Biomaterials xxx (2012) 1–8

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Hydrogels derived from central nervous system extracellular matrix

Christopher J. Medberry [a,c,1], Peter M. Crapo [c,1], Bernard F. Siu [a,c], Christopher A. Carruthers [a,c], Matthew T. Wolf [a,c], Shailesh P. Nagarkar [d], Vineet Agrawal [c,e], Kristen E. Jones [c], Jeremy Kelly [c], Scott A. Johnson [c], Sachin S. Velankar [d], Simon C. Watkins [f], Michel Modo [a,c], Stephen F. Badylak [b,c,*]

[a] Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15219, USA
[b] Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15213, USA
[c] McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA
[d] Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15261, USA
[e] Medical Scientist Training Program, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA
[f] Department of Cell Biology and Physiology, Center for Biological Imaging, University of Pittsburgh, Pittsburgh, PA 15261, USA

ARTICLE INFO

Article history:
Received 29 August 2012
Accepted 25 October 2012
Available online xxx

Keywords:
ECM (extracellular matrix)
Hydrogel
Scaffold
Central nervous system
Regenerative medicine
Tissue engineering

ABSTRACT

Biologic scaffolds composed of extracellular matrix (ECM) are commonly used repair devices in preclinical and clinical settings; however the use of these scaffolds for peripheral and central nervous system (CNS) repair has been limited. Biologic scaffolds developed from brain and spinal cord tissue have recently been described, yet the conformation of the harvested ECM limits therapeutic utility. An injectable CNS-ECM derived hydrogel capable of in vivo polymerization and conformation to irregular lesion geometries may aid in tissue reconstruction efforts following complex neurologic trauma. The objectives of the present study were to develop hydrogel forms of brain and spinal cord ECM and compare the resulting biochemical composition, mechanical properties, and neurotrophic potential of a brain derived cell line to a non-CNS-ECM hydrogel, urinary bladder matrix. Results showed distinct differences between compositions of brain ECM, spinal cord ECM, and urinary bladder matrix. The rheologic modulus of spinal cord ECM hydrogel was greater than that of brain ECM and urinary bladder matrix. All ECMs increased the number of cells expressing neurites, but only brain ECM increased neurite length, suggesting a possible tissue-specific effect. All hydrogels promoted three-dimensional uni- or bi-polar neurite outgrowth following 7 days in culture. These results suggest that CNS-ECM hydrogels may provide supportive scaffolding to promote in vivo axonal repair.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Biologic scaffolds composed of extracellular matrix (ECM) can facilitate the constructive remodeling of numerous tissues including esophagus [1,2], lower urinary tract [3,4], muscle and tendon [5,6], and myocardium [7,8], among others. Although the mechanisms by which ECM scaffolds promote a constructive and functional remodeling response are only partially understood, recruitment of endogenous multipotent progenitor cells [9,10], modulation of the innate immune response [11,12], scaffold degradation with the generation of bioactive molecular cues [13–15], and innervation [16] have all been shown to be important events in this process. The contribution of the innate three-dimensional ultrastructure, unique surface ligand distribution, or molecular composition to constructive, functional remodeling is largely unknown. However, hydrogel formulations of matrix scaffolds lack the native three-dimensional ultrastructure of the source tissue but still possess in vitro and in vivo biologic activity [17–22], suggesting that the molecular composition of these materials is an active factor in remodeling events. There have also been reports that suggest tissue-specific biologic scaffold materials have properties that enhance greater site-appropriate phenotypic cell differentiation compared to ECM scaffolds derived from non-homologous tissue sources [23–26].

The use of biologic scaffold materials within either the central or peripheral nervous system has not been extensively investigated [27]. However, it has been shown that innervation of remodeled scaffold materials is an early event when such materials are placed in several different anatomic locations and represents a predictor of

* Corresponding author. McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA. Tel.: +1 412 235 5144; fax: +1 412 235 5110.
E-mail address: badylaks@upmc.edu (S.F. Badylak).
[1] These authors contributed equally to this work.

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.10.062

Please cite this article in press as: Medberry CJ, et al., Hydrogels derived from central nervous system extracellular matrix, Biomaterials (2012), http://dx.doi.org/10.1016/j.biomaterials.2012.10.062

constructive and functional outcomes [16,28,29]. It has also been shown that innervation is a critical event in robust regenerative responses that occur in species such as the newt and axolotl [30–32]. Methods for the isolation of central nervous system (CNS) ECM have recently been described. The objectives of the present study were to develop a method to create hydrogel forms of brain and spinal cord ECM, examine the biomolecular composition and mechanical properties of the resulting hydrogels, and evaluate the in vitro neural cytocompatibility and neurotrophic potential of CNS-ECM hydrogels versus a hydrogel prepared from a non-CNS-ECM; specifically, porcine urinary bladder matrix.

