Acta Biomaterialia 52 (2017) 92–104

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Human placenta hydrogel reduces scarring in a rat model of cardiac ischemia and enhances cardiomyocyte and stem cell cultures ☆

Michael P. Francis [a,b,*], Erick Breathwaite [a], Anna A. Bulysheva [c], Frency Varghese [c,d], Rudy U. Rodriguez [a], Sucharita Dutta [b], Iurii Semenov [c], Rebecca Ogle [a], Alexander Huber [a], Alexandra-Madelaine Tichy [e], Silvia Chen [a], Christian Zemlin [c,d]

[a] LifeNet Health Institute of Regenerative Medicine, Virginia Beach, VA, United States
[b] Eastern Virginia Medical School, Norfolk, VA, United States
[c] Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA, United States
[d] Department of Electrical and Computer Engineering, Old Dominion University, Norfolk, VA, United States
[e] FH Campus Wien, Applied Life Sciences, Wien, Austria

ARTICLE INFO

Article history:
Received 27 August 2016
Received in revised form 7 December 2016
Accepted 9 December 2016
Available online 10 December 2016

Keywords:
Placenta
Extracellular matrix
Ischemia
Cardiomyocyte culture
Hydrogel
Decellularized

ABSTRACT

Introduction: Xenogeneic extracellular matrix (ECM) hydrogels have shown promise in remediating cardiac ischemia damage in animal models, yet analogous human ECM hydrogels have not been well development. An original human placenta-derived hydrogel (hpECM) preparation was thus generated for assessment in cardiomyocyte cell culture and therapeutic cardiac injection applications.
Methods and results: Hybrid orbitrap-quadrupole mass spectrometry and ELISAs showed hpECM to be rich in collagens, basement membrane proteins, and regenerative growth factors (e.g. VEGF-B, HGF). Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes synchronized and electrically coupled on hpECM faster than on conventional cell culture environments, as validated by intracellular calcium measurements. *In vivo*, injections using biotin-labeled hpECM confirmed its spatially discrete localization to the myocardium proximal to the injection site. hpECM was injected into rat myocardium following an acute myocardium infarction induced by left anterior descending artery ligation. Compared to sham treated animals, which exhibited aberrant electrical activity and larger myocardial scars, hpECM injected rat hearts showed a significant reduction in scar volume along with normal electrical activity of the surviving tissue, as determined by optical mapping.
Conclusion: Placental matrix and growth factors can be extracted as a hydrogel that effectively supports cardiomyocytes *in vitro*, and *in vivo* reduces scar formation while maintaining electrophysiological activity when injected into ischemic myocardium.

Statement of Significance

This is the first report of an original extracellular matrix hydrogel preparation isolated from human placentas (hpECM). hpECM is rich in collagens, laminin, fibronectin, glycoproteins, and growth factors, including known pro-regenerative, pro-angiogenic, anti-scarring, anti-inflammatory, and stem cell-recruiting factors. hpECM supports the culture of cardiomyocytes, stem cells and blood vessels assembly from endothelial cells. In a rat model of myocardial infarction, hpECM injections were safely deliverable to the ischemic myocardium. hpECM injections repaired the myocardium, resulting in a significant reduction in infarct size, more viable myocardium, and a normal electrophysiological contraction profile. hpECM thus has potential in therapeutic cardiovascular applications, in cellular therapies (as a delivery vehicle), and is a promising biomaterial for advancing basic cell-based research and regenerative medicine applications.

☆ Part of the Special Issue on Extracellular Matrix Proteins and Mimics, organized by Professor Katja Schenke-Layland.

* Corresponding author at: 700 West Olney Rd., Norfolk, VA 23507, United States.
E-mail addresses: mpf3b@virginia.edu (M.P. Francis), erick_breathwaite@lifenethealth.org (E. Breathwaite), abulyshe@odu.edu (A.A. Bulysheva), fvarg001@odu.edu (F. Varghese), rudy_rodriguez@lifenethealth.org (R.U. Rodriguez), duttasm@evms.edu (S. Dutta), Rebecca_ogle@lifenethealth.org (R. Ogle), alexander_huber@lifenethealth.org (A. Huber), czemlin@odu.edu (C. Zemlin).

http://dx.doi.org/10.1016/j.actbio.2016.12.027
1742-7061/

1. Introduction

Regenerative medicine-related therapeutic approaches to heart disease have ranged from the use of cellular and gene therapies, to complex extracellular matrix (ECM) hydrogel injections, each with varying degrees of success [1]. Cell therapy with cardiac progenitor cells injections, for example, has shown early promise [2–4]. However, cellular therapy studies show injected cells alone may not remain at the injection site long-term [5,6], suggesting that cells may act by mechanisms unrelated to the maintenance and long-term function, integration, or survival of the cells at the injection site. Rather, injected cells seemingly act via a paracrine effect, via growth factor secretions and extracellular matrix remodeling events [7,8]. If growth factor or ECM remodeling-related effects are in fact the causative therapeutic agent in myocardial cell therapies, alternative therapeutic approaches may be possible clinically that are more defined, reproducible, and controllable, which may provide a better treatment option compared to use of extraordinarily complex and thus far poorly defined cellular therapeutic strategies.

