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Mechanically- and Chemically-Tunable Cell Culture System to Study Myofibroblast Phenotype

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Abstract

Cell culture systems for studying the combined effects of matrix proteins and mechanical forces on the behavior of soft tissue cells have not been well developed. Here, we describe a new biomimetic cell culture system that allows for the study of mixtures of matrix proteins while controlling mechanical stiffness in a range that is physiological for soft tissues. This system consists of layer-by-layer (LbL)-assembled films of native matrix proteins atop mechanically tunable soft supports. We used hepatic stellate cells, which differentiate to myofibroblasts in liver fibrosis, for proof-of-concept studies. By culturing cells on collagen and lumican LbL-modified hydrogels, we demonstrate that this system is noncytotoxic and offers a valid control substrate, that the hydrogel determines the overall system mechanics, and that the addition of lumican to collagen influences the stellate cell phenotype. LbL-modified hydrogels offer the potential to study the influence of complex environmental factors on soft-tissue cells in culture.

Keywords

lumican, hepatic stellate cell, hydrogel, polyacrylamide, elastic modulus, liver fibrosis, layer-by-layer assembly

Disciplines

Cell and Developmental Biology | Medical Neurobiology | Neurology | Neuroscience and Neurobiology

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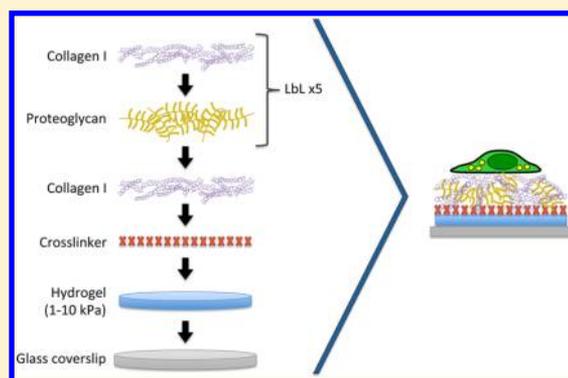
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ABSTRACT: Cell culture systems for studying the combined effects of matrix proteins and mechanical forces on the behavior of soft tissue cells have not been well developed. Here, we describe a new biomimetic cell culture system that allows for the study of mixtures of matrix proteins while controlling mechanical stiffness in a range that is physiological for soft tissues. This system consists of layer-by-layer (LbL)-assembled films of native matrix proteins atop mechanically tunable soft supports. We used hepatic stellate cells, which differentiate to myofibroblasts in liver fibrosis, for proof-of-concept studies. By culturing cells on collagen and lumican LbL-modified hydrogels, we demonstrate that this system is noncytotoxic and offers a valid control substrate, that the hydrogel determines the overall system mechanics, and that the addition of lumican to collagen influences the stellate cell phenotype. LbL-modified hydrogels offer the potential to study the influence of complex environmental factors on soft-tissue cells in culture.



INTRODUCTION

Interactions between cells and their surrounding environment are key determinants of phenotype. A large body of literature has documented the role of cell interactions with matrix proteins, and there are increasing numbers of studies demonstrating that mechanical forces are critical factors in driving cell behavior.¹ This has been well demonstrated for stem cells, cancer cells, and fibrogenic myofibroblasts in wound healing and fibrosis.^{2–6}

Cell culture systems for studying the combined effect of matrix proteins and mechanical forces on cell phenotype, however, are inadequate. Although mechanically tunable substrates have been developed and differential effects of elasticity and matrix coatings on fibroblast phenotype have recently been reported,⁷ most culture systems permit only limited study of the cell response to chemical features of the matrix environment. Similarly, matrix proteins are rarely studied in complex mixtures and are often overlaid on tissue culture plastic or glass such that mechanical interactions with the underlying nonphysiologically stiff substrate predominate over cell–matrix interactions. The lack of appropriate cell culture systems has particularly hindered the study of minor components of the extracellular matrix (such as proteoglycans) that have important effects on cell behavior by virtue of their chemical and mechanical interactions with more abundant matrix proteins such as collagens.

Films generated by the layer-by-layer (LbL) deposition of positively and negatively charged polymers onto a substrate are an attractive system for studying mixtures of natural polyelectrolytes such as proteins.^{8–11} LbL assemblies of proteins offer the advantage of controlled and uniform charge-based interactions between constituent molecules, without chemical cross-linking, and for the potential to generate thick protein mixtures. This technique has previously been used to study the role of proteoglycans in determining cell phenotype. Chen et al. mixed the small leucine-rich proteoglycan decorin with collagen I and used this mixture to coat synthetic LbL films, demonstrating differences in hepatocyte metabolic function depending on the presence or absence of decorin.⁸ These synthetic LbL films were assembled atop glass slides and were mechanically tunable, enabling the study of cell behavior in response to varying substrate stiffness (although stiffnesses were suprphysiological). Mhanna et al. extended this technique, taking advantage of the opposite charges of collagen and glycosaminoglycans at certain pH values to generate LbL systems consisting entirely of collagen and chondroitin sulfate (a common glycosaminoglycan modifying proteins).⁹ This study was novel because, unlike other LbL studies, it bypassed the use of synthetic, non-

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biological polymers. However, it did not evaluate the cellular response to underlying substrate stiffness or potential changes in stiffness due to the LbL assembly. Other groups have attempted to extend these LbL techniques by generating multilayers atop substrates softer than glass or tissue culture plastic. Gaudière et al., for example, generated a biomimetic system of six bilayers composed of the biopolymers poly-L-lysine and chondroitin-4-sulfate atop PDMS. These LbL-modified PDMS substrates had stiffnesses in the MPa range; preosteoblast behavior on these substrates varied according to their stiffness.¹² Most of these studies have used poly-(dimethylsiloxane) (PDMS) as the foundation, with a few notable exceptions.¹³ Although the stiffness of PDMS can be tuned, the range over which its modulus can be varied ($G' \approx$ kPa–MPa) is significantly higher than the modulus of soft tissues ($G' \approx$ Pa–kPa).

Our goal was to develop a cell culture system combining LbL-assembled films of native matrix proteins with mechanically tunable soft supports and to demonstrate its relevance to cell phenotype in proof-of-concept studies. As a model cell type, we used hepatic stellate cells, which differentiate into fibrogenic myofibroblasts in the setting of liver injury, leading to liver fibrosis.¹⁴ This phenotypic change is typically and easily monitored by the acquisition of α -smooth muscle actin (α -SMA) expression and an increase in cell area. We have previously demonstrated that stellate cell differentiation is sensitive to the stiffness of the underlying support, with cells on soft supports ($G' < 1$ kPa) exhibiting a quiescent, non-myofibroblastic phenotype and cells on stiff supports ($G' > 8$ kPa) exhibiting a fibrogenic, myofibroblastic phenotype.⁵ As model matrix proteins, we used collagen I and the small leucine-rich proteoglycan lumican. The deposition of both proteins increases significantly in liver fibrosis, and they are known to interact in physiological settings, with lumican contributing to the proper organization of collagen fibrils.¹⁵ Additionally, recent studies using lumican null mice have demonstrated that lumican is necessary for the development of liver fibrosis in animal models.¹⁶

Because collagen and lumican are oppositely charged proteins, they can be used in LbL assembly to set up a matrix more closely mimicking their in vivo interactions than is possible in a thin layer resulting from the use of cross-linked proteins. We built these LbL-assembled protein mixtures on top of mechanically tunable polyacrylamide hydrogels to form chemically and mechanically relevant cell culture substrates we term LbL-modified hydrogels. We report here the successful use of this system to demonstrate a role for both the matrix environment and substrate stiffness in hepatic stellate cell myofibroblastic differentiation.

■ EXPERIMENTAL SECTION

Layer-by-Layer Assembly on Glass Slides. For initial experiments, LbL-assembled films of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) were assembled on glass slides as previously described, adjusting the pH to control the mechanical stiffness as defined by E .¹⁷ Because the average layer thickness is inversely proportional to pH, films built from polymer solutions of pH 2.0 and 4.0 went through a total of 11 and 15 dipping cycles, respectively, in order to maintain a constant film thickness.⁸ To complete one LbL assembly cycle, glass slides were dipped into a 10 mM solution of PAH for 30 min, followed by three rinse–bath dippings for 2, 1, and 1 min in DI H₂O and then were dipped into a 10 mM solution of PAA for 30 min, followed by the same three-bath rinse. The films were then coated with solutions of 100 μ g/mL

collagen I (BD Biosciences) or 100 μ g/mL collagen I and 25 μ g/mL lumican (R&D Systems, Inc.) for 2 to 3 h at 37 °C. LbL assemblies are typically described by the cation/anion pair and total number of bilayers. For example, (PAH/PAA)_{*n*} indicates an assembly of *n* bilayers composed of one layer of PAH followed by one layer of PAA.

Layer-by-Layer-Modified Hydrogel Preparation. Polyacrylamide hydrogels with variable concentrations of bis-acrylamide, resulting in variable elastic moduli, were constructed on 25 mm circular glass coverslips as described.^{6,18–20} 0.05% Sulfo-SANPAH (Thermo Scientific) in 20 mM HEPES pH 8.0 was pipetted onto the top of each gel and then UV cross-linked for 2 min. The gels were washed for 10 min in 20 mM HEPES pH 8.0, and the cross-linking and washing step was repeated once. Each cross-linked gel was then incubated overnight at 4 °C on 1 drop of 100 μ g/mL collagen I in 250 mM HEPES pH 8.0. Following the incubation, the gels were UV cross-linked for 2 min and stored in ice-cold water while the solutions for LbL were prepared: 200 μ g/mL collagen I (BD Biosciences), 500 μ g/mL poly-D-glutamic acid (PGA, Sigma-Aldrich), and 1 μ g/mL lumican (R&D Systems, Inc.), all of which, including the deionized rinsewater, were titrated to pH 4.2 to allow for optimal LbL assembly with collagen.²¹ Using a programmable slide stainer (HMS series programmable slide stainer, Carl Zeiss, Inc. or Microm DS-50), LbL assembly was used to create 10 alternating layers of either polyglutamic acid (PGA) and collagen (Col/PGA bilayers) or lumican and collagen I (Col/Lum bilayers) atop the initial cross-linked collagen I layer, producing 5.5 bilayers in all. Immediately following the deposition of the bilayers, the LbL-modified hydrogels were incubated with 1:100 ethanolamine (Sigma-Aldrich) in 50 mM HEPES pH 8.0 for 30 min at 4 °C to prevent the adhesion of other molecules to the substrate. To prepare for cell culturing, the gels were transferred to sterile Petri dishes with serum-free media and exposed to UV for sterilization. They were then incubated in the sterilized media for at least 2 h at 37 °C.

Atomic Force Microscopy-Based Nanoindentation. Atomic force microscopy (AFM)-based nanoindentation was carried out on the uncoated as well as Col/PGA- and Col/Lum-coated polyacrylamide hydrogels (both 1 and 10 kPa) in 1× PBS at room temperature using a Dimension Icon AFM (Bruker Nano) and a colloidal spherical tip (radius $R \approx 12.5$ μ m). The spherical tip was prepared by manually gluing a polystyrene colloid (PolySciences) onto a tipless silicon nitride cantilever (tip A, nominal spring constant $k \approx 0.2$ N/m, AIO, BudgetSensors) with M-Bond 610 epoxy (Micro-Measurements) using the AFM. At each indentation location, the probe tip was programmed to indent the hydrogel at a 1 μ m/s constant z -piezo displacement rate (approximately equals the indentation depth rate) up to an ~ 30 nN maximum indentation force (corresponding to ~ 3 and 0.7 μ m maximum indentation depths for 1 and 10 kPa hydrogels, respectively). For each specimen, indentation was performed on relatively flat regions (surface roughness < 40 nm for 5 μ m \times 5 μ m contact mode surface scans) to minimize the impact of surface roughness. At least eight different indentation locations were tested on each sample. For each indentation curve, the cantilever deflection (in volts) and z -piezo displacement (in μ m) were converted to an indentation force (in nN) and depth (in μ m) through calibrating the cantilever deflection sensitivity (nm/V) by indenting on a hard mica substrate and a spring constant (nN/nm) via thermal vibration.²² The initial tip–sample contact point was determined via an algorithm reported previously for soft materials in the absence of attractive interactions.²³ The loading portion of the curve at each location was fit to the elastic Hertz model via least-squares linear regression to calculate the effective indentation modulus at the given indentation rate, E_{ind} (Figure 4),

$$F = \frac{4}{3} \frac{E_{\text{ind}}}{(1 - \nu^2)} R^{1/2} D^{3/2}$$

where F and D are the indentation force and depth, respectively, R is the colloidal tip radius, and ν is Poisson's ratio of the hydrogel ($\nu = 0.49$ for fully swollen hydrogels²⁴). In this model, the polystyrene spherical colloid was assumed to have an infinite modulus (~ 4 GPa)

