Biomaterials 59 (2015) 39–52

Contents lists available at ScienceDirect

Biomaterials

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

Three-dimensional, soft neotissue arrays as high throughput platforms for the interrogation of engineered tissue environments

Michael Floren, Wei Tan*

Department of Mechanical Engineering, University of Colorado at Boulder, Boulder, CO 80309, USA

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form
17 April 2015
Accepted 21 April 2015
Available online 15 May 2015

Keywords:
Stem cell differentiation
3-Dimensional cell culture
High-throughput screening
Extracellular matrix

ABSTRACT

Local signals from tissue-specific extracellular matrix (ECM) microenvironments, including matrix adhesive ligand, mechanical elasticity and micro-scale geometry, are known to instruct a variety of stem cell differentiation processes. Likewise, these signals converge to provide multifaceted, mechanochemical cues for highly-specific tissue morphogenesis or regeneration. Despite accumulated knowledge about the individual and combined roles of various mechanochemical ECM signals in stem cell activities on 2-dimensional matrices, the understandings of morphogenetic or regenerative 3-dimenstional tissue microenvironments remain very limited. To that end, we established high-throughput platforms based on soft, fibrous matrices with various combinatorial ECM proteins meanwhile highly-tunable in elasticity and 3-dimensional geometry. To demonstrate the utility of our platform, we evaluated 64 unique combinations of 6 ECM proteins (collagen I, collagen III, collagen IV, laminin, fibronectin, and elastin) on the adhesion, spreading and fate commitment of mesenchymal stem cell (MSCs) under two substrate stiffness (4.6 kPa, 20 kPa). Using this technique, we identified several neotissue microenvironments supporting MSC adhesion, spreading and differentiation toward early vascular lineages. Manipulation of the matrix properties, such as elasticity and geometry, in concert with ECM proteins will permit the investigation of multiple and distinct MSC environments. This paper demonstrates the practical application of high through-put technology to facilitate the screening of a variety of engineered microenvironments with the aim to instruct stem cell differentiation.

1. Introduction

There is abundant evidence suggesting that local signals from tissue-specific extracellular matrix microenvironments significantly affect cellular differentiation, phenotypic expression and maintenance [1–3]. Substrate biophysical signals, such as soluble factors [3], cell–ligand interactions [4], matrix elasticity [5,6] and geometry [7] play critical roles in a diversity of biological events including cell adhesion, growth, differentiation, and apoptosis [7,8]. Together these signals converge to provide a multifaceted, complex mechanochemical signaling environment for highly-specific tissue morphogenesis and regeneration. Despite accumulated knowledge regarding individual and combined roles of various mechanochemical ECM signals in stem cell activities, the intricacy exhibited by cellular microenvironments poses a considerable challenge in resolving the mechanisms ascribed to stem cell behavior and fate determination processes. This complexity mandates a systemic approach whereby integrative studies must be expanded to capture a more comprehensive understanding of the determinants which direct stem cell differentiation toward desired cell type and function. Conventional methods to elucidate these mechanisms have traditionally been executed in large scale, two-dimensional tissue culture platforms which are often limited by combinatorial brevity, substrate production, and reagent supply. Furthermore, these signals, matrix and biophysical, are often observed independently to differentiate cells on 2-dimensional substrates, an environment vastly different from the way cells are presented naturally *in vivo*, i.e. a 3-dimensional tissue context which elicits multiple signal inputs to regulate cell fate.

High through-put approaches have emerged in recent years to circumvent the limitations of traditional low through-put techniques (i.e. conventional cultureware), with the promise to develop complex platforms for combined biomolecule/substrate discovery. The salient features of microarray technology include the

* Corresponding author. Department of Mechanical Engineering, 427 UCB University of Colorado at Boulder, CO 80309-0427, USA.
E-mail address: wtan@colorado.edu (W. Tan).

http://dx.doi.org/10.1016/j.biomaterials.2015.04.036
0142-9612/

reproducibility and screening of multiple microenvironments with significantly less reagent and substrate requirements than traditional methods, while lending improved deconstruction of complex multivariable studies [9]. Several reports have demonstrated ECM protein microarrays [10], soluble factor screening [11], biomaterial chemistry screening [12,13], and multiple signal integration arrays (i.e. elasticity and chemical factor) with encouraging results [14,15]. However, despite the versatility afforded by current microarray technologies, the incorporation of multiple signals within engineered microarrays remain limited. Meanwhile the integration of current combinatorial microarray technologies in three-dimensions, coupled with other biophysical properties, such as tunable stiffness and geometry, have yet to reach fruition. Capturing complex, multifaceted 3-dimensional environments in high-throughput with combinatorial signaling will likely prove instrumental towards the design of future tissue regeneration biomaterial platforms.

To resolve the mechanisms associated with complex matrix signals and stem cell behavior and fate decisions, we established a high-throughput ECM platform based on soft, fibrous matrices meanwhile highly-tunable in elasticity and 3-dimensional geometry. The technology we demonstrate here is amenable to manipulation of several matrix properties, such as elasticity and geometry, in concert with customizable ECM protein micro-dot combination. Furthermore, selective cellular adhesion and isolation afforded by ECM microarrays permits the investigation of multiple and distinct cellular microenvironments in the presence of specific ECM signaling. Altogether, we demonstrate the practical adaptation of high-throughput technology to facilitate the screening of various tunable mechano-ligand microenvironments in three dimensions with the aim to optimize stem cell fate decisions.

