Advanced Drug Delivery Reviews 96 (2016) 77–82

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

Decellularized myocardial matrix hydrogels: In basic research and preclinical studies☆

Raymond M. Wang, Karen L. Christman *

Department of Bioengineering, Sanford Consortium of Regenerative Medicine, University of California, San Diego, 2880 Torrey Pines Scenic Drive, La Jolla, CA 92037, USA

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 19 May 2015
Accepted 2 June 2015
Available online 6 June 2015

Keywords:
Myocardial infarction
Biomaterial
Hydrogel
Tissue engineering
Regenerative medicine

ABSTRACT

A variety of decellularized materials have been developed that have demonstrated potential for treating cardiovascular diseases and improving our understanding of cardiac development. Of these biomaterials, decellularized myocardial matrix hydrogels have shown great promise for creating cellular microenvironments representative of the native cardiac tissue and treating the heart after a myocardial infarction. Decellularized myocardial matrix hydrogels derived from porcine cardiac tissue form a nanofibrous hydrogel once thermally induced at physiological temperatures. Use of isolated cardiac extracellular matrix in 2D and 3D *in vitro* platforms has demonstrated the capability to provide tissue specific cues for cardiac cell growth and differentiation. Testing of the myocardial matrix hydrogel as a therapy after myocardial infarction in both small and large animal models has demonstrated improved left ventricular function, increased cardiac muscle, and cellular recruitment into the treated infarct. Based on these results, steps are currently being taken to translate these hydrogels into a clinically used injectable biomaterial therapy. In this review, we will focus on the basic science and preclinical studies that have accelerated the development of decellularized myocardial matrix hydrogels into an emerging novel therapy for treating the heart after a myocardial infarction.

Contents

Abbreviations: DMMB, dimethylmethylene blue assay; ECM, extracellular matrix; EDV, end-diastolic-volume; EF, ejection fraction; ESV, end-systolic-volume; H&E, hematoxylin and eosin; hESC, human embryonic stem cell; HF, heart failure; LV, left ventricular; MI, myocardial infarction; MMP, matrix metalloproteinase; PCL, polycaprolactone; PEG, poly(ethylene glycol); SDS, sodium dodecyl sulfate; sGAG, sulfated glycosaminoglycan.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Tissue engineering of the heart: from in vitro models to regenerative solutions".

* Corresponding author.
E-mail address: christman@eng.ucsd.edu (K.L. Christman).

http://dx.doi.org/10.1016/j.addr.2015.06.002
0169-409X/

1. Introduction

Advancements in cardiac tissue engineering have demonstrated great promise in the pursuit of regenerative medicine for the treatment of cardiovascular diseases. Ischemic heart disease leading to myocardial infarction (MI) and subsequent heart failure (HF) is both the leading cause of death in the western world [1] and worldwide with a projected increase to 13.4% of overall annual deaths by 2030 [2]. After suffering a MI, the adult human heart lacks the regenerative capabilities to restore damaged myocardium leading to progressive pathophysiological remodeling such as extracellular matrix (ECM) degradation by matrix metalloproteinases (MMPs), fibrosis by increased collagen deposition, hypertrophy, and tissue necrosis that ultimately leads to HF [3–5]. Currently, no treatment is available that can prevent HF post-MI and options for end-stage HF are limited to whole heart transplant and left ventricular (LV) assist devices. However, these procedures put heavy strain on patients' quality of life and medical resources [1]. In response, research in cardiac tissue engineering is focused on investigating whether restoration of function and possibly regeneration of the damaged myocardium can be achieved by novel methods of therapy.

An advancement that has particularly accelerated the field of cardiac tissue engineering is the isolation of the underlying ECM scaffold from native cardiac tissue first pioneered by decellularization techniques used by Ott *et al.* [6]. The ECM provides cells with tissue specific biochemical cues which have been demonstrated to be important for cellular migration [7,8], differentiation [9,10], and development [11]. Later work by Singelyn *et al.* first demonstrated that decellularized porcine cardiac tissue could be processed into an injectable biomaterial therapy that forms a nanofibrous porcine myocardial matrix hydrogel when thermally induced or injected *in vivo* [12]. Application of the hydrogel in post-MI animal models has resulted in improved LV function, increased cardiac muscle, and induced cellular recruitment into the treated infarct [13,14]. By creating a biomaterial representative of the native cardiac tissue, decellularized myocardial matrix hydrogels have quickly demonstrated their promising applications as a novel cell study platform and biomaterial therapy.

In this review, we will describe characteristics of decellularized myocardial matrix hydrogels, methods to modulate their material properties, demonstrated uses in creating *in vitro* models of the cardiac microenvironment, and significant progress in preclinical studies that have demonstrated their therapeutic potential (Fig. 1). Additional studies, which utilize the native architecture of the decellularized cardiac tissue scaffold either as a whole heart construct [6,15–18] or as pieces of cardiac ECM [19–27], are not covered since these methods provide a spatial and mechanical environment that have distinct advantages and disadvantages compared to hydrogel methods.