2. Materials and methods

2.1. Overview of experimental design

Following decellularization of porcine brain and spinal cord, the resulting brain and spinal cord ECM (B-ECM and SC-ECM, respectively) were solubilized. The ECM materials were analyzed for collagen and sulfated glycosaminoglycan content, ultrastructure, and hydrogel mechanical properties. A commonly used neural cell line for examining neurite extension, N1E-115 [33,34], was used to identify the neurotrophic potential of ECM hydrogels in two- and three-dimensional culture. The results were compared to an ECM hydrogel manufactured from a non-CNS source, porcine urinary bladder matrix (UBM-ECM) [20].

2.2. ECM biologic scaffold production

Porcine brain, spinal cord, and urinary bladder were obtained from market weight animals (Tissue Source, Lafayette, IN). Tissues were frozen immediately after harvesting at −80 °C, thawed before use, and processed by tissue-specific methods described previously (Table 1A) [23]. In brief, CNS tissue was agitated (spinal cord tissue at 200 rpm; brain tissue at 120 rpm unless otherwise stated) in the following decellularization baths: deionized water (16 h at 4 °C; 60 rpm), 0.02% trypsin/0.05% EDTA (60 min at 37 °C; 60 rpm; Invitrogen Corp., Carlsbad, CA, USA), 3.0% Triton X-100 (60 min; Sigma–Aldrich Corp., St. Louis, MO, USA), 1.0 M sucrose (15 min; Fisher Scientific, Pittsburgh, PA, USA), water (15 min), 4.0% deoxycholate (60 min; Sigma), 0.1% peracetic acid (Rochester Midland Corp., Rochester, NY, USA) in 4.0% ethanol (v/v; 120 min), PBS (15 min; Fisher), deionized water (twice for 15 min each rinse), and PBS (15 min). Each bath was followed by rinsing the remaining tissue through a strainer with deionized water. Decellularized B-ECM and SC-ECM were lyophilized and stored dry until use.

UBM-ECM was prepared as previously described [35]. In brief, connective tissue was removed from the serosal surface of the bladder. The tunica serosa, tunica submucosa, and majority of the tunica muscularis mucosa were mechanically delaminated, which left the basement membrane and tunica propria intact. Luminal urothelial cells were dissociated from the basement membrane by soaking the UBM-ECM in deionized water. The UBM-ECM was then agitated in 0.1% peracetic acid in 4.0% ethanol (v/v; 120 min; 300 rpm) followed by a series of PBS and deionized water rinses and lyophilization.

2.3. ECM digestion and solubilization

Lyophilized and comminuted B-ECM (20 mesh), SC-ECM (20 mesh or hand cut), and UBM-ECM (20 mesh or hand cut; 400–1000 μm largest particle dimension as measured by mesh diameter or ruler) were separately placed into a 0.01 N HCl solution containing 1 mg/mL pepsin (Sigma) at a concentration of 10 mg ECM/mL and stirred at room temperature for 48 h as previously described (Table 1B) [20]. After 48 h, B-ECM, SC-ECM, and UBM-ECM were completely digested and formed a pre-gel solution (pH ~ 2). The pre-gel ECM solution was brought to pH 7.4 using 0.01 N NaOH and diluted to the desired volume/salt concentration using 10× and 1 × PBS. Pepsin is irreversibly inactivated at pH above 7.5 [36].

Table 1
Methods for decellularization and solubilizing B-ECM, SC-ECM, and UBM-ECM.

A. Decellularization methods		
Step	B-ECM and SC-ECM (120 and 180 rpm respectively)	UBM-ECM (300 rpm)
1.	Deionized water soak (18–24 h)	Mechanical delamination
2.	0.025% Trypsin (1 h)	
3.	3% Triton × 100 (1 h)	
4.	1 M Sucrose (30 min.)	
5.	Deionized water soak (30 min.)	
6.	4% Deoxycholic acid (1 h)	
7.	0.01% Peracetic acid	0.01% Peracetic acid

B. Methods for solubilizing and digesting			
	B-ECM	SC-ECM	UBM-ECM
Particle size	<400 μm	400–1000 μm	400–1000 μm
Solubilization	0.01 N HCl	0.01 N HCl	0.01 N HCl
Digestion	1 mg/mL Pepsin	1 mg/mL Pepsin	1 mg/mL Pepsin

