Methods 84 (2015) 53–59

Contents lists available at ScienceDirect

Methods

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

Fabrication and characterization of injectable hydrogels derived from decellularized skeletal and cardiac muscle

J.L. Ungerleider, T.D. Johnson, N. Rao, K.L. Christman *

Department of Bioengineering, Sanford Consortium for Regenerative Medicine, University of California, San Diego, La Jolla, CA, USA

ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form 24 March 2015
Accepted 26 March 2015
Available online 2 April 2015

Keywords:
Decellularization
Hydrogel
Extracellular matrix
Myocardial infarction
Peripheral artery disease

ABSTRACT

Biomaterials, which can contain appropriate biomechanical and/or biochemical cues, are increasingly being investigated as potential scaffolds for tissue regeneration and/or repair for treating myocardial infarction, heart failure, and peripheral artery disease. Specifically, injectable hydrogels are touted for their minimally invasive delivery, ability to self-assemble *in situ*, and capacity to encourage host tissue regeneration. Here we present detailed methods for fabricating and characterizing decellularized injectable cardiac and skeletal muscle extracellular matrix (ECM) hydrogels. The ECM derived hydrogels have low cellular and DNA content, retain sulfated glycosaminoglycans and other extracellular matrix proteins such as collagen, gel at physiologic temperature and pH, and assume a nanofibrous architecture. These injectable hydrogels are amenable to minimally invasive, tissue specific biomaterial therapies for treating myocardial infarction and peripheral artery disease.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Cardiovascular disease is the leading cause of death in the United States [1]. One consequence of cardiovascular disease, myocardial infarction (MI), afflicts more than 900,000 Americans annually and can lead to heart failure and death. MI is caused by acute occlusion of a coronary artery, leading to myocardial ischemia, cardiomyocyte necrosis, collagen scar formation, and subsequent diminished pump function [2]. The only treatments for heart failure post-MI are either a heart transplant or a mechanical left ventricular (LV) assist device, and no current therapy prevents negative left ventricular remodeling and heart failure. As such, the five-year mortality rate post-MI is 50% [1]. Another manifestation of cardiovascular disease, peripheral artery disease (PAD) is a cardiovascular condition that afflicts 12–20% of Americans over age 65 [3]. Its most common cause is atherosclerosis, but it predominantly afflicts smokers, diabetics, and African Americans. The disease first presents as pain during exercise due to chronic plaque build-up and reduced blood flow in peripheral arteries, most commonly in the legs [1]. Over time, the plaque occludes more of the major arteries and leads to critical limb ischemia (CLI), the most severe form of the disease. CLI affects about a quarter of the overall PAD population [1]. Currently, the only effective treatment is surgical revascularization; however, few patients are eligible for this therapy, and it carries a high failure rate due to restenosis, which is why amputation rates due to PAD have remained relatively unchanged in the last 30 years [4,5]. In both instances, atherosclerosis leads to acute or chronic muscle ischemia, followed by negative remodeling and fibrosis. Thus, an ideal therapy would encourage positive remodeling post-ischemia and prevent chronic ischemia and inflammation from leading to overall tissue damage and failure over time.

Direct injection of growth factors [6,7], cells [8], and gene therapy [9,10] are some examples of tissue engineering approaches used to stimulate angiogenesis in the ischemic limb or heart. Recently, biomaterial scaffolds have begun to be used for their potential in prolonging the release of angiogenic factors and for their inherent ability to encourage tissue-scale regeneration. Naturally-derived [6,7,11,12] and synthetic [13,14] hydrogel scaffolds have been tested for their ability to recruit endogenous progenitor cells and promote tissue remodeling post-ischemia. While promising results have been seen with material delivery of growth factors or stem cells [5,15–17], the expensive nature of preparing such a combination product and sub-optimal clinical trial results

Abbreviations: PAD, peripheral artery disease; CLI, critical limb ischemia; MI, myocardial infarction; ECM, extracellular matrix; SDS, sodium dodecyl sulfate; PenStrep, penicillin/streptomycin; IPA, isopropyl alcohol; PBS, phosphate-buffered saline; dsDNA, double-stranded DNA; sGAG, sulfated glycosaminoglycan.

* Corresponding author at: Department of Bioengineering, Sanford Consortium for Regenerative Medicine, University of California, San Diego, 2880 Torrey Pines Scenic Drive, La Jolla, CA 92037, USA.

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

http://dx.doi.org/10.1016/j.ymeth.2015.03.024
1046-2023/© 2015 Elsevier Inc. All rights reserved.

have halted these therapies' progression to market [18]. In contrast, material-alone therapies have significant potential for many translational reasons, including minimally invasive delivery and reduced costs compared to a cell- or growth factor-based therapy [19]. In particular, injectable hydrogels derived from decellularized muscle ECM have shown significant potential for treating both MI and PAD. Singelyn et al. first showed the capability of a decellularized cardiac ECM hydrogel to increase vascular cell migration *in vitro* and vessel density *in vivo* [20]. The material has since shown efficacy to increase cardiac muscle, reduce fibrosis, and improve cardiac function post-MI in small and large preclinical animal models [21,22] and is now planned for testing in a Phase I clinical trial (clinicaltrials.gov: NCT02305602). DeQuach et al. showed skeletal muscle progenitor recruitment and neovascularization due to injection of a skeletal muscle ECM hydrogel alone in a preclinical model of hindlimb ischemia, thus indicating the potential for ECM hydrogels to be used alone to treat PAD and regenerate ischemic damaged skeletal muscle [12].

In this article, we present detailed methods for fabricating injectable hydrogels derived from either decellularized cardiac or skeletal muscle extracellular matrix (ECM). We also present methods, which we recommend should be performed on each batch of material prior to *in vitro* or *in vivo* use, to ensure limited batch-to-batch variability and more consistent results.

