Biomaterials 89 (2016) 114–126

Contents lists available at ScienceDirect

Biomaterials

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

Perfusion-decellularized skeletal muscle as a three-dimensional scaffold with a vascular network template☆

Jian Zhang [a, b, 1], Zhi Qian Hu [a, 1], Neill J. Turner [c, 1], Shi Feng Teng [a, 1], Wen Yue Cheng [b, 1], Hai Yang Zhou [a], Li Zhang [c], Hong Wei Hu [d], Qiang Wang [a, b, d, *], Stephen F. Badylak [c, e, **]

[a] *Department of Surgery, Shanghai Chang Zheng Hospital, Second Military Medical University, Shanghai 200003, PR China*
[b] *Department of Regenerative Medicine, Shanghai Zhabei District Central Hospital, Shanghai 200072, PR China*
[c] *McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA*
[d] *Department of General Surgery, Shanghai Zhabei District Central Hospital, Shanghai 200072, PR China*
[e] *Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15219, USA*

A R T I C L E I N F O

Article history:
Received 21 December 2015
Received in revised form
7 February 2016
Accepted 23 February 2016
Available online 26 February 2016

Keywords:
Extracellular matrix
Perfusion decellularization
Skeletal muscle
Three-dimensional scaffold
Volumetric muscle loss
Abdominal defect repair

A B S T R A C T

There exists a great need for repair grafts with similar volume to human skeletal muscle that can promote the innate ability of muscle to regenerate following volumetric muscle loss. Perfusion decellularization is an attractive technique for extracellular matrix (ECM) scaffold from intact mammalian organ or tissue which has been successfully used in tissue reconstruction. The perfusion-decellularization of skeletal muscle has been poorly assessed and characterized, but the bioactivity and functional capacity of the obtained perfusion skeletal muscle ECM (pM-ECM) to remodel *in vivo* is unknown. In the present study, pM-ECM was prepared from porcine rectus abdominis (RA). Perfusion-decellularization of porcine RA effectively removed cellular and nuclear material while retaining the intricate three-dimensional microarchitecture and vasculature networks of the native RA, and many of the bioactive ECM components and mechanical properties. *In vivo*, partial-thickness abdominal wall defects in rats repaired with pM-ECM showed improved neovascularization, myogenesis and functional recellularization compared to porcine-derived small intestinal submucosa (SIS). These findings show the biologic potential of RA pM-ECM as a scaffold for supporting site appropriate, tissue reconstruction, and provide a better understanding of the importance maintaining the tissue-specific complex three-dimensional architecture of ECM during decellularization and regeneration.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Skeletal muscle injuries resulting from traumatic accidents, tumor ablation, or degenerative disease result in volumetric muscle loss (VML) with limited treatment options and high morbidity. Although adult skeletal muscle has robust ability to regenerate in response to injury, this regenerative response is dependent upon the severity of muscle insult [1,2]. Generally, if more than 20% of the muscle is lost, the regenerative process will fail and repair with an accumulation of scar tissue occurs with a concomitant loss of function [1–3]. There exists a need for therapeutic options that can promote the innate ability of skeletal muscle to regenerate and restore function following severe trauma.

A variety of mammalian tissues and organs, including the small intestinal submucosa (SIS), pericardium, urinary bladder, arterial vasculature, heart valves, fascia lata and dermis, have been decellularized using a number of different approaches. The resulting extracellular matrix (ECM) has been utilized as biologic scaffolds to support the functional reconstruction of injured or missing tissues [2,4–9]. The ECM represents a complex network of structural proteins such as collagen, elastic fibers, heparin, proteoglycans, and associated non-matrix molecules such as growth factors. In a clinical case study of five patients with VML treated with an ECM-

☆ The content is the responsibility of the authors alone and does not necessarily reflect the views of any commercial organization.

* Corresponding author. Department of Surgery, Shanghai Chang Zheng Hospital, Second Military Medical University, 415 Feng Yang Road, Shanghai 200003, PR China.

** Corresponding author. McGowan Institute for Regenerative Medicine, 450 Technology Drive Suite 300, University of Pittsburgh, Pittsburgh, PA 15219, USA.

E-mail addresses: wang2929@hotmail.com (Q. Wang), badylaks@upmc.edu (S.F. Badylak).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2016.02.040
0142-9612/© 2016 Elsevier Ltd. All rights reserved.

derived biologic scaffold, all patients showed signs of new muscle and blood vessels six months following implantation with associated improvement in limb strength during physical therapy [10]. However, other preclinical animal studies have noted only a limited amount of vascularized muscle (<10% of the defect area) when non-tissue specific ECM scaffolds were used [4]. It is plausible that site specific ECM, such as, skeletal muscle ECM (M-ECM) may contain a biochemical composition, that represents a favorable environment for the formation of new skeletal muscle and promote positive remodeling characteristics and myogenesis *in vivo* [11,12].

The anatomically distinct biochemical composition of the ECM is susceptible to the decellularization techniques used. Insufficient decellularization will leave behind cellular debris that will promote a pro-inflammatory response. Conversely, aggressive decellularization techniques may strip the ECM of essential growth factors, and crosslink or denature the structural proteins. Antegrade or retrograde perfusion decellularization is an attractive technique for ECM scaffold preparation initially used at the whole organ scale, which largely preserves chemical and architectural features of the tissue of origin, including the vascular bed [13–24]. To date, perfusion-decellularized heart [13], liver [14–16], lung [17,18], kidney [19,20], pancreas [21], limb [22] and intestinal [23,24] bio-scaffolds have been manufactured from rodent, porcine or primate organs, and the perfusion-decellularized heart has been shown to facilitate extensive functional recellularization [13]. Inspired by these works both at the whole organ [13–21] and sub-organ scale [23,24], a new perfusion-decellularization of skeletal muscle (pM-ECM) from porcine rectus abdominis (RA) was evaluated because of its similarities with human organ architecture and volume.

