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Construction of scaffolds composed of acellular cardiac extracellular matrix for myocardial tissue engineering

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ABSTRACT

High rates of mortality and morbidity stemming from cardiovascular diseases unveil extreme limitations in current therapies despite enormous advances in medical and pharmaceutical sciences. Following myocardial infarction (MI), parts of myocardium undergo irreversible remodeling and is substituted by a scar tissue which eventually leads to heart failure (HF). To address this issue, cardiac patches have been utilized to initiate myocardial regeneration. In this study, a porous cardiac patch is fabricated using a mixture of decellularized myocardium extracellular matrix (ECM) and chitosan (CS). Results of rheological measurements, SEM, biodegradation test, and MTT assay showed that the scaffold composed of 3.5% (w/w) CS and 0.5% ECM has the best potential in providing cardiac progenitor cells (CPCs) with a suitable microenvironmental condition for both attachment and growth. This study demonstrates that the fabricated scaffold is capable of transmitting both mechanical and chemical cues that is native to myocardial tissue and supports efficient growth of the CPCs.

1. Introduction

Being responsible for almost half a million deaths per year, cardiovascular diseases are the leading cause of human mortality in the United States [1]. Myocardial infarction, a condition happening following a heart attack, results in substantial loss of myocardium due to prolonged lack of oxygen supply to the tissue. Irreversibility of myocardial degeneration [2], shortage of donors for heart transplantation, and limited success of current therapies have led to development of several tissue engineering approaches for fabrication of novel biomaterials to be used for induction of myocardium regeneration [3]. Thus far, cardiac tissue engineers have fabricated various types of scaffolds including microspheres [4], electrospun sheets [5,6], thermosensitive injectable materials [7,8] and cardiac patches [9,10], among which the last type and electrospun sheets benefits from an interconnected porous structure shown to improve oxygen and nutrition transfer as well as extent of cell infiltration [11].

In designing cardiac patches, important parameters including porosity, mechanical properties, and chemical components have to be taken into account in order to reach an optimum cell infiltration as well as oxygen and nutrition transfer. Moreover, an ideal patch resembles the host tissue mechanical properties and provides cells with right chemical cues to induce tissue regeneration [3]. To this purpose, various materials including synthetic and natural polymers have been used to fabricate cardiac patches via different techniques [12]. Synthetic materials such as poly-(L-lactide-co-caprolactone) (PLCL) [13], Poly-(ester carbonate urethane) urea (PECUU) [14] and Poly-(Ethylene glycol) (PEG) [15] are usually chosen due to their strong mechanical properties, enabling the patch to endure varying ranges of stress that heart beats will apply to it. On the other hand, natural materials such as type I collagen [16,17], fibrin [18], matrigel [19,20], and acellular extracellular matrix (ECM) [21,22] of the myocardial tissue are used due to their strong capability to support cells with natural biochemical cues and vital signals.

Among aforementioned natural materials, decellularized cardiac ECM has shown to be highly effective in mimicking the structural and compositional complexity of the natural heart environment [23]. ECM is a complex and interconnected network of fibrous proteins, proteoglycans, glycosaminoglycans (GAGs), and bioactive molecules including cytokines and growth factors, incorporation of which into a biomaterial can improve cell viability, proliferation, and differentiation by providing necessary ligands to the seeded cells [24]. Decellularizing cardiac tissue and immediate use of it as a scaffold [23,25] can provide cells with preserved heart vasculature and ECM architecture [26].

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However, the unprocessed acellular matrix has shown to possess lower mechanical strength after decellularization [27]. To address this issue, solubilization of the decellularized ECM followed by incorporating it into a composite mixture to fabricate a cardiac patch is suggested. Among many available synthetic and natural materials to mix with acellular soluble ECM, in this work, chitosan (CS) is selected due to its biocompatibility and biodegradability [21,28–30]. Although, chitosan does not have cell binding cites [31], it has been widely used for cardiac tissue engineering purposes [32], mostly in composite structures, due to its aforementioned properties. Moreover, it is shown that the mechanical properties of CS/ECM ECM/chitosan composites are improved compared to pure ECM [21].

In this study, with the idea of developing a novel ECM-based cardiac patch, a CS/ECM composite scaffold is fabricated via lyophilization. Further, by varying the ratio of CS/ECM, we successfully showed that mechanical properties, biodegradability and cell viability can be optimized. Results of this study highly suggests the use of CS/ECM composite scaffolds for further cardiovascular tissue engineering studies and myocardium regeneration.

2. Material and methods

2.1. Materials

Medium molecular weight chitosan powder with Mw = 280 KDa and DD = 75–85% was purchased from Sigma-Aldrich. Sodium hydroxide, hydrochloric acid, acetic acid and pepsin were obtained from Merck. 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) solution, dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), trypsin-EDTA, penicillin streptomycin, and lysozyme from chicken egg white were supplied by Sigma-Aldrich. Sodium dodecyl sulfate (SDS), Triton X-100 and glutaraldehyde were obtained from Dae Jung.

