed by injection molding (Fig. 1a,g). The heart valve shaped collecting surface was connected with a high voltage generator, and the "shield" component was mechanically connected to the motor providing the rotation. A second motor controlled the translational rastering speed [34,35]. The conductive component diameter was selected to be 40 mm based on echocardiography data by Ring et al. [36] of the anterior-posterior diameter D and septal-lateral diameter of human tricuspid valves throughout the cardiac cycle. The height h was calculated based on the diameter utilizing a h/D ratio of 1.6 utilized in the Sapien 3 prosthesis (Carpentier-Edwards, Irvine, CA). Leaflet profile was modeled as described with detail in Ref. [37], a design method that minimizes the central opening of the valve, targeting a central open area <1% of the orifice area (Figs. 1a,g and 2).

2.2. Double component fiber deposition (DCD) processing conditions

Poly(ester urethane) urea (PEUU) was synthesized as described in Ref. [38], and biodegradable valves were fabricated with electrospinning [39] using the developed mandrel for DCD and the following process variables: polymer voltage 11 kV, mandrel voltage −5 kV, polymer gap 15.5 cm, polymer flow rate 1.5 mL/h, polymer 12% w/v in HFIP, mandrel tangential velocities 0.3–3 m/s, mandrel rastering velocities 0–2.5 cm/s (Figs. 1–7, and 10 and Supplemental video 1–3). DCD capacity to tune microstructure and duplicate native leaflet fiber bundles diameter or pore size (Fig. 8f and g) was assessed by comparing leaflets fabricated under different conditions (polymer - mandrel voltage difference: 4–32 kV, gap: 5.5–7.5 cm, polymer/solvent concentration: 4–12%) with decellularized porcine valve leaflet tissue (Fig. 9).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.biomaterials.2017.10.011.

2.3. Engineered leaflet thickness measurements

In order to assess the capacity of DCD to reproduce native valve leaflet thickness for the four valve types, the relationship between leaflet thickness and processing time was characterized. Native and electrospun leaflets ($n \geq 3$/group) were dissected from the whole valve, and thickness measurements were taken with a dial indicator gage (Starrett, Athol, MA) on five locations spanning from the free edge to the belly and the commissural regions. The experimental error due to the valve leaflet wet content was minimized by performing each measurement 5 min after the leaflet was positioned on the gage [40,41]. The mean thickness values of the electrospun valves were plotted against the polymer deposition time for four independent fabrication times and then compared with the native tricuspid porcine valve. Based on the linear interpolation of these experimental points, the deposition time required to generate leaflets with native tricuspid porcine valve thickness was identified and tricuspid engineered valves with tailored leaflets thickness were fabricated (Fig. 1f). Engineered valve leaflets fabricated with these conditions were further tested ($n=4$) to assess the capacity of the design paradigm to be used in controlling the average leaflet thickness. Last, in order to further characterize the quality of the DCD in terms of surface homogeneity, maps for engineered and native valves were obtained by biquintic numerical interpolation (Matlab, MathWorks, Natick, MA) of thicknesses experimentally measured at fifteen different leaflet locations described above and on equally distributed locations around the leaflets edges (Figs. 1h–i and 3).