Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 2929–2938
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Influence of ECM proteins and their analogs on cells cultured on 2-D hydrogels for cardiac muscle tissue engineering

Stephanie M. LaNasa, Stephanie J. Bryant *

Department of Chemical and Biological Engineering, University of Colorado at Boulder, Boulder, CO 80309, USA

Received 28 January 2009; received in revised form 26 April 2009; accepted 13 May 2009
Available online 18 May 2009

Abstract

This study assessed the role of immobilized cell adhesion moieties on controlling the cellular attachment, adhesion and phenotype of cardiac muscle cells towards developing scaffolds for cardiac muscle tissue engineering. Collagen I, laminin and the cell-adhesive oligopeptide, arginine-glycine-aspartic acid (RGD) at concentrations of 0.5 and 5 mM were covalently bound to flexible two-dimensional hydrogels. A robust skeletal myoblast cell line demonstrated good bioactivity for the modified hydrogels, resulting in myoblast attachment and development of an intracellular contractile network after 1 day. Primary neonatal rat ventricular myocytes cultured for up to 7 days, however, were more sensitive to the different modified substrates. Although total cardiomyocyte DNA content did not vary significantly with surface modification, immunostaining for the contractile protein Troponin I and focal adhesion protein vinculin revealed marked improvements in spreading and intracellular contractile protein deposition for cells attached to protein-modified hydrogels over those modified with RGD, regardless of RGD concentration. On the RGD-modified surfaces, cardiomyocytes self-associated, forming aggregates that exhibited a disorganized cytoarchitecture. Cardiomyocyte maturation was assessed through the fetal gene program where expression for atrial natriuretic peptide decreased and sarco(endo)plasmic reticulum Ca^{2+} increased with culture time for the protein-modified surfaces, indicating a trend towards maturation, while the α/β-myosin heavy-chain ratio remained near fetal expression levels for all surfaces. Overall, our findings suggest that whole proteins, collagen and laminin, are effective in promoting cardiomyocyte interaction with hydrogels and cardiomyocyte maturation while RGD does not provide adequate extracellular matrix cues for cardiomyocytes.
© 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Keywords: Cardiomyocyte; Skeletal myoblast; Hydrogel; Cell adhesion; Fetal gene program

1. Introduction

Nearly eight million Americans each year experience a myocardial infarction [1] and must cope with the side effects of compromised heart muscle function. Cell transplantation therapies have shown promise for improving heart function after myocardial infarction [2], but have several shortcomings. Most notably, only a small fraction of transplanted cells survive long term, the overall success of cell transplantation is varied and highly dependent on the cell type used, and the results have been less than optimal in humans [3,4]. The use of a three-dimensional (3-D) cell carrier may help overcome many of these shortcomings. By combining the benefits of cell transplantation with the structural guidance of a 3-D scaffold, myocardial tissue engineering offers a promising and exciting alternative therapy for creating a patch of living cardiac muscle.

Towards engineering the myocardium, several scaffold chemistries have been investigated, from naturally occurring systems, including alginate [5], collagen type I [6,7–9], gelatin [10], fibrin [11,12] and Matrigel™ [6], to synthetic systems, including polyurethane [13,14], poly(glycolic acid) [15,16], poly(lactide) co-polymers [17] and poly(glycerol sebacate) [18]. Synthetic scaffolds offer many advantages over natural scaffolds as their mechanical properties and

* Corresponding author. Tel.: +1 303 735 6714.
E-mail address: stephanie.bryant@colorado.edu (S.J. Bryant).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.05.011

degradation rates can be tailored. For synthetic-based scaffolds, a number of studies have focused on designing scaffolds with elastomeric properties that mimic the mechanical properties of the heart [18–20]. These materials are often hydrophobic where cell attachment is driven by nonspecific protein adsorption [21]. However, studies have reported that cardiomyocyte adhesion and organization into a contractile tissue have been far superior on natural scaffolds compared to synthetic scaffolds [6,22]. To circumvent this shortcoming, suspending cells in Matrigel™, collagen or fibrin gel within the pores of synthetic materials has shown improved cardiomyocyte performance within synthetic hydrogels [23,24].

Interestingly, there have been few efforts focused on immobilizing bioactive groups to synthetic scaffolds for cardiac muscle tissue engineering. A few studies have shown that proteins present in the basement membrane of the myocardium, e.g. laminin, promote cardiomyocyte adhesion in 2-D cultures [13,25]. An alternative to using whole proteins to promote cell adhesion to synthetic scaffolds is to incorporate cell-adhesive oligopeptides, enabling precise control over ligand presentation and density. The oligopeptide arginine-glycine-aspartic acid (RGD) is the most commonly used cell adhesion peptide because it is found in a number of adhesion proteins, including fibronectin, vitronectin, laminin and collagen type I [26]. Many cell types demonstrate good adhesion to synthetic scaffolds immobilized with RGD and often show comparable results to the full protein [27].