2.2. Decellularization of bovine myocardial tissue

The bovine heart was maintained on ice while transported to the laboratory to prevent cellular lysis and damage to the matrix. Next, myocardium tissue was cut into pieces of about 2 mm in thickness and decellularized as previously described [8]. Briefly, tissue was rinsed with deionized water and then stirred in phosphate buffered saline (PBS) containing 1% (w/v) SDS for 4–5 days, until the tissue was decellularized. The tissue was then stirred in Triton X-100 (1% v/v) for 30 min for final cell removal. Finally, decellularized cardiac tissue were stirred overnight in deionized water to ensure removal of detergents. Following the described decellularization procedure, the ECM was frozen overnight at −20 °C and lyophilized (Lyotrap-Plus) for 24 h and milled to create a fine-grained powder.

2.3. Evaluation of the decellularization process

2.3.1. Histological characterization

Parts of the decellularized bovine myocardium tissue were fixed for 24 h in 10% neutral buffered formalin solution in PBS (pH 7.4) at room temperature. Subsequently the samples were washed, dehydrated in graded series of ethanol, embedded in paraffin, and sectioned with a 5 µm thickness using a microtome. Hematoxylin and eosin (H&E, Sigma-Aldrich) staining was performed after deparaffinization by xylene and rehydrating to validate the cell's removal from decellularized tissue.

2.3.2. DNA quantification

Total DNA content was quantified in samples of native and decellularized bovine myocardial tissue. Samples were completely homogenized and solubilized in 1 ml lysis buffer (50 mM tris-HCl, 50 mM EDTA, 1% SDS, 10 mM NaCl, pH 8.0) and subsequently digested in the presence of proteinase K at 65 °C, followed by a phenol/chloroform extraction. DNA was precipitated from the aqueous phase with 100% ethanol, after which the extracts were subsequently washed with 70% ethanol. After dissolving the resultant pellet in water, spectrophotometer was used to determine the concentration of DNA at 280 nm. The amount of DNA was expressed as mg/mg dry weight of samples.

2.4. Preparation of the porous scaffold

To fabricate a porous composite scaffold composed of myocardium ECM and chitosan, the decellulaized matrix was first solubilized, as reported in a previously published protocol for the same tissue [8]. Briefly, fine milled myocardium powder was allowed to digest in pepsin at 1 mg/ml that was dissolved in 0.1 M HCl for about 60 h under constant stirring. Afterwards, the solution was brought to pH 7.4 by addition of 1 M NaOH and 10X PBS. After preparing solubilized ECM and chitosan (CS) solution, they were mixed in certain quantities and then stirred for 2 h to reach a homogenous mixture. Then, 1 ml of the prepared mixture was poured to each chamber of the 24-well plates, frozen at −20 °C and lyophilized overnight. The dried porous scaffolds were then chemically crosslinked with a sterile solution of glutaraldehyde (2% v/v). Following 4 h crosslinking, the scaffolds were rinsed under deionized water and lyophilized for 24 h to be thoroughly dried.

In these experiments, two groups of scaffold were prepared from separate CS and ECM solutions (Table 1), to have the optimum properties in terms of suitable handling as well as cell attachment, proliferation rate and mechanical strength. The pure decellularized bovine myocardial ECM and pure CS were used as controls.

2.5. Characterization of the scaffolds

2.5.1. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra were recorded using ABB Bomem MB-100 FTIR spectrophotometer. Absorbance resolution was set to 2 cm^{-1} and the spectra were recorded in the range of 4000 to 400 cm^{-1}.

2.5.2. Rheological measurements

A Physica MCR 301 rheometer (Anton Paar) with 25-mm parallel plates at 25 °C was used to characterize the specimens' oscillatory shear deformation. The lyophilized specimens were put into PBS solution for 48 h to reach the thermodynamic equilibrium prior to analysis. The dynamic viscoelastic parameters such as the dynamic shear storage modulus G′ and loss modulus G″ were measured as a function of frequency at the constant temperature 25 °C. The test was done at 37 °C, a gap of 80 µm and the value of the strain amplitude was fixed at 0.8 to ensure that all the measurements were carried out within the linear viscoelastic regime, where the G′ was independent of strain amplitude.

Table 1
Preparation data of CS-ECM scaffolds.

Sample	Scaffold having various CS concentration			Scaffold having various ECM concentration		
	CS2.5-ECM0.5	CS3-ECM0.5	CS3.5-ECM0.5	CS3.5-ECM0.25	CS3.5-ECM0.33	CS3.5-ECM0.5
CS solution in 2% acetic acid (w/v)	2.5%	3%	3.5%	3.5%	3.5%	3.5%
ECM solution in pepsin (w/v)	0.5%	0.5%	0.5%	0.25%	0.33%	0.5%