The objective of the current study was two-fold: First, generate a pM-ECM scaffold from perfusion decellularized porcine RA, and characterize the structure, composition and bioactivity of the resulting pM-ECM; and second, determine the effectiveness of this pM-ECM at reconstructing a partial-thickness abdominal wall defect in rats.

2. Materials and methods

2.1. Overview of study design

Porcine RA was harvested and decellularized by enzymatic and detergent perfusion through the associated artery and vein. The biochemical and structural composition, mechanic strength, and bioactivity *in vitro* were then assessed in the resulting RA pM-ECM. *In vivo* evaluation compared the remodeling characteristics of pM-ECM to a non-homologous ECM (SIS) in a rodent partial-thickness abdominal wall defect. All animal experiments were conducted in accordance to Second Military Medical University Institutional Animal Care and Use Committee (IACUC) regulations and guidelines. Research was conducted in compliance with the Animal Welfare Act and followed the guidelines set forth by the Guide for the Care and Use of Laboratory Animals, NRC publications, 1986 edition. All procedures were reviewed and approved by the Institution's Animal Care and Use Committee and were performed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

2.2. Porcine RA retrieval

Complete details of the porcine RA retrieval are elaborated in Data in Brief article [25]. Following systemic heparinization at a dose of 10000UI per animal administrated through the auricular marginal vein, whole RA (right or left) below the umbilical point and above the pubic symphysis and the pubic bone crest was collected through a long extra-peritoneal midline incision under sterile conditions from anesthetized adult female Yorkshire pigs. The external iliac artery, the femoral artery and the pudendal epigastricus trunk, which is formed by inferior epigastric artery, epigastric caudalis superficialis artery and pudenda external artery, together with their accompanying veins were carefully isolated and skeletonized, after pulling the peritoneum cranially with partial exposure of the retroperitoneal space (Fig. 3A in Data in Brief). The RA was then infused with 0.9% saline with 50UI/ml heparin through the external iliac artery and the inferior epigastric artery at a speed of 30–50 ml/min by a pump for 5 min. The intra-arterial pressure was maintained at 110–150 mmHg during infusion (Fig. 3B in Data in Brief). As soon as the RA turned white and infused liquid flowing out from veins became colorless, the RA was then harvested by detaching the posterior sheath from the peritoneum and transverse abdominis, dissecting along the surface of anterior sheath, and transecting all the anterior perforating branches to external, internal oblique muscle and all the posterior perforating branches to transverse muscle as well as the posterior perforating vessels to the inferior border of costal arch. The RA was then carefully dissected free from that platysmal aponeurosis along the linea semilunaris. The perforator vessels on the RA surface were identified and ligated separately with surgical threads (Fig. 3C in Data in Brief). The distal half of RA was chosen because of its similarities with humans in terms of fewer perforator vessels and linea transversae compared with upper part of RA (Fig. 3D in Data in Brief).

2.3. Preparation of pM-ECM and SIS-ECM

The obtained porcine RA was decellularized by continuous perfusion using a series of chemical and enzymatic treatments via the inferior epigastric artery and vein in a perfusion bioreactor (Text and Tab.1 in Data in Brief [25]). Briefly, 0.02% Trypsin/0.05% ethylene glycol-bis-(β-amino-ethyl ether) N, N, N′, N′-tetra-acetic acid (EGTA) (at 37 °C, pH = 7.8) was infused via the artery for 1.75 h followed by 0.25 h via the vein. This was followed by 12 h perfusion (11 h via artery and 1 h via vein) of 0.1% sodium dodecyl sulphate (SDS, Sigma, St Louis MO) in deionized water, 12 h perfusion (11 h via artery and 1 h via vein) of 1% Triton-X 100 (Sigma), a 2 h perfusion via artery of 0.1% peracetic acid (PAA)/4% ethanol (ETOH) and a 0.5 h perfusion via artery of DNase (40 U/ml, Sigma)/α-galactosidase (10 U/ml, Sigma). The next step involved extensive perfusion with sterilized deionized water, via the artery for up to 7 days to remove any residual detergent. The perfusion pressure within supporting artery was maintained at 110–150 mmHg. The resultant pM-ECM scaffolds were kept in sterilized 1× PBS solution, or lyophilized, and/or powdered for further analysis.

Porcine jejunum was obtained from 6 month old female Yorkshire pigs and prepared as SIS-ECM by the method described previously [12]. After gentle cleansing, the majority of the mucosa and the entire serosa, and muscularis external layers were mechanically delaminated from the jejunum. The remaining submucosa, muscularis mucosa and stratum compactum layer underwent 2 freeze–thaw cycles and was disinfected with 0.1% (v/v) peracetic acid/4% (v/v) ETOH for 2 h, and followed by 4 alternating washes in deionized water and PBS for 15 min. The SIS-ECM was then vacuum pressed into 8-layer laminates.

2.4. Integrity assessment and vascular corrosion casting

Integrity test in pM-ECM was performed by dye continuous infusion with pressure under 300 mmHg. Corrosion casting was carried out for graft vasculature using Batson's No. 17 Plastic Replica and Corrosion Kit (Polysciences, Inc.) following the manufacturer's instructions. Detailed procedures are mentioned in Data in Brief [25]. Briefly, the pigments were added to base solution prior to

Download English Version:

https://daneshyari.com/en/article/6484997

Download Persian Version:

https://daneshyari.com/article/6484997

Daneshyari.com