2. Materials and methods

2.1. Fabrication of injectable hydrogels

2.1.1. Day 0 – initial tissue processing

Tissue specific injectable hydrogels were derived from either porcine myocardium or skeletal muscle. In order to fabricate a sterile material, all steps in the protocol were conducted with sterile solutions and autoclaved beakers or in a biosafety cabinet where possible. Decellularization was accomplished with a 1% wt/vol sodium dodecyl sulfate (SDS) solution, made by adding appropriate volumes of 20× PBS, 10× SDS, and ultrapure water. The psoas muscle or heart was harvested from Yorkshire farm pigs weighing 30–45 kg. Note that larger animals or other sources of skeletal muscle are more likely to have greater interstitial adipose tissue within the muscle, which interferes with tissue processing. The skeletal muscle was obtained and isolated from skin, superficial fat, and fascia, leaving only the homogenous skeletal muscle tissue behind. For cardiac ECM fabrication, the left ventricle (LV) free wall and septum were isolated from the right ventricular free wall, atria, and valves by blunt dissection and cleared of any fat or fascia. Papillary muscles and chordae tendinae in the LV lumen were also removed, leaving only myocardium remaining. Muscle was cut into regularly sized cubes approximately 3–5 mm (skeletal muscle, Fig. 1A) or 2 mm (cardiac muscle) per side at the smallest, as tissue is prone to degradation and collapse during decellularization. A larger piece of muscle was set aside for histological analysis as a "before decellularization" sample. Tissue was weighed and divided into 1 L autoclaved beakers with 20–35 g of tissue in each beaker, and ultrapure water was added to a total volume of 800 mL and spun with a stir bar at 125 rpm for 30–45 min. Tissue was strained in an autoclaved fine mesh strainer, rinsed under ultrapure water, and returned to the beaker. Previously mixed 1% SDS solution was added to the beaker so that the total volume of tissue and SDS was 800 mL and was stirred at 125 rpm for 2 h as an initial rinse. Again, after 2 h the tissue was rinsed in the fine mesh strainer with ultrapure water and returned to the beaker, also rinsed with ultrapure water. Fresh 1% SDS was added to the beaker to a final volume of 800 mL. Four mL of 10,000 U penicillin/streptomycin (PenStrep) was then added to each beaker, giving a final working concentration of 50 U PenStrep in 1% SDS. The beaker was kept sealed with a square of parafilm and the tissue was spun at 125 rpm for 24 h.

2.1.2. Day 1–5 – SDS solution changes

Tissue was strained and the beaker/stir bar were thoroughly rinsed with ultrapure water. On the first day only, larger pieces of tissue were more finely cut into smaller pieces to ensure consistent rates of decellularization (larger pieces tended to have a deeper red or pink center after the first day of decellularization, Fig. 1B). Tissue was returned to the beaker and fresh 1% SDS was added to 800 mL with 4 mL 10,000 U PenStrep. Through this process, beakers were kept covered with parafilm whenever possible to reduce the risk of contamination. Rinses and solution changes were repeated every 24 h until the tissue was completely white, usually 3–4 days (Fig. 1C). Remaining ECM was spun for an extra 24 h period to ensure full decellularization. Additional days of solution changes were minimized once tissue was fully white to avoid degradation and loss of ECM proteins. Cardiac ECM was then processed starting with the water rinse step (Section 2.1.4), while skeletal muscle was processed first with the IPA lipid removal step (Section 2.1.3).

2.1.3. IPA lipid removal (skeletal muscle ECM only)

The presence of lipids after decellularization could inhibit subsequent gelation of the digested material, and therefore an isopropyl alcohol (IPA) lipid removal step was implemented only for fattier skeletal muscle tissue. After final SDS solution change, ECM was rinsed in the mesh strainer with ultrapure water and the beaker and stir bar were rinsed thoroughly to remove any trace SDS. ECM was spun in ultrapure water at 800 mL total volume for 2 h to remove residual SDS. Up to 5 beakers of rinsed ECM were placed into a single clean 1 L beaker with a clean stir bar. In a fume hood, IPA was added up to 400 mL total volume. The beaker was sealed with parafilm and spun at 125 rpm for 12–24 h.

2.1.4. Water rinse, freezing, milling

ECM was rinsed in ultrapure water and for skeletal muscle ECM, used IPA was properly disposed of as hazardous waste. Both skeletal and cardiac muscle ECM was spun in 800 mL ultrapure water at

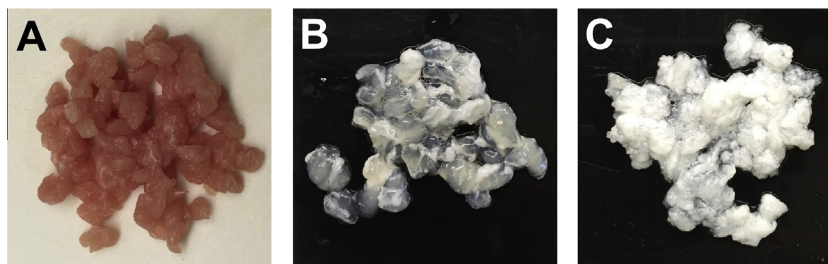

Fig. 1. Decellularization process. (A) Images of freshly cubed porcine skeletal muscle. (B) Tissue after the first day in 1% SDS. (C) Final ECM after complete decellularization. Note larger pieces of ECM maintain an opaque, pink-tinted center after the first day of SDS rinsing (B), indicating incomplete decellularization.

Download English Version:

https://daneshyari.com/en/article/1993265

Download Persian Version:

https://daneshyari.com/article/1993265

Daneshyari.com