Materials Science and Engineering C 51 (2015) 263–273

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Engineering of a polymer layered bio-hybrid heart valve scaffold

S. Jahnavi [a,b,1], T.V. Kumary [b,2], G.S. Bhuvaneshwar [c], T.S. Natarajan [d,3], R.S. Verma [a,*]

[a] Stem Cell and Molecular Biology Laboratory, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, TN, India
[b] Tissue Culture Laboratory, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Trivandrum, Kerala 695012, India
[c] Trivitron Innovation Centre, Department of Engineering Design, Indian Institute of Technology Madras, Chennai 600036, TN, India
[d] Conducting Polymer laboratory, Department of Physics, Indian Institute of Technology, Madras, Chennai 600036, TN, India

ARTICLE INFO

Article history:
Received 13 November 2014
Received in revised form 13 January 2015
Accepted 9 March 2015
Available online 11 March 2015

Keywords:
Heart valve
Tissue engineering of heart valve (TEHV)
Bio-Hybrid
Decellularized bovine pericardium extracellular matrix (DBPECM) and Polycaprolactone-Chitosan (PCL-CH)

ABSTRACT

Current treatment strategy for end stage valve disease involves either valvular repair or replacement with homograft/mechanical/bioprosthetic valves. In cases of recurrent stenosis/ regurgitation, valve replacement is preferred choice of treatment over valvular repair. Currently available mechanical valves primarily provide durability whereas bioprosthetic valves have superior tissue compatibility but both lack remodelling and regenerative properties making their utility limited in paediatric patients. With advances in tissue engineering, attempts have been made to fabricate valves with regenerative potential using various polymers, decellularized tissues and hybrid scaffolds. To engineer an ideal heart valve, decellularized bovine pericardium extracellular matrix (DBPECM) is an attractive biocompatible scaffold but has weak mechanical properties and rapid degradation. However, DBPECM can be modified with synthetic polymers to enhance its mechanical properties. In this study, we developed a Bio-Hybrid scaffold with non-cross linked DBPECM in its native structure coated with a layer of Polycaprolactone-Chitosan (PCL-CH) nanofibers that displayed superior mechanical properties. Surface and functional studies demonstrated integration of PCL-CH to the DBPECM with enhanced bio and hemocompatibility. This engineered Bio-Hybrid scaffold exhibited most of the physical, biochemical and functional properties of the native valve that makes it an ideal scaffold for fabrication of cardiac valve with regenerative potential.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Congenital heart valve disease is one of the most common abnormalities and currently available mechanical or tissue valve substitutes do not have the ability to grow, repair or remodel [1–5]. Though, mechanical valves display high mechanical strength and durability, they have drawbacks such as haemolysis, thromboembolism, and sub-optimal flow dynamics and require lifelong anticoagulation therapy. Bioprosthetic valves (BVs) exhibit better hemodynamics and hemocompatibility but often fail due to weak mechanical properties, structural deterioration and calcification [6]. The mechanical properties of these BVs are currently improved using gluteraldehyde (GA) cross linking [7]. However, the cross linking treatment increases the oxidative stress levels, which promotes susceptibility to collagenase and ultimately leads to valve failure [8,9]. Despite these drawbacks, GA is still considered the gold standard since it provides resistance from enzymatic degradation and durability to the tissues [10,11]. Considering all the drawbacks, efforts are being made to improve the BVs without cross linking to attain adequate mechanical properties with growth and regenerative potential [12].

Recently, tissue engineering (TE) has emerged as a promising area to develop BVs suitable for replacement in paediatrics [13]. In the last two decades, TE scaffolds intended to replace BVs include polymeric (both synthetic and natural), decellularized tissues with or without cross linking and combination of polymers and natural extracellular matrix (ECM) [14–16]. Different human cell sources such as blood vessels, bone marrow, umbilical cord tissue and blood, and chorionic villi have been tested for their suitability for ECM production in these TE valve scaffolds [17]. The recent approach of "Hybrid Tissue Engineering (HTE)"combines synthetic polymers with natural ECM that maintains a balance between the rate of scaffold degradation and the generation of new ECM to match the features of the native valvular tissue [16,18]. Considering the advantages of HTE, we intended to improve the biomechanical properties of bovine pericardium (BP) which is cheap, durable, easily available off the shelf and currently used as a template for fabrication of commercially available BVs [19]. DBPECM is less immunogenic when compared to BP and retains the bio-matrix components such as

