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Biological and mechanical evaluation of a Bio-Hybrid scaffold for autologous valve tissue engineering

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ABSTRACT

Major challenge in heart valve tissue engineering for paediatric patients is the development of an autologous valve with regenerative capacity. Hybrid tissue engineering approach is recently gaining popularity to design scaffolds with desired biological and mechanical properties that can remodel post implantation. In this study, we fabricated aligned nanofibrous Bio-Hybrid scaffold made of decellularized bovine pericardium: polycaprolactone-chitosan with optimized polymer thickness to yield the desired biological and mechanical properties. $CD44^+$, αSMA^+, $Vimentin^+$ and $CD105^-$ human valve interstitial cells were isolated and seeded on these Bio-Hybrid scaffolds. Subsequent biological evaluation revealed interstitial cell proliferation with dense extra cellular matrix deposition that indicated the viability for growth and proliferation of seeded cells on the scaffolds. Uniaxial mechanical tests along axial direction showed that the Bio-Hybrid scaffolds has at least 20 times the strength of the native valves and its stiffness is nearly 3 times more than that of native valves. Biaxial and uniaxial mechanical studies on valve interstitial cells cultured Bio-Hybrid scaffolds revealed that the response along the axial and circumferential direction was different, similar to native valves. Overall, our findings suggest that Bio-Hybrid scaffold is a promising material for future development of regenerative heart valve constructs in children.

1. Introduction

Heart valve pathologies such as stenosis and/or regurgitation are prevalent worldwide due to various cardiovascular risk factors, genetic predisposition and rheumatic fever [1–2]. In patients with end stage valve disease, native valve replacement with either mechanical or bioprosthetic valve still remains the ultimate treatment option [3–4]. Although commercially available heart valves (HVs) are suitable for replacement, they are not ideal for paediatric patients in view of their inability to grow and regenerate [4–5]. Hence, there is a need for better design and fabrication of a heart valve scaffold that is mechanically robust and has the ability to remodel [6–10].

Tissue engineering (TE) has been a promising approach for fabrication of a living tissue-engineered heart valve (TEHV) substitute with ideal properties such as adequate mechanical strength, pliability, elasticity, durability, unidirectional and central flow with minimal turbulence, low mass inertia with minimal resistance, non-thrombogenicity and minimal immunological activity [10–12]. Various strategies for TEHV have been reported so far and detailed review articles [13–21] summarize the approaches to mimic the native valve structure that include: decellularized tissue matrices with or without cells, biodegradable synthetic polymers, biological protein based polymers, in vivo TEHV, hybrid tissue engineering (HTE) strategies combining different approaches and 3D printing of the valve. Each approach has specific advantages and drawbacks, but the main limitations that remain are calcification, degradation of the scaffold versus remodelling rate, contraction of the construct and mechanical behaviour of the prosthesis several months after implantation [22–24].

HTE has been recently explored as a simple yet versatile method in fabricating polymer nanofibers (NFs) coated biological scaffolds via electrospinning that mimic the trilayered architecture and mechanical properties of the native HV [25–29]. HTE based biological scaffolds are promising in overcoming some of the limitations of currently available TEHV. HTE scaffolds demonstrate an overall increase in uniaxial strength and stiffness owing to the addition of polymer to tissue [30–31]. A HTE scaffold that combined decellularized bovine pericardium (DBP) and polycaprolactone-chitosan (PCL-CH) nanofibers was

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reported to have adequate uniaxial mechanical strength [32]. PCL NFs contributed to adequate uniaxial mechanical strength required for valve TE and its unique chemistry with CH helped in adhesion of the polymer blend to the tissue. The fabricated Bio-Hybrid scaffold supported fibroblast and endothelial cell proliferation with potential remodelling capacity [32]. Uniaxial testing to characterize HTE scaffolds like Bio-Hybrid may be inadequate because the actual state of stress in the valve is at least biaxial and hence the experimental loading is not physiological [33]. Further, to develop constitutive relations systematically to evaluate and develop TEHV [34–35], one needs biaxial data, which are not adequately available [30–31]. Moreover, these HTE tissue based scaffolds like Bio-Hybrid scaffold have not been tested with valve specific cells in simulated physiological conditions that contribute to the overall mechanical response of the valve tissue, which can be directly correlated to heart valve function and regenerative ability [9,26].

In order to further improve the Bio-Hybrid scaffold and make it more suitable for TEHV, it was hypothesized that polymeric NFs when aligned along the collagen fibrils present in bovine pericardium (BP) would primarily enhance the mechanical properties of the scaffold [36–38]. Conducting biaxial tests on such HTE scaffolds would facilitate the development of the constitutive relation for the TEHV. This constitutive relation would then aid in deciding the orientation of the scaffold in the TEHV construct, so that its performance could be made more similar to the native heart valve. Further, it is also envisaged that the TEHV scaffold should have optimum biological properties to support human heart valve cell adhesion and proliferation that will facilitate in vivo remodelling upon implantation in future.

Therefore, the objectives for this study are: (1) to modify our previously reported Bio-Hybrid scaffold and improve it with aligned NFs along axial direction of BP to enhance mechanical properties suitable for TEHV [32]; (2) carryout a detailed investigation of the biaxial mechanical properties of modified Bio-Hybrid scaffold in order to decide the orientation of scaffold and further develop a constitutive relation to design TEHV; 3) isolate and culture hVICs to induce functionality and cellular alignment along the polymeric NFs for fabrication of a suitable HV construct [39–40]; and (4) study the Bio-Hybrid scaffold's suitability for autologous TEHV using heart valve interstitial cells (hVICs).

2. Materials and methods

The studies involving human HV cells were performed in accordance with the institutional guidelines approved by the Institute Ethics Committees of Indian Institute of Technology Madras, Chennai (ISCREC/IITM/003/2013) and Institute of Cardiovascular Diseases, Madras Medical Mission, Chennai. For this study, institution review board approval was taken and followed the principles outlined in the Declaration of Helsinki for all human and animal experimental investigations performed. In addition, for investigations involving human subjects i.e. for collection of regurgitant valves from patients whose native valves were being replaced with artificial valves, informed consent was obtained from the participants involved.

2.1. Bovine pericardial sample preparation and orientation

Pericardia were collected from a local slaughter house (Perambur slaughterhouse, Chennai, TN, India) and immediately transported to the laboratory in ice-cold PBS (Phosphate Buffer Saline, pH 7.4) without Ca^{2+} and Mg^{2+} ($PBS^{-/-}$). Adherent fat was removed from the pericardium over the ventral aspect of the heart. The aortic root (base) and the apex sides were marked with suture threads. A 10 cm^2 of pericardium from base to apex was excised along with the sutures that helped to identify the anatomical landmarks for orienting the primary axes of the samples (as depicted in Fig. 1). Samples for testing were cut from the left anterior section of the pericardium, which is most homogenous [41]. The pericardium for testing was cut along the desired orientation and was labelled as axial (base to apex) or circumferential (perpendicular to axial).

2.2. Fabrication of non-cross-linked Bio-Hybrid (DBP-PCL-CH) scaffold

The BP was decellularized using 1% deoxycholic acid and Bio-Hybrid scaffold was fabricated using decellularized BP as previously reported [32]. Briefly, 10% PCL (440,744, Sigma-Aldrich, USA, MW 70,000–90,000) was blended with 1% CH (417,963, Sigma-Aldrich, USA, MW > 1,00,000) in common solvent mixture of Trifluoroacetic acid (TFA; 2,029,282, SISCO Research Laboratories Pvt Ltd. India) and Dichloromethane (DCM; 0422123, SISCO Research Laboratories Pvt Ltd. India) in the ratio of 80:20. DBP was placed on hydrated 2% lyophilized agarose gel and PCL-CH blend was electrospun at room temperature (27–32 °C) and a voltage of 15 kV using a customized electrospinning device (Holmarc Opto-Mechatronics Pvt Ltd., India; Model: HO-NFES-040). Flow rate was set to 0.5 mL/h using a 22G blunt stainless steel needle at a distance of 15 cm between the needle-tip and collector. The polymer coated DBP samples were then neutralized in 0.5 M NaOH for 10 min to fully regenerate the free amine form of CH so that it can interact with DBP and avoid any CH dissolution. We modified the Bio-Hybrid scaffold and aligned the PCL-CH nanofibers along the axial direction of the BP in order to enhance the mechanical properties. To fabricate aligned scaffolds, a customized XY stage was used as a collector, while for the random fibers a stationary aluminium plate was used. The speed of the XY plate while electrospinning polymer fibers aligned in the direction of the collagen fibers was set to 100 cm/min. This speed of the continuous movement of the XY plate in the X direction was found to result in uniform deposition of polymeric NFs in the direction parallel to the pericardial fibers without interruption throughout the electrospinning cycle. In order to define the optimum polymer thickness for TEHV, the electrospinning was carried out for 1, 2 and 3 h durations. Random and aligned scaffolds were labelled as Bio-Hybrid (R) and Bio-Hybrid (A) respectively. DBP-PCL-CH Bio-Hybrid scaffold with varying polymer coating thickness were prepared and washed thoroughly with distilled water until neutral physiological pH. They were stored in 70% ethanol prior to further characterization and biological experiments.

2.3. Surface characterization of Bio-Hybrid scaffold

The surface topology of DBP, PCL-CH fibers and Bio-Hybrid scaffolds (R & A) were characterized using scanning electron microscopy (SEM). Samples ($n = 3$) were sputter-coated in vacuum with an electrically conductive 5 nm thick layer of Gold/Palladium alloy using a Precision Etching Coating system (Gatan, PA, USA;Model 682). The high resolution SEM (FEI, Hillsboro, OR, USA; Quanta 400 FEG) images were used to analyse and measure the fiber and pore diameter of the scaffolds using Image J software (ImageJ: open source image processing program; imagej.nih.gov/ij). The biological samples (DBP and Bio-Hybrid) before and after cell seeding were rinsed with 0.1 M Phosphate Buffer (0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4), pH − 7.2 and fixed with 2.5% glutaraldehyde overnight. They were then serially dehydrated from 30% to 100% ethanol and air dried for imaging.

2.4. Mechanical characterization of Bio-Hybrid scaffold

2.4.1. Determination of uniaxial tensile strength and Young's modulus

The uniaxial mechanical properties of the DBP, Bio-Hybrid (R) and Bio-Hybrid (A) scaffolds after 1, 2 and 3 h of polymer coating were evaluated by tensile testing technique (BISS-Nano Plug and Play, Universal Testing Machine). Longitudinal strips of the samples for testing were cut in the axial direction while viewing the orientation of pericardial fibers under an illuminator. Twenty samples each of DBP, Bio-Hybrid (R) and Bio-Hybrid (A) of size 10 mm × 20 mm were tested. All the samples were soaked in Dulbecco's Phosphate Buffered Saline (DPBS), pH 7.4