2. Methods

2.1. Materials

Polyethylene glycol dimethacrylate (PEGDM) with a molecular weight of 750 and polyethylene oxide (PEO) (MW 400 kDa) were purchased from Sigma (St. Louis, MO). The photointiator Irgacure® 2959 was purchased through Ciba Specialty Chemicals Corp. (Tarrytown, NY). (3-trimethoxysilyl)propyl methacrylate (TMPMA) was purchased through Sigma. Rhodamine-methacrylate was supplied by Polysciences, Inc. (Warrington, PA). Albumin-Cy3 and streptavidin-Cy5 protein conjugates we acquired through Life Technologies (Grand Island, NY). Microarray print buffer components, glycerol, triton X-100, were purchased through Sigma. Collagen I extracted from rat tail was supplied by Sigma. Collagen III and collagen IV were extracted by human placenta and provided by Sigma. Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane was acquired through Sigma. Fibronectin purified from human plasma was obtained through EMD Millipore Corp. (Temecula, CA.). α-elastin extracted from bovine ligament was purchased from Elastin Products Co (Owensville, Missouri). Anti-collagen I, anti-collagen III, anti-collagen IV, anti-laminin, anti-fibronectin and anti-elastin primary antibodies were obtained from EMD Millipore Corp. Secondary antibody Cy3 conjugate was purchased through EMD Millipore Corp. Primary rat pulmonary arterial smooth muscle cells (PASMCs) were maintained in DME-F12 (Hyclone, Logan, UT), with 10% fetal bovine serum (FBS, Atlanta Biologicals, Flowery Branch, GA) and 1% Pen/Strep (Hyclone, Logan, Ut). Rat mesenchymal stem cells were maintained in DMEM (Corning, Corning, New York) with 10% defined FBS (Hyclone) for MSCs and 1% Penn/Strep (Hyclone). Bovine serum albumin (BSA) was obtained from Sigma. (4′,6-diamidino-2-phenylindole) DAPI nuclear stain and Alexa488-phallodin cytoskeleton stain were purchased through Invitrogen, Inc. (Eugene, OR). Primary anti-PECAM antibody was supplied through Novus Biologicals (Littleton, CO). Secondary anti-rabbit IgG antibody conjugated with Alexa 555 was acquired through Invitrogen, Inc. Vectashield hard mount mounting media was obtained through Vector Laboratories, Inc. (Burlingame, CA).

2.2. Fabrication of PEGDM soft matrices

An electrospinning solution composed of 3.2% wt PEGDM 750, 3.4% wt PEO, 0.4% wt of Irgacure 2959 and 93% DI H_2O was mixed for 30 min with magnetic stir bar. PEGDM 750 photopolymerizable soft matrices were fabricated by electrospinning on a custom setup comprised of a high voltage power supply (Gamma High Voltage Research, Ormond Beach, FL), grounded collecting surface, motorized syringe pump (NE-300 New Era Pump Systems, Farmingdale, NY), and a 14 mm syringe. The solution (2 ml) was spun at a distance of 26 cm from the stationary collecting surface, at the voltage of 30 kV, and a flow rate of 1.10 ml/h. Electronspun matrices were deposited onto standard glass slides (25 mm × 75 mm, Fischer Scientific Inc.) that were pretreated with TMPMA to present methacrylate groups that can bond the matrices to the glass. PEGDM matrices were subsequently introduced into an inert argon environment to remove oxygen, and then were stabilized with polymerization under UV exposure (352 nm light) with an average intensity of 5 mW/cm^2 for predetermined time durations.

2.3. Characterization of PEGDM soft matrices

2.3.1. FTIR analysis

PEGDM electrospun samples were first loaded into a sealed liquid-cell (Sigma), in the presence of an inert argon environment to prevent oxygen contamination during IR acquisition. The double bond conversion in PEGDM was evaluated using a real-time mid-range Fourier transform infrared spectroscopy (FTIR) (Nicolet 4700, Thermo Fisher Scientific, Waltham, MA) by examining the disappearance of the C═C peak within the methacrylate group (at ~1635 nm) over time during polymerization under UV light (5 mW/cm^2). To account for sample and background variation, data were normalized with the C═O peak located in the range from 1650 to 1726 cm^{-1}.

2.3.2. Scanning electron microscopy imaging

Scanning electron microscopy (FESEM, JSM-7401F, Jeol Ltd, Tokyo, Japan) was used to examine the microstructure of the electrospun PEGDM substrates in both dry and hydrated states. For hydrated samples, substrates were photopolymerized for 15 min and rinsed in DI H_2O for 24 h. To prepare for imaging, rinsed samples were shock frozen in liquid nitrogen (−195 °C), and lyophilized for approximately 24 h. ImageJ was used to analyze changes in fiber diameter and porosity.

2.3.3. Fluorescent imaging

To image the structure of PEGDM soft matrices in their hydrated state, rhodamine-methacrylate was introduced into the electrospun fibers and subsequently stabilized with UV exposure to provide fluorescence of the fibrous structure. Matrices with PEGDM-rhodamine conjugates were then visualized using either a fluorescent microscope or a confocal laser scanning microscope.

2.3.4. Rheology

Changes in the storage modulus (G′) of PEGDM substrates with respect to photopolymerization time were characterized using a rheometer, (ARES TA rheometer, TA Instruments, New Castle, DE).