Cell Motility and the Cytoskeleton 65: 332–341 (2008)

Cell Shape Regulates Collagen Type I Expression in Human Tendon Fibroblasts

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Understanding the relationship between cell shape and cellular function is important for study of cell biology in general and for regulation of cell phenotype in tissue engineering in particular. In this study, microcontact printing technique was used to create cell-adhesive rectangular and circular islands. The rectangular islands had three aspect ratios: 19.6, 4.9, and 2.2, respectively, whereas circular islands had a diameter of 50 μm. Both rectangular and circular islands had the same area of 1960 μm^2. In culture, we found that human tendon fibroblasts (HTFs) assumed the shapes of these islands. Quantitative immunofluorescence measurement showed that more elongated cells expressed higher collagen type I than did less stretched cells even though cell spreading area was the same. This suggests that HTFs, which assume an elongated shape in vivo, have optimal morphology in terms of expression of collagen type I, which is a major component of normal tendons. Using immunohistochemistry along with cell traction force microscopy (CTFM), we further found that these HTFs with different shapes exhibited variations in actin cytoskeletal structure, spatial arrangement of focal adhesions, and spatial distribution and magnitude of cell traction forces. The changes in the actin cytoskeletal structure, focal adhesion distributions, and traction forces in cells with different shapes may be responsible for altered collagen expression, as they are known to be involved in cellular mechanotransduction. Cell Motil. Cytoskeleton 65: 332–341, 2008. © 2008 Wiley-Liss, Inc.

Key words: cell shape; collagen type I expression; cell traction force; actin cytoskeleton; focal adhesion

INTRODUCTION

In culture, cells attach to and spread on a substrate. Previous studies have shown that cell attachment and spreading is related to gene expression and protein production, implying a cell shape-based regulation of cellular function [Singhvi et al., 1994; McBeath et al., 2004; Lee et al., 2006]. Understanding how cell shape influences cellular function is important for regulating cell phenotype and developing engineered tissues for medical applications [Langer and Vacanti, 1993; Liu and Chen, 2005].

Soft lithography, a technique derived from microfabrication technology in the semiconductor industry, provides a powerful approach for studying cell shape or

Contract grant sponsor: NSF; Contract grant number: ECS-0401083; Contract grant sponsor: NIH; Contract grant numbers: AR049921, AR113046.

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Received 2 July 2007; Revised 3 November 2007; Accepted 20 December 2007

Published online 31 January 2008 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/cm.20263

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spreading in regulation of cell functions [Singhvi et al., 1994; Mrksich et al., 1996; Chen et al., 1997; Whitesides et al., 2001]. This technique allows fabrication of adhesive patterns on substrate at micro- to sub-micrometer scales. Confining cells to these adhesive patterns with various shapes and sizes can precisely control cell shape and spreading area. Micropatterning substrates by soft lithