ARTICLE IN PRESS

Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Designing a fibrotic microenvironment to investigate changes in human liver sinusoidal endothelial cell function

Andrew J. Ford [a], Gaurav Jain [b], Padmavathy Rajagopalan [a,b,c,*]

[a] *Department of Chemical Engineering, Virginia Tech, Blacksburg, VA 24061, United States*
[b] *School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA 24061, United States*
[c] *ICTAS Center for Systems Biology of Engineered Tissues, Virginia Tech, Blacksburg, VA 24061, United States*

A R T I C L E I N F O

Article history:
Received 12 December 2014
Received in revised form 8 June 2015
Accepted 23 June 2015
Available online xxxx

Keywords:
Liver sinusoidal endothelial cells
Kupffer cells
Fibrosis
Fenestrae
Matrix stiffness

A B S T R A C T

The deposition of extracellular matrix (ECM) proteins by hepatic cells during fibrosis leads to the stiffening of the organ and perturbed cellular functions. Changes in the elasticity of liver tissue are manifested by altered phenotype in hepatic cells. We have investigated changes in human liver sinusoidal endothelial cells (hLSECs) that occur as the elastic modulus of their matrix transitions from healthy (6 kPa) to fibrotic (36 kPa) conditions. We have also investigated the role played by Kupffer cells in the dedifferentiation of hLSECs. We report the complete loss of fenestrae and the expression of CD31 at the surface as a result of increasing elastic moduli. LSECs exhibited a greater number of actin stress fibers and vinculin focal adhesion on the stiffer substrate, as well. A novel finding is that these identical trends can be obtained on soft (6 kPa) substrates by introducing an inflamed microenvironment through the addition of Kupffer cells. hLSEC monocultures on 6 kPa gels exhibited fenestrae that were 140.7 ± 52.6 nm in diameter as well as a lack of surface CD31 expression. Co-culturing hLSECs with rat Kupffer cells (rKCs) on 6 kPa substrates, resulted in the complete loss of fenestrae, an increase in CD31 expression and in a well-organized cytoskeleton. These results demonstrate that the increasing stiffness of liver matrices does not solely result in changes in hLSEC phenotype. Even on soft substrates, culturing hLSECs in an inflamed microenvironment can result in their dedifferentiation. Our findings demonstrate the interplay between matrix elasticity and inflammation in the progression of hepatic fibrosis.

© 2015 Published by Elsevier Ltd. on behalf of Acta Materialia Inc.

1. Introduction

Liver fibrosis is a leading cause of death [1–3]. This health condition can lead to hepatic carcinomas, renal failure, toxin-induced comas, bleeding, and a host of metabolic disorders [1]. Hepatic fibrosis distorts liver architecture causing resistance to blood flow resulting in organ dysfunction [1–3]. At its core, hepatic fibrosis is an uncontrolled wound healing mechanism. Alcohol abuse, obesity, diabetes or hepatitis C viral infections are some initiating events that induce fibrosis [2]. When hepatic cells experience altered signaling they respond by secreting inflammatory molecules and extra-cellular matrix (ECM) proteins [1,4,5]. These secretions change the rigidity and chemical properties of liver matrices that cells are exposed to, thereby resulting in altered cellular phenotypes [1]. As hepatic fibrosis occurs, an abundance of ECM proteins are produced by hepatic stellate cells (HSCs) and accumulate in the region between the sinusoidal endothelium and the liver parenchyma, known as the Space of Disse. This buildup of ECM causes changes in the liver microarchitecture and can result in as much as a 6-fold increase in liver stiffness [6,7].

Liver sinusoidal endothelial cells (LSECs) are specialized endothelial cells and play a critical role in maintaining normal liver homeostasis [2]. LSECs line the blood vessels in the liver forming a semi-permeable barrier between the blood and liver parenchyma [8,9]. They play a vital role in the balance of lipids and cholesterol [2,8,10]. Through their scavenging properties, these cells eliminate components of connective tissues such as proteins and proteoglycans as well as debris from cell turnover [11]. LSECs exhibit pores called fenestrae that are approximately 100–200 nm in diameter [2,9]. In LSECs, fenestrae enable the transport of nutrients and other molecules contained in blood between the sinusoidal lumen and the liver parenchyma. Under healthy conditions, LSECs do not exhibit the surface marker cluster of differentiation 31 (CD31) [12].

Associated with hepatic fibrosis is the process of capillarization, in which a complete basement membrane is formed underneath

* Corresponding author at: Department of Chemical Engineering, School of Biomedical Engineering and Sciences, ICTAS Center for Systems Biology of Engineered Tissues, Virginia Tech, Blacksburg, VA 24061, United States.

E-mail addresses: ford89@vt.edu (A.J. Ford), jaing@vt.edu (G. Jain), padmar@vt.edu (P. Rajagopalan).

http://dx.doi.org/10.1016/j.actbio.2015.06.028
1742-7061/© 2015 Published by Elsevier Ltd. on behalf of Acta Materialia Inc.

Please cite this article in press as: A.J. Ford et al., Designing a fibrotic microenvironment to investigate changes in human liver sinusoidal endothelial cell function, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.06.028

the sinusoidal endothelium, hindering the transport of macromolecules [13,14]. During capillarization, LSECs also experience a decrease in the size and number of fenestrae and eventually their complete disappearance [15,16]. Although, it is well known that the presence of fenestrae and their characteristics are reliant on the health of liver tissues, few studies have focused upon the relationship between LSECs and substrate rigidity [17]. There have been detailed investigations on how LSEC phenotype changes in response to being cultured on different types ECM [18,19], however these studies do not elaborate on the mechanical properties of these ECM substrates. To the best of our knowledge, only one study has investigated the changes LSECs experience in response to differing rigidities [20]. Juin et al. examined the ability of LSECs to form podosomes (actin-rich protrusions involved in cell adhesion, migration, invasion and extracellular matrix degradation), when seeded on polyacrylamide hydrogels ranging in Young's Moduli from 1.75 to 20 kPa.

Critical to the progression of hepatic fibrosis, are the complex interactions between the parenchymal (hepatocytes) and non-parenchymal cells of the liver such as LSECs, Kupffer cells (KCs) and HSCs [3]. KCs, the resident macrophages of the liver, are known to secrete a number of inflammatory cytokines including tumor necrosis factor α (TNF-α), transforming growth factor β (TGF-β), interleukin 1α (IL-1α), and interleukin 6 (IL-6) [11]. The release of these cytokines can lead to the activation of HSCs, which in response secrete large amounts of ECM and continue the progression of fibrosis [1–3].

There have been reports in the literature that have shown the effects of other hepatic cell types, such as hepatocytes and HSCs, on LSEC phenotype [15,19,21,22]. There are a few studies conducted *in vivo* that have examined how KCs may regulate LSEC behavior. It has been shown that in response to bile duct ligation in a rat model there is an increase in the number of KCs present in the liver correlating with an increase in the degree of fibrosis [23]. The study by Hutchins et al. [24] reported that in response to cecal ligation and puncture surgery to induce sepsis in mouse models KCs acted as potentiators of LSEC injury. The authors reported an increase in the interaction of programmed death receptor 1 (PDR1) on KCs with programmed death ligand 1 on LSECs leading to the decline of normal endothelial function. However, changes in fenestrae, signaling molecules and the effects of KCs were not investigated. To the best of our knowledge, there is no other study that has investigated the role of KCs on hLSEC behavior in a fibrotic microenvironment.

The onset and progression of liver fibrosis is a consequence of membrane rigidity, the secretion of inflammatory molecules and altered cellular signaling. In this study, we have examined the effect of matrix rigidity alone on phenotypic changes in human LSEC (hLSEC) monocultures. We have designed collagen gels exhibiting Young's moduli of 6 and 36 kPa to closely mimic the stiffness found in a healthy and fibrotic liver, respectively [6,7]. To recapitulate the inflammatory microenvironment, we have co-cultured hLSECs with rat KCs (rKCs). We report significant changes in hLSEC fenestrae, CD31 expression, actin organization and focal adhesions as well as in the levels of vascular endothelial growth factor (VEGF) and TNF-α as a result of a fibrotic and inflamed microenvironment.

2. Materials and methods

2.1. Materials

Glutaraldehyde (25%v/v) was purchased from Electron Microscopy Sciences, Hatfield, PA. Bovine calf serum (BCS) was purchased from Hyclone, Logan, UT. Insulin was purchased from MP Biomedicals, Santa Ana, CA. Medium 199, endothelial cell growth supplement, L-glutamine, fetal bovine serum (FBS), penicillin–streptomycin, hexamethyldisilazane (HMDS), sodium-cacodylate, osmium tetroxide solution (4%v/v), chloroform, and β-mercaptoethanol were purchased from Sigma Aldrich, St. Louis, MO. Dulbecco's Modified Eagle Medium (DMEM) and phosphate buffered saline (PBS) were purchased from Invitrogen Life Technologies, Carlsbad, CA. All other chemicals and supplies were purchased from Thermo Fisher Scientific, Waltham, MA unless otherwise specified.

2.2. Casting collagen gels

Type I collagen solutions were diluted in 1X PBS to obtain solutions at two different concentrations. The final concentration of each solution was either 1.1 or 4.4 mg/mL. Prior to casting gels, the diluted collagen solutions were mixed with 10X DMEM at a ratio of 9:1. The pH of collagen solutions was maintained between 6.9 and 7.0 for all experiments. Glass coverslips (18 mm, Fisher Scientific) were activated using previously published procedures [25]. Briefly, coverslips were coated with NaOH and 3-aminopropyl tri-ethoxysilane for 10 min each and placed in glutaraldehyde (8%v/v) overnight. The activated coverslips were coated with collagen (0.3 mL/coverslip) and incubated at 37 °C for 1 h to promote crosslinking.

2.3. Profilometry

A Veeco Dektak 150 (Bruker, Billerica, MA) profiler was used to determine the hydrated thickness of the collagen gels. The thickness of the gels was determined by scanning across the gels at five different locations on three samples. The height of the gel was calculated by the difference in height between the surface of the gel and the underlying coverslip. Prior to taking measurements, the gels were placed in culture medium for a minimum of 2 h to ensure hydration. Thickness measurements were taken within 10 min of removing the gels from culture medium to prevent dehydration.

2.4. Atomic force microscopy (AFM)

Young's modulus (YM) measurements of collagen hydrogels were obtained on hydrated samples using a Veeco MultiMode AFM (DNP-10, Veeco, Santa Barbara CA) equipped with a liquid cell chamber. The liquid cell chamber provided an enclosed environment with a watertight seal to enable measurements on hydrated samples. All measurements were conducted in contact mode using pyramidal SiN cantilever tips (Bruker AFM Probes, Camarillo, CA) with a spring constant of 0.06 N m^{-1} (minimum value = 0.03 N m^{-1}, maximum value = 0.12 N m^{-1}). Force–distance curves were obtained at 1 Hz for a Z-scan distance of 1 μm using blunted tips with a half open angle of 18°. The elastic modulus was obtained by fitting the raw data to a modified Hertz cone model using Eqs. (1) and (2) [25].

$$F = k(d - d_o) \quad (1)$$

$$F = \frac{2\tan\alpha}{\pi}\left[\frac{E}{1-\nu^2}\right]\delta^2 \quad (2)$$

where F = applied force, α = 18°, E = YM, k = spring constant of the cantilever, υ = Poisson's ratio (constant = 0.25) [26], d = deflection of the cantilever, d_o = deflection point during contact, and δ = indentation. Force distance curves were fit for indentations up to 10% of the overall hydrogel thickness to eliminate any contribution from the rigid glass coverslip.

Please cite this article in press as: A.J. Ford et al., Designing a fibrotic microenvironment to investigate changes in human liver sinusoidal endothelial cell function, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.06.028

Download English Version:

https://daneshyari.com/en/article/6483532

Download Persian Version:

https://daneshyari.com/article/6483532

Daneshyari.com