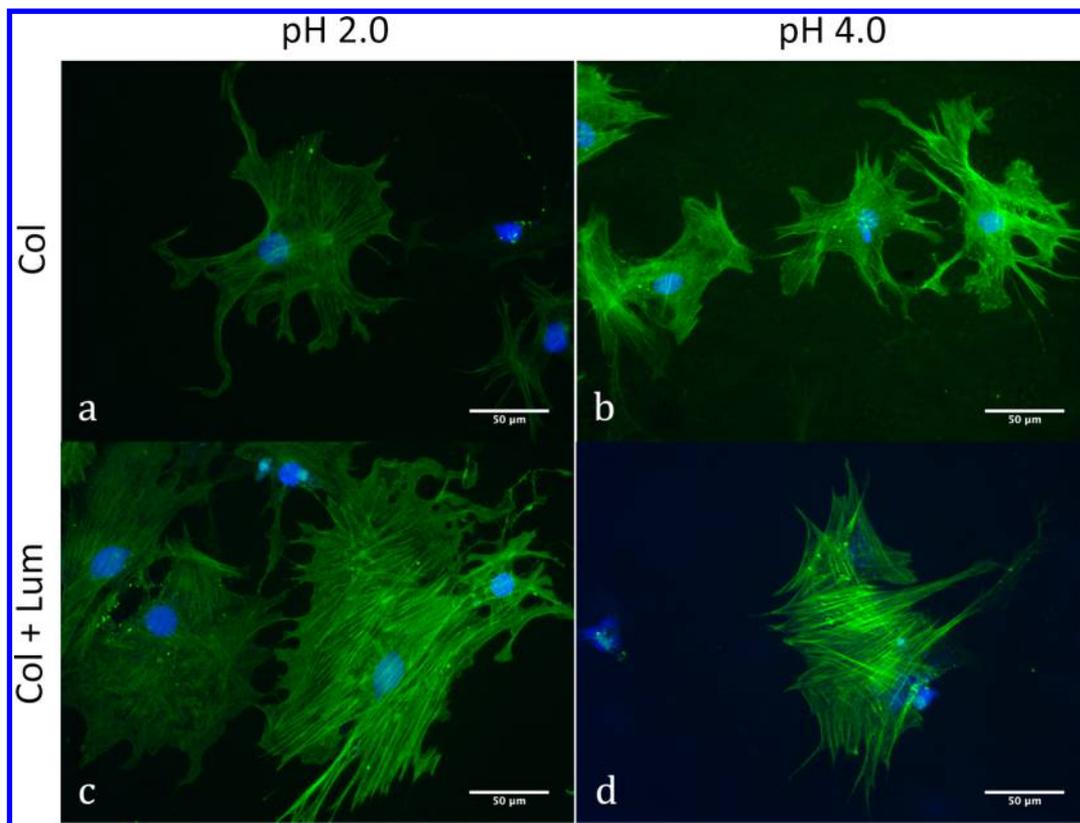


Figure 1. Hepatic stellate cells cultured for 7 days on collagen I (Col) (a, b) and Col plus lumican (Lum) (c, d) deposited on (PAH/PAA)_n at pH 2.0 (a, c) and pH 4.0 (b, d); cells stained with antibodies against myofibroblast marker α -SMA (green) and with nuclear marker DAPI (blue). $n = 11$ for pH 2.0 and 15 for pH 4.0. Bar, 50 μ M.

compared to that of the hydrogels.²⁵ In addition, the uncoated PAA gel has a thickness of ~ 100 μ m, which is orders of magnitude higher than the maximum indentation depth. We thus expect the substrate constraints effect to be negligible.²⁶ To avoid assuming a data normal distribution and homoscedasticity, one-way analysis of variance on the global rank transforms of the actual data²⁷ followed by a Tukey–Kramer posthoc test was applied to compare the values of E_{ind} between different samples. A p value of <0.05 was taken as statistically significant.

Immunostaining. Cells were fixed in 4% paraformaldehyde in PBS for 15 min, permeabilized with 0.1% Triton X for 10 min, and blocked with 1% BSA in PBS for 30 min. The cells were then incubated with primary antibody for 1 h at room temperature and overnight at 4 $^{\circ}$ C, followed by a 2 h incubation at room temperature with secondary antibody and a 10 s incubation with DAPI for nuclear staining. Primary antibodies were monoclonal mouse anti- α -smooth muscle actin (α -SMA, Sigma, 1:500), polyclonal rabbit antidesmin (Abcam, 1:80), and antihuman lumican (R&D Systems, 1:400). Secondary antibodies were cy2 donkey antimouse (1:200), cy3 donkey antirabbit (1:200), and cy3 donkey antigoat (1:200), all from Jackson ImmunoResearch Laboratories.

Scanning Electron Microscopy. Samples were washed three times in 50 mM Na-cacodylate buffer and fixed in 2% glutaraldehyde in 50 mM Na-cacodylate buffer (pH 7.3) for 2 h. The samples were then dehydrated first in a graded series of ethanol concentrations through 100% over a period of 1.5 h and then three times in 100% ethanol. Following dehydration, the samples were immersed in 100% hexamethyldisilazane (Sigma-Aldrich) twice for 10 min and left to air dry for 30 min as described previously.²⁸ In preparation for imaging, specimens were then mounted on stubs and sputter coated with gold and palladium. Sample observation and imaging was done using a Philips XL20 scanning electron microscope (FEI) at a 10 kV acceleration voltage.