* Corresponding author at: 201, Stem Cell and Molecular Biology Laboratory, Bhupat and Jyoti Mehta School of Life Sciences, Department of Biotechnology, Indian Institute of Technology, Madras, Chennai, TN 600036.
E-mail addresses: jani84@gmail.com (S. Jahnavi), tvkumary@yahoo.com (T.V. Kumary), gs.bhuvnesh@gmail.com (G.S. Bhuvaneshwar), tsniit@gmail.com (T.S. Natarajan), vermars@iitm.ac.in (R.S. Verma).
[1] Stem Cell and Molecular Biology Laboratory, Bhupat and Jyoti Mehta School of Life Sciences, Department of Biotechnology, Indian Institute of Technology, Madras, Chennai, TN 600036.
[2] Tissue Culture Laboratory, Biomedical Technology Wing, Sree Chitra Tirunal Institute of Medical Sciences and Technology, Poojappura, Thiruvananthapuram, KL, India 695012.
[3] Conducting Polymer Laboratory, Department of Physics, Indian Institute of Technology, Madras, Chennai, TN 600036.

http://dx.doi.org/10.1016/j.msec.2015.03.009
0928-4931/© 2015 Elsevier B.V. All rights reserved.

collagen, elastin and GAG but has weak mechanical properties which makes it unsuitable for tissue engineering of heart valve (TEHV) [20, 21]. In the past few years, modification of DBPECM with synthetic polymers has been demonstrated to improve its non thrombogenicity, mechanical strength, cell adhesion and proliferation [22]. Polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polyhydroxyalkanoate (PHA), and PCL have been the main materials of choice as they have suitable properties for TEHV [23]. Earlier studies reported that PGA resulted in high degradation rate while PHA showed increased risk of premature mechanical failure [24]. Other commonly used polymers for TEHV such as poly (ester urethane) urea (PEUU), poly-hydroxybutyrate (PHB) and its co polymers combined with natural ECM corroborated better biomechanical properties [16,25,26]. These studies displayed superior mechanical properties but did not provide evidence for retention of tissue in its native hydrated form. In this study, we report the efforts to fabricate a Bio-Hybrid scaffold using DBPECM with an adhesive layer of PCL-CH, to provide superior biomechanical properties in its native hydrated structure that mimicked the native valve.

2. Materials and Methods

The studies involving human umbilical cord tissue and vein derived endothelial cells were performed in accordance with the institutional guidelines approved by the Institute Ethics Committees at Indian Institute of Technology Madras, Chennai (ISCREC/IITM/003/2013) and Sree Chitra Tirunal Institute of Medical Sciences, Trivandrum, India. For this study, institution review board approval was taken and/ or has followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations performed. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

2.1. Decellularization of bovine pericardium

The BP was obtained from local slaughter house and transported to the laboratory in ice-cold PBS (Phosphate Buffer Saline, pH 7.4) without Ca^{2+} and Mg^{2+} ($PBS^{-/-}$). The tissue was immediately cleaned by excising adherent fat and myocardial tissue. The remaining clear BP was stored at 4 °C. The BP was decellularized using 1% DCA (D2510-100G; Sigma-Aldrich, MO, USA) for 24 h with continuous stirring at 37 °C in $PBS^{-/-}$ followed by DNAase (D4527; Sigma-Aldrich, MO, USA) and RNAase (R6513; Sigma-Aldrich, MO, USA). The DBPECM was thoroughly washed in PBS, 60% ethanol to remove any DCA remnants and finally stored in 70% ethanol at room temperature. The DBPECM samples were processed for histological analysis and paraffin embedded sections were taken at 5 μm using microtome (Leica Wetzlar, Germany, model: RM2245) onto polylysine coated glass slides. The DBPECM was confirmed for acellularity by i) routine hematoxyline and eosin (H&E) staining, ii) nuclear specific 4′,6-diamidino-2-phenylindole (DAPI) staining, and iii) DNA estimation by an optimized protocol followed by agarose gel electrophoresis. Briefly, 100 mg BP was homogenized in lysis buffer (10 mM Tris HCl;pH 8.0, 0.1 M EDTA and 0.5% SDS) and Proteinase K (20 μg/ml, P2308; Sigma-Aldrich, MO, USA). The homogenate was then incubated in a water bath at 50 °C overnight till it became a clear viscous solution. Equal volume of Tris saturated phenol:chloroform:isoamyl alcohol (24:24:1) was added to the homogenate and centrifuged at 12,000 rpm at 4 °C for 10 min. The upper aqueous phase was carefully transferred to a fresh tube and finally precipitated in ice cold absolute ethanol at −20 °C overnight. The amount of DNA was measured spectrophotometrically. This was followed by demonstration of extracted DNA by 1% agarose gel electrophoresis carried out in 1X TAE buffer at 110 V for 45 min. The tissue sections were also stained for Masson's trichrome used for evaluating the preservation of collagen, Verhoeff's Van Gieson for elastin and Alcian blue for GAG. Thermal stability of the scaffolds ($4 \times 4\ mm^2$) was demonstrated by DSC in a NETZSCH DSC 204 using Pan Ag crucibles with about 3.380 mg of samples, under dynamic nitrogen atmosphere (50 and 30 mL.min-1) and heating rate of 10 K.min-1 in temperature range from −25.0 to 500.0 (K/min).