One such an alternative approach is seen in the delivery of ECM extracts to the ischemic heart, without the additional introduction of cells [9,10]. The use of ECM from the heart (from pericardium or myocardium) or as extracted from the Engelbreth-Holm-Swarm tumor [11] has surprisingly shown effectiveness in reducing damage from cardiac ischemia, including a reduction in scar formation, improved animal survival and overall health. These materials, derived from porcine and murine tissue origins, while effective in rodent and porcine ischemia models, have concerns of transmitting xenozoonoses clinically. Non-human animal tissues also poses risks of immune rejection from the cellular debris, or foreign (possibly tumorigenic) DNA, and also immunogenic epitopes remaining in any decellularized matrix of non-human animal tissue, such as alpha-galactosyl [12,13]. Such foreign material, if injected into the human heart, may stimulate immune reactions that further degrade cardiac health. These risks would be avoidable if the ECM hydrogels were produced from quality controlled, pathogen-screened human tissues with minimal cellular debris (e.g. DNA). Attempts to produce hydrogel from human myocardium have been made, however they failed to form gels and did not appear to persist at the injection sites [14,15], thus limiting clinical utility.

A human placenta tissue-derived ECM hydrogel biomaterial for cardiovascular cell culture and potential therapeutic applications was therefore designed and tested in this work, presenting a clinically-relevant material source from an abundantly available human donor tissue. ECM and growth factor-rich placenta-derived therapeutics (e.g. amniotic dressings) have been used clinically as a matrix to induce cellular migration and proliferation [16] and have been shown to promote the wound healing process [17]. Placenta tissue has been shown to act to reduce inflammation [18] and prevent scar tissue formation [19], while also being non-immunogenic [20]. Placenta products have been used clinically as wound dressings in the eye, heart, skin, bone, and other indications [17,19–23]. However, the use of a placenta-derived ECM preparation formulated as a hydrogel for applications *within* the heart for possibly treating and remediating ischemic injuries, along with use for human cell culture and additional applications, has yet to be explored.

2. Materials and methods

2.1. Extracellular matrix (ECM)

Human placentas were procured from 9 donors with research authorization through LifeNet Health's organ and tissue procurement service and stored frozen. Whole chorionic plate was ground, washed in water, then decellularized for 16–24 h using 2% N-lauryl sarcosine (Sigma Aldrich, St. Louis, MO). Detergent and cell debris was eluted in water with exchanges every hour until clear, after which a sample was taken for gross histological analysis with H&E staining to validate cell removal (processed at Bons Secours DePaul Medical Center, Norfolk, Virginia). The decellularized tissue was then collected by centrifugation at 500*G*, homogenized in 0.1 M hydrochloric acid with pepsin (Sigma-Aldrich, St. Louis, MO) and digested for 64–72 h, followed by irreversible inactivation of the pepsin at pH 8.0. The resulting liquid product of human placenta extracellular matrix (hpECM) was confirmed to form a gel at 37 °C while remaining liquid from room temperature to 4 °C. The total protein concentration in the hpECM was determined by the modified Lowry Protein Assay Kit (Pierce, Grand Island, NY) as described by the manufacturer's recommendations. The final hpECM preparations were confirmed to be free from mycoplasma (MycoAlert™ Plus, Lonza), contained <0.5 EU/ml of endotoxin (PyroGene™ Recombinant Factor C Endotoxin Detection Assay kit, Lonza), assessed for residual DNA contamination by Quant-iT PicoGreen™ (ThermoFisher Scientific, Carslbad, CA), with all tests performed per manufacturer instructions. Lots were also tested for sterility by direct liquid media inoculations.

For use, hpECM preparations were thawed on ice at 4 °C overnight or at room temperature for approximately an hour, then diluted in phosphate buffered saline to normalize protein concentrations (5 mg/ml). Liquid hpECM was handled without the use of pre-chilled tools and equipment and used immediately to prepare hydrogels (formed at 37 °C for 15–30 min in a humidified incubator with 5% CO_2), or used for cell delivery experiments in liquid form then cast at 37 °C after delivery. Cast hydrogels were used immediately for cell culture experiments or stored for further analysis. Other ECM substrates including Matrigel® Basement Membrane Matrix HC (354262, Corning Life Sciences, Tewksbury MA), fibronectin (F2006, Sigma Aldrich, St. Louis MO), and gelatin (G1890, Sigma Aldrich, St. Louis MO) were used according to manufacturer's recommendations.

2.2. Mass spectrometry

Protein concentration of hpECM was determined using a DTT compatible BCA assay (Thermo Fisher Scientific, San Jose CA) and 100 μg of extracted protein sample was run on a NuPAGE reducing gel (4–12% Bis-Tris Gel) (Life Technologies, Carlsbad CA) with NuPAGE MOPS SDS 1X buffer run at 200 V for about 10 min. The remaining protocol for complex protein clean up; protein alkylation with Iodoacetamide; protein cysteine bond reduction via Dithiothreitol and trypsin digestion was carried out following published protocols from our group [24]. Post tryptic digestion, peptides were analyzed on the Q-Exactive (Thermo Fisher Scientific, Waltham, MA) mass spectrometer as described previously [24].

Data informatics on the sample was carried out by applying the label-free precursor ion detection method (Proteome Discoverer, version 1.3, Thermo Fisher Scientific, Waltham, MA). The Sequest algorithm was used to identify peptides from the resulting MS/MS spectra by searching against the combined Human Protein Database (a total of 28,000 sequences) extracted from Swissprot (version 57) using taxonomy "Human." Searching parameters for parent and fragment ion tolerances were set as 15 ppm and 60 mmu for the Q-Exactive MS. Other parameters used were a fixed modification of carbamidomethylation–Cys, variable modifications of Phosphorylation (S,T,Y) and oxidation (Met). Trypsin was set as the primary protease and pepsin was set as the secondary cleavage enzyme since we used this enzyme in our workflow to generate the original biological material. We considered a maximum of 2 missed cleavages. Only those proteins that had >2 peptides identified (or >50% of protein covered by peptides) were included in the