2.4. Collagen and sGAG quantification

Collagen concentration of the pre-gel ECM solution was determined for samples from each production batch with the Sircol Assay Kit (Biocolor Ltd., UK) following the manufacturer's recommended protocol ($n = 4$ in duplicate or triplicate). Sulfated glycosaminoglycan (sGAG) concentrations were determined using digested ECM at a concentration of 50 mg ECM/ml with 0.1 mg/ml proteinase K (Sigma) in buffer (10 mM Tris–HCl, pH 8.0, 100 mM NaCl, 25 mM EDTA for 48–72 h at 50 °C) using the Blyscan Sulfated Glycosaminoglycan Assay Kit (Biocolor Ltd., UK) and following the manufacturer's recommended protocol ($n = 3$ in duplicate or triplicate).

2.5. Scanning electron microscopy

Scanning electron microscopy was used to examine the surface topography of brain, spinal cord, and UBM-ECM hydrogels. Five hundred micron thick hydrogels were prepared and then fixed in cold 2.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA) for 24 h followed by three 30 min washes in 1× PBS. Hydrogels were dehydrated in a graded series of alcohol (30, 50, 70, 90, 100% ethanol) for 30 min per wash, and then placed in 100% ethanol overnight at 4 °C. Hydrogels were washed 3 additional times in 100% ethanol for 30 min each and critical point dried using a Leica EM CPD030 Critical Point Dryer (Leica Microsystems, Buffalo Grove, IL, USA) with carbon dioxide as the transitional medium. Hydrogels were then sputter-coated with a 4.5 nm thick gold/palladium alloy coating using a Sputter Coater 108 Auto (Cressington Scientific Instruments, UK) and imaged with a JEOL JSM6330f scanning electron microscope (JEOL, Peabody, MA, USA).

2.6. Turbidity gelation kinetics

Turbidimetric gelation kinetics were determined as previously described [37]. The pre-gel solution was diluted to 6 mg/mL and maintained on ice at 4 °C until placed into a 96 well plate (100 μL/well). The plate was immediately transferred to a spectrophotometer (Molecular Devices) preheated to 37 °C, and absorbance was measured at 405 nm every 2 min for 50 min. Normalized absorbance was calculated using Equation (1) and then used to calculate the following parameters: time required to reach 50% and 95% maximum absorbance was denoted as $t_{1/2}$ and t_{95}, respectively, the lag phase, t_{lag}, calculated by extrapolating the linear portion of the curve, and the turbidimetric speed, S, of gelation was determined by calculating the growth portion slope of the curve normalized to absorbance [20]. The assay was repeated three times with independent samples in triplicate.

$$\text{Normalized Absorbance} = \frac{A - A_0}{A_{max} - A_0} \quad (1)$$

2.7. Rheological measurements

The pH of the ECM digest was neutralized to 7.4 and diluted to 4, 6, or 8 mg ECM/ml. The diluted pre-gel solution was then placed on a 40 mm parallel plate rheometer (AR 2000, TA Instruments) at 1 Pa stress and 10 °C to ensure even distribution and the liquidity of the pre-gel solutions between the plates. A dynamic time sweep was run with the parameters of 5% strain (with the exception of spinal cord ECM gel at 8 mg/mL, which was run with 0.5% strain), 1 rad/s (0.159 Hz) and rapidly increasing temperature from 10 °C to 37 °C to induce gelation as indicated by a sharp increase and plateauing of the storage modulus (G'), and the loss modulus (G'') ($n = 3$ per gel per concentration).

2.8. N1E-115 ECM cytocompatibility and two-dimensional neurite extension

N1E-115 mouse neuroblastoma cells (ATCC No. CRL 2263), a commonly used experimental cell line to examine neurotrophic potential and differentiation [33,34], were cultured in DMEM (Sigma) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham MA, USA)/1% pen/strep (Sigma) at a concentration of 100,000 cells per well in 12 well plate prior to the addition of ECM. B-ECM, SC-ECM, or UBM-ECM digest was added after cell attachment at a concentration of 100 μg ECM/mL. Following 18–24 h in culture with ECM, the medium was removed and 4 μM calcein-AM and 4 μM ethidium homodimer-1 was added to each well to evaluate cytotoxicity. Live cells that hydrolyze membrane-permeable calcein-AM, but not ethidium homodimer-1, fluoresce in green and dead cells that bind and activate ethidium homodimer-1, but not calcein-AM, fluoresce in red.

Download English Version:

https://daneshyari.com/en/article/10229292

Download Persian Version:

https://daneshyari.com/article/10229292

Daneshyari.com