Cell Isolation and Culture. Hepatic stellate cells were isolated from 500 to 700g Sprague–Dawley rats by sequential in situ digestion of the liver with 0.4% Pronase (Roche Diagnostics) and 0.04% type II collagenase (Worthington), followed by density gradient centrifugation over 9% Histodenz (Sigma-Aldrich), as described.²⁹ Freshly isolated cells were plated on sterilized PEMs or LbL-modified hydrogels and cultured for 7 days at 37 $^{\circ}$ C.

RESULTS

As an initial experiment to explore the effects of lumican on stellate cell differentiation in culture, before we developed our protein LbL-modified hydrogels we constructed synthetic LbL films and overlaid them with either collagen I alone or a mixture of collagen I and lumican, akin to the experimental system of Chen et al. using collagen I and decorin.⁸ LbL films composed of cationic PAH and anionic PAA were assembled on standard glass slides. The assembly pH of the (PAH/PAA) LbL films determines their mechanical stiffness, with stiffness increasing with increasing pH such that (PAH/PAA) LbL films assembled at pH 2.0 had an E of approximately 10^5 Pa and those at pH 4.0 had an E of approximately 10^6 Pa under physiological conditions.^{8,17} (PAH/PAA) LbL films coated with collagen I or a mixture of collagen I and lumican were used as culture substrates for freshly isolated rat hepatic stellate cells. After 7 days of culturing, we found that α -SMA-positive stress fibers, a marker of myofibroblastic differentiation, were more prominent in hepatic stellate cells cultured on collagen I and lumican than on collagen I alone (Figure 1) and that stellate cells cultured on collagen I alone displayed lamellipodia, which were absent in cells cultured on collagen I and lumican. As would be expected,⁵ given the extremely high stiffnesses of the

substrates, no difference was observed between HSC cultured on LbL films assembled at pH 2.0 compared to those assembled at pH 4.0.

The phenotypic differences we observed using synthetic LbL films motivated us to study the effects of lumican on hepatic stellate cells in more depth using a system that was more mechanically and chemically physiological. We used polyacrylamide hydrogels as the basis for our system since these are tunable over a range of stiffnesses typical of normal and fibrotic soft tissues. Collagen, which is positively charged, was used as the polycation, and lumican, which as a proteoglycan is negatively charged, was the polyanion for an LbL film built on top of the hydrogels. PGA was used as the control polyanion. For these pilot studies, the initial collagen layer was cross-linked at 4 °C, and LbL deposition was carried out at room temperature. In the future, however, it will be important to vary the LbL deposition temperature and determine in detail the effect of temperature on collagen fibril organization and topography in the LbL system.

To show that lumican was successfully incorporated into the (Col/Lum)_{5,5} hydrogel system, the (Col/Lum)_{5,5} gels were immunostained to demonstrate the presence of lumican (Figure 2). During the assembly process, a portion of the gel

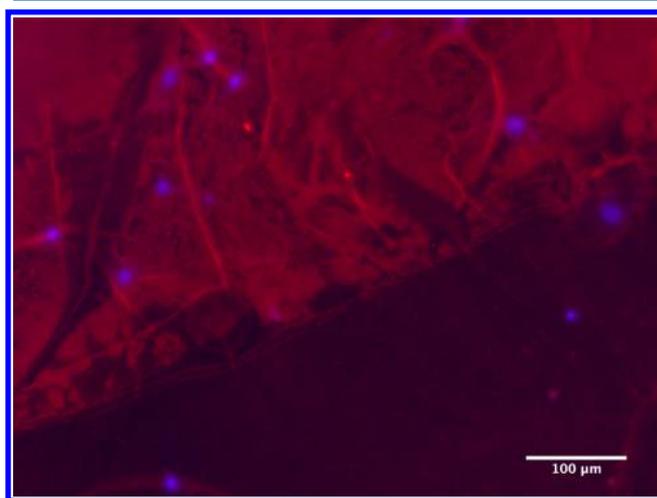


Figure 2. Hepatic stellate cells cultured for 7 days on (Col/Lum)_{5,5} gel; cell stained for lumican (red) and with nuclear marker DAPI (blue). Scale bar, 100 μm.

(lower half of the image) was not submerged in polymer solution, and this unsubmerged portion is clearly demonstrated in the staining. Control (Col/PGA)_{5,5} gels (not shown) showed no staining for lumican.

LbL-modified polyacrylamide hydrogels were compared to hydrogels with a single layer of cross-linked matrix proteins (either collagen I alone or collagen I plus lumican) using scanning electron microscopy (Figure 3). The organization of collagen in the presence of lumican in an extensive, organized network did not appear to be significantly different between the single-layer cross-linked system and the (Col/Lum) LbL system. For collagen I in the absence of lumican, there were multiple small clumps visualized for the (Col/PGA)_{5,5}-modified hydrogel, but visible fibers were absent for the hydrogel with a single layer of cross-linked protein.