2.2. Fabrication of PCL-CH polymeric nanofibers

PCL (440744, Sigma-Aldrich, MO, USA) solutions (4, 6, 8 and 10%) and CH (417963, Sigma-Aldrich, MO, USA) solutions (1, 2, 4 and 6%) were prepared in common solvent mixture of Trifluroacetic acid (TFA; 2029282, SISCO Research Laboratories Pvt Ltd. India) and Dichloromethane (DCM; 0422123, SISCO Research Laboratories Pvt Ltd. India) in the ratio of 80:20. PCL-CH solutions were prepared in different combinations varying from 4 to 10% and 1 to 6% respectively. All the polymers were mixed well for 3 h using magnetic stirrer. All the concentrations of PCL-CH were electrospun at room temperature using a customized device (Holmarc Opto-Mechatronics Pvt Ltd, India; Model: HO-NFES-040) using a 5 ml syringe and 22 G nozzle.

2.3. Fabrication of Bio-Hybrid scaffold

DBPECM was placed on hydrated 2% lyophilized agarose gel to maintain the tissue in its native 3D structure. This was placed on stationary aluminium target and the PCL-CH blend was electrospun for 2 h at a voltage of 15 kV. The polymer coated pericardial samples were then neutralized in 0.5 M NaOH solution for 10 min to fully regenerate the free amine form of CH so that it can interact with DBPECM and avoid any CH dissolution. The Bio-Hybrid scaffolds were washed with deionized water till neutral physiological pH and then preserved in 70% ethanol for further use.

2.4. Surface characterization

The surface morphology of electrospun scaffolds (PCL, CH, PCL-CH) were observed by SEM. The samples were air dried and 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). Images were then recorded with a High Resolution SEM (FEI, Hillsboro, OR, USA; Quanta 400 FEG). The BP, DBPECM and Bio-Hybrid scaffolds were also analysed using SEM for confirming decellularization, deposition of PCL-CH nanofibers and cell attachment onto these scaffolds. Tissue samples (BP, DBPECM and Bio-Hybrid) before and after cell seeding were rinsed with 0.1 M, pH-7.2 Phosphate Buffer (0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4) and fixed with 2.5% GA overnight. They were then serially dehydrated up to 100% ethanol and air dried for imaging.

2.5. Functional characterization

Surface chemistry and functional groups of PCL, CH, PCL-CH, DBPECM and Bio-Hybrid samples were assessed using Attenuated Total Reflectance spectra (ATR-FTIR; Perkin-Elmer Spectrum One, MA,USA). Spectra were taken in the wavelength region 400 cm^{-1} to 2000 cm^{-1}.

2.6. Assessment of hydrophilicity

Contact angle was measured using goniometer (Easy DROP, KRUSS, Hamburg, Germany) for BP, DBPECM and Bio-Hybrid samples to assess hydrophilicity. One drop of water was placed on the sample and the contact angle was measured using the video setup and software provided with the instrument.

Download English Version:

https://daneshyari.com/en/article/7869693

Download Persian Version:

https://daneshyari.com/article/7869693

Daneshyari.com