The goal of this study was to assess the role of immobilized cell adhesion moieties in controlling cellular attachment, adhesion and phenotype of cardiac muscle cells. Cells were grown on flexible 2-D hydrogel substrates based on poly(2-hydroxyethyl methacrylate) (poly(HEMA)) and poly(ethylene glycol) (PEG) immobilized with full proteins or oligopeptides. Hydrogels formed from poly(HEMA) and PEG are particularly attractive because they do not promote protein adsorption or cell adhesion, thereby acting as a blank platform into which bioactive groups may be incorporated in a highly controlled manner. Specifically, we explored two proteins which are present in the native myocardium—collagen type I, an interstitial protein, and laminin, a basement membrane protein—and the oligopeptide RGD, which is a cell adhesion site present in both collagen type I and laminin. Hydrogels were initially screened with a skeletal myoblast cell line, which served as a robust type of muscle cell to test cell attachment to the bioactive hydrogels and from which to compare the more sensitive primary cardiomyocytes. Cell attachment was assessed by total DNA content, while cell adhesion and phenotype were examined by immunofluorescence for contractile and focal adhesion proteins. Cardiomyocyte phenotype was further assessed through gene expression for proteins involved in the fetal gene program, which has been used as an indicator of both cell maturity as well as a pathological phenotype [28]. Findings from this study will aid in designing suitable synthetic hydrogel scaffolds for cardiac muscle tissue engineering.

2. Materials and methods

2.1. Fabrication of 2-D hydrogel films

Hydrogel films were fabricated by two different methods depending on the incorporation of oligopeptides (i.e. RGD) or full proteins (i.e. collagen I or laminin).

For hydrogels immobilized with RGD, poly(ethylene glycol) diacrylate (PEGDA, MW 3000) was synthesized as previously described [29] and verified through ^{1}H nuclear magnetic resonance spectroscopy (Varian YVR-500S). The peptide sequence, NH_3–Tyr–Arg–Gly–Asp–Ser–COOH (YRGDS, BaChem), was reacted with acryloyl-PEG-NHS (MW 3400, Nektar Therapeutics) in a 50 mM sodium bicarbonate buffer, pH 8.4, for 2 h at room temperature. The product, acryloyl-PEG-RGD, was dialyzed and lyophilized for 48 h. Percent conjugation was determined by use of a fluoraldehyde reagent (Pierce Biotechnology). The acryloyl-PEG-RGD macromer at concentrations of 0.5 or 5 mM was incorporated into a solution comprised of 20% w/w PEGDA and 0.12% (w/w) photoinitiator (1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one, Irgacure 2959, Ciba Specialty Chemicals) in distilled, deionized water. The solution was poured between two glass slides spaced 0.8 mm apart, polymerized under 365 nm UV light (5 mW cm^{-2}) for 10 min, and rinsed in a deionized water-bath overnight. Individual 5 mm diameter discs were punched from the hydrogel films, soaked in 70% ethanol for 2 h, then rinsed four times in sterile phosphate-buffered saline (PBS). The moist hydrogels were stored in a sterile, sealed container at 4 °C until use.

For protein-modified hydrogels, a macromer solution was prepared by combining 6.7% (w/w) poly(ethylene glycol) dimethacrylate (MW 3000), which was synthesized as described elsewhere [29], 81.5% (w/w) 2-hydroxyethyl methacrylate (HEMA, Polysciences, Inc.), 0.17% photoinitiator (1-hydroxy-cyclohexyl-phenyl-ketone, Irgacure 184, Ciba Specialty Chemicals) in a 57% deionized water/43% ethylene glycol solution. HEMA was chosen because it contains a pendant hydroxyl group within each repeat unit, which can be readily modified with proteins after hydrogel fabrication [30]. The solution was poured between two glass slides coated with a thin layer of glycerol to prevent the hydrogel from adhering to the glass surface, spaced 0.8 mm apart. The solution was polymerized under 365 nm UV light (5 mW cm^{-2}) for 15 min. The hydrogel was soaked in a deionized water-bath overnight. Individual 5 mm discs were punched from the hydrogel films, sterilized with 70% ethanol for 2 h, and dried under high vacuum for 2 h. The free hydroxyl groups were reacted with 100 mM CDI in dry acetone, as described previously [30]. Collagen Type I (rat tail, Upstate Cell Signaling Solutions, 3.56 mg ml^{-1}) or laminin (Upstate Cell Signaling Solutions, 1.6 mg ml^{-1}) was diluted in sterile PBS to create a protein solution at a concentration of 667 μg ml^{-1}. Hydrogel discs were each placed in 0.8 ml chilled protein solutions for 48 h on an orbital shaker. Hydrogels were transferred to sterile PBS and stored at 4 °C until use.

ID	Title	Pages
1104	Influence of ECM proteins and their analogs on cells cultured on 2-D hydrogels for cardiac muscle tissue engineering	10

Download Full-Text Now

http://fulltext.study/article/1104

FullText.study

Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search

Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price

Free Downloadable Articles
In each journal some of the articles are available to download for free

Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

http://FullText.Study