We attempted to measure the thickness of the protein layers on hydrogels using ellipsometry in solution, but the results did not prove reliable, likely due to minimal contrast in the

refractive indices of the hydrogel and the protein layers. Thus, we relied on SEM imaging and immunofluorescence microscopy to confirm the deposition of the proteins, although this method admittedly does not provide quantitative information and does not address the potential for differences in hydration of the polyelectrolytes used to generate the LbL films. While it would be possible to carry out the LbL deposition of collagen and lumican (or PGA) on a silicon wafer and to carry out ellipsometry measurements, it is not clear that this would be relevant to the system that is the focus of this article.

LbL films are commonly used as substrates in stiffness-sensitive studies because the thin films are more pliant than glass slides or plastic dishes and their stiffness can be adjusted based on the polymers used, the solution pH, and the number of bilayers. In our studies, the goal was to use polyacrylamide gels as the mechanically tunable substrate and LbL films as a system to mimic in vivo protein–protein interactions. Because the LbL films are atop the hydrogel and are the surface to which cells attach, we characterized the stiffness of the LbL-modified hydrogels to determine whether the cells would sense the stiffness of the hydrogel (as we predicted given the thinness of the 5.5 bilayer LbL films) or of the LbL films. We found that for both 1 and 10 kPa polyacrylamide hydrogels neither Col/PGA nor Col/Lum bilayers had a significant effect on the indentation modulus, E_{ind} ($p > 0.05$, Figure 4). In comparison, we found the expected significant differences in E_{ind} between the 1 and 10 kPa hydrogels ($p < 0.0001$, Figure 4).

To rule out cytotoxicity associated with the LbL-modified hydrogel system, hepatic stellate cells were cultured on single cross-linked layers of collagen I on 1 and 10 kPa polyacrylamide gels as controls and on (Col/PGA)_{5,5} PEMs on 1 and 10 kPa polyacrylamide gels. Cells remained healthy through at least day 7 (data not shown). Additionally, cell spreading was as predicted based on the stiffnesses of the underlying substrates.⁵ This suggested that PGA was a reasonable choice for the control polyanion.

The effect of lumican on the myofibroblastic differentiation of stellate cells was then tested using the LbL-modified hydrogel system. HSC were cultured on (Col/Lum)_{5,5} or (Col/PGA)_{5,5} on 1 and 10 kPa polyacrylamide gels and then immunostained for the expression of α -SMA and hepatic stellate cell marker desmin. (Note that desmin is used to confirm stellate cell identity but is not a reliable indicator of the activation state.) Stellate cells cultured on (Col/Lum)_{5,5} expressed more α -SMA (in green) and are more spread; this is particularly noticeable on the 1.0 kPa gel, where there is almost no α -SMA visible in the cells cultured on (Col/PGA)_{5,5} (Figure 5). Cells on 10 kPa gels demonstrated a myofibroblastic phenotype regardless of the matrix proteins in the system but, consistent with the behavior of cells on single layers of matrix proteins (Figure 1), demonstrated fewer lamellipodia and more organized stress fibers when lumican was added to collagen I. Stellate cells were significantly more spread when cultured on collagen I in the presence of lumican as opposed to PGA, especially on the 1 kPa hydrogel.

DISCUSSION

We describe here the development of a new cell culture system that enables the study of complex mixtures of matrix proteins in a mechanically tunable setting. Our system is novel for two reasons: first, it enables matrix mixtures to be studied simultaneously with, but independently of, mechanical stiffness, and second, to our knowledge this is the first LbL assembly

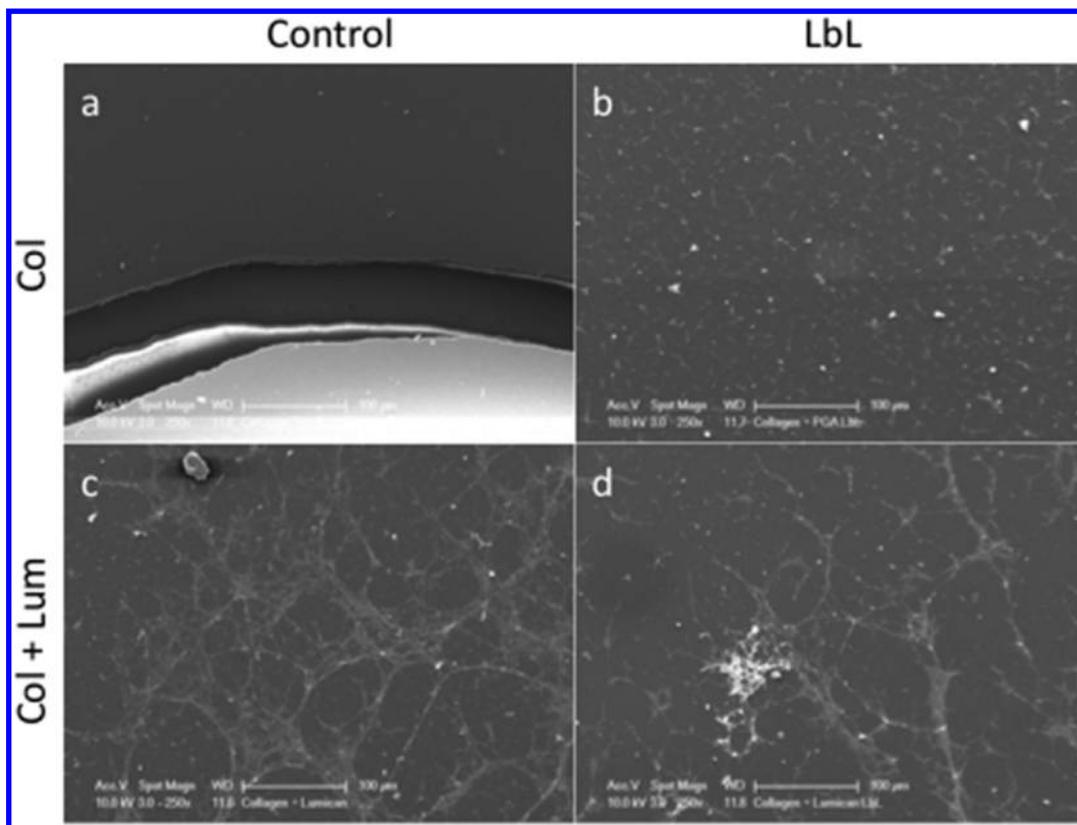


Figure 3. Scanning electron microscopy images of polyacrylamide hydrogels with (a) cross-linked collagen I (crack in gel across the lower half of the image highlights the location of the gel), (b) (Col/PGA)_{5,5}, (c) a cross-linked collagen I–lumican mixture, and (d) (Col/Lum)_{5,5}. Scale bar, 100 μm .

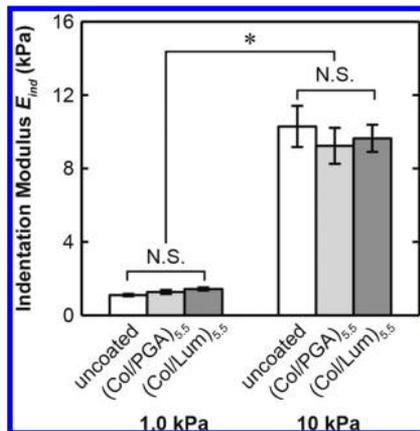


Figure 4. Indentation modulus, E_{ind} , for six coated and uncoated polyacrylamide hydrogels measured via AFM-based nanoindentation in PBS (*: $p < 0.0001$ between the groups of 1 and 10 kPa hydrogels).

incorporating a collagen and a proteoglycan. Our studies with hepatic stellate cells demonstrate that this system is non-cytotoxic, that the control anion (PGA) has no effect on cells, and that the underlying hydrogel determines the overall system mechanics, at least for the thin bilayer films used here. Additionally, we demonstrate clearly in a cell culture system that the addition of lumican to collagen has phenotypic implications. The absence of lamellipodia in hepatic stellate cells cultured in the presence of lumican in initial studies indicates that lumican participates in the regulation of motility, although as noted below we cannot separate the effects of lumican on collagen organization from its direct effect on cells. The prominent α -SMA-positive stress fibers in these cells

indicate a myofibroblastic phenotype, suggesting that lumican is involved in the differentiation of stellate cells into highly adherent myofibroblasts, which are typically less motile as stress fibers become more prominent. Our method will enable future studies of the effects of multiple proteoglycans (including other small leucine-rich proteoglycans such as fibromodulin and decorin) on cell behaviors including myofibroblastic differentiation, fibrogenesis, and motility.