2. Generation and characterization of decellularized myocardial matrix hydrogels

2.1. Generation of decellularized myocardial matrix hydrogels

To create decellularized myocardial matrix hydrogels, myocardium is first cut into smaller pieces and decellularized by agitation in a 1% sodium dodecyl sulfate (SDS) solution [12]. Other decellularization techniques using mechanical, chemical, and enzymatic methods have also been described for the heart [6,15,29]. Following complete decellularization and water rinses to remove detergent, the decellularized ECM is lyophilized and milled into a fine powder. ECM powder is re-suspended and partially digested with pepsin at a tenth the ECM concentration for 48 h in a 0.1 M HCl solution. After enzymatic treatment, pepsin is inactivated by increasing the pH to 7.4 with NaOH solutions and the digested ECM is diluted to a desired concentration in a 1× PBS solution. The digested ECM can then be lyophilized, stored at −80 °C, and simply re-suspended with sterile water prior to use [30].

Depending on the source material, additional processing might be required to isolate the ECM such as isopropyl alcohol rinses for removing excess lipid content, which was needed for processing human heart tissue [31]. Material from multiple hearts should be collected into a single batch to reduce batch-to-batch variability. After generating a new batch of material, characterization should be performed to confirm quality of processing and consistency between each batch. These characterization steps include hematoxylin and eosin (H&E) staining to observe lack of nuclei indicating successful decellularization, DNA isolation and quantification to further confirm removal of cellular content, protein separation by gel electrophoresis to verify a similar pattern of ECM peptides between batches, a dimethylmethylene blue assay (DMMB) assay to determine sulfated glycosaminoglycan (sGAG) content, and a demonstration of material *in vitro* gelation [30]. Images of material generation are displayed in Fig. 2 and video instruction demonstrating pericardial tissue processing into a decellularized injectable hydrogel can be found online [32]. Although the pericardial tissue has different material properties such as ECM composition [33], the processing is identical to the porcine myocardial matrix procedure with the exception of the time necessary for decellularization and digestion.

2.2. Properties of decellularized myocardial matrix hydrogels

Decellularized cardiac ECM composition by mass spectroscopy has shown retention of an array of ECM proteins such as collagen, elastin, and fibronectin [28,31], and analysis by the DMMB assays has indicated retention of sGAGs. Based on complex viscosity analysis, liquid myocardial matrix is shear thinning, which supports its capability to be

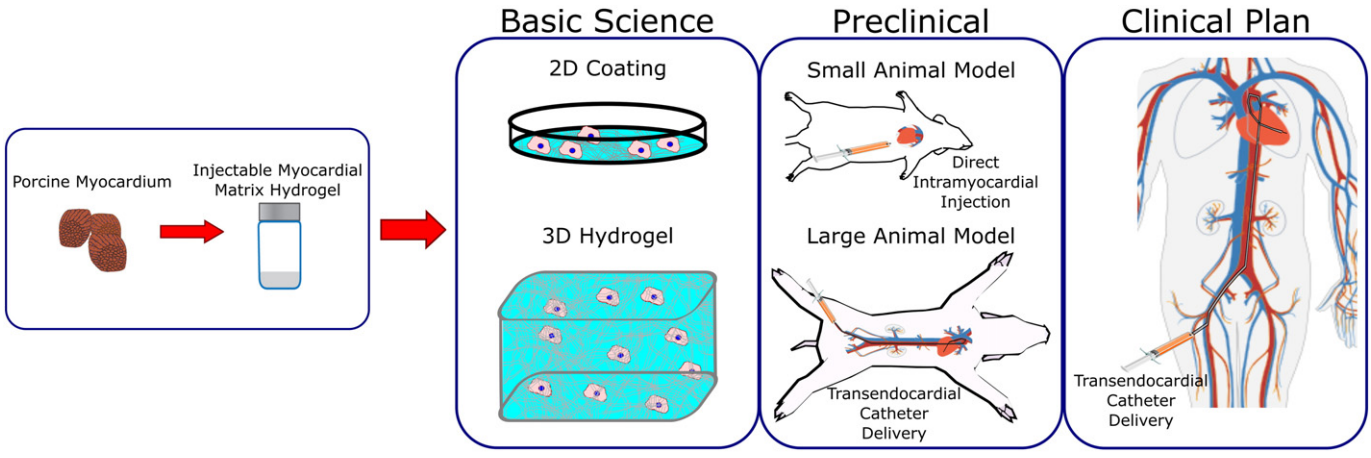

Fig. 1. Summary of the use of injectable decellularized myocardial matrix hydrogels for basic science and preclinical studies along with future plans for translation. Selected images reprinted from [28].