The small clumps of collagen and the extensive organized fibrous matrix of collagen in the presence of lumican in Figure 3 indicate a clear difference in surface topography, raising the question of whether the phenotypic differences we see result from the cellular reaction to different surface topographies. Previous studies have demonstrated that extracellular matrix topography can have effects on cellular differentiation on the nanoscale and, less certainly, the microscale.^{30,31} This does not discount our conclusion that lumican has a phenotypic effect on HSCs but raises the possibility that lumican exerts its influence indirectly by altering the organization of collagen fibers in a way that affects cellular differentiation in hepatic stellate cells. This is consistent with previous findings that demonstrate a role for lumican in organizing the collagen matrix in vivo.¹⁵ However, to our knowledge, no studies have assessed the influence of topography on the myofibroblastic differentiation of hepatic stellate cells, and the topographical effect is known to be dependent on the cell type.³¹ Lumican has been shown in vivo through the use of knockout mice to enhance stellate cell myofibroblastic differentiation, suggesting that it has a significant impact on myofibroblasts regardless of whether it is direct or indirect.¹⁶ While the specific effects of surface topography on hepatic stellate cell differentiation are relevant,

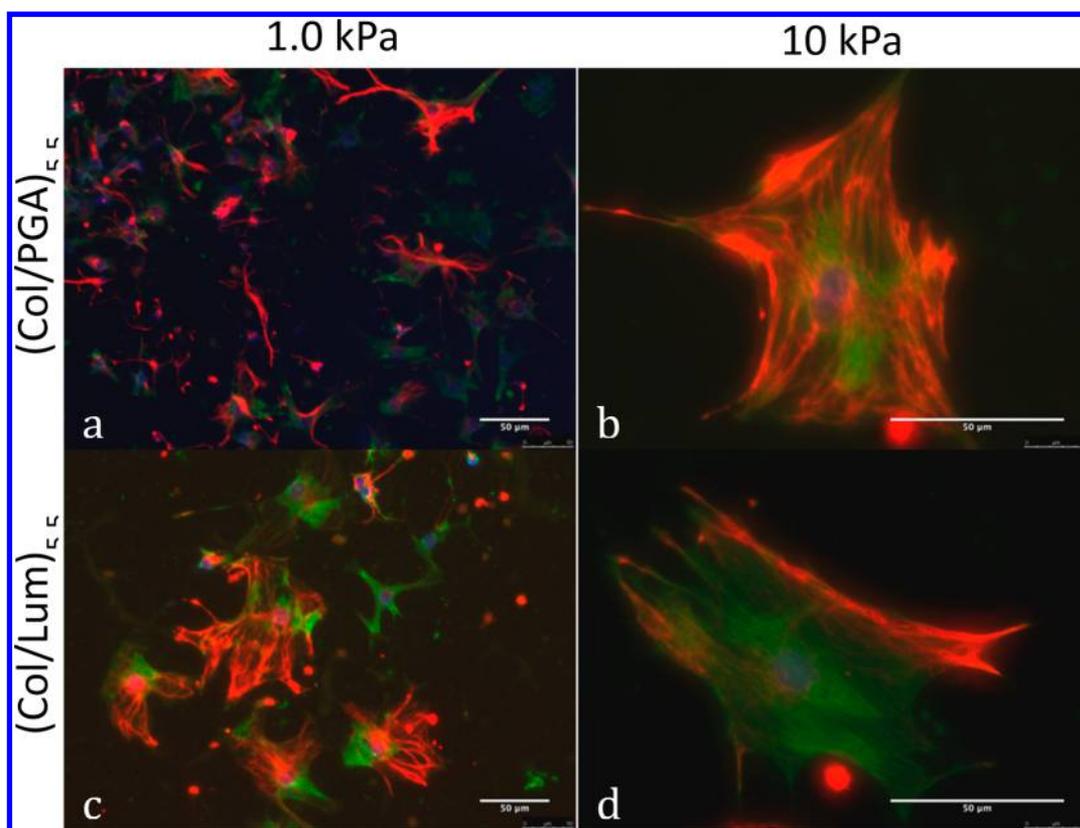


Figure 5. Day 7 HSC on $(\text{Col}/\text{PGA})_{5,5}$ gels (a, b) and $(\text{Col}/\text{Lum})_{5,5}$ gels (c, d) of 1 kPa (a, c) and 10 kPa (b, d); staining for myofibroblast activation marker α -SMA (green), stellate cell marker desmin (red), and nuclear stain DAPI (blue). Scale bar, 50 μm . Note the difference in magnification between the right and left panels.

this is outside the scope of this work and will require detailed study in the future. The results together may provide a reason to incorporate the control of surface topography into our mechanically and chemically tunable system.

OUTLOOK

We have established a new method for studying cells in culture. The mechanical tunability and LbL matrix organization of these culture substrates offer the potential to study soft-tissue cells in an environment that more closely mimics that of their natural environment than standard tissue culture substrates and may be relevant to tissue engineering.

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Notes

The authors declare no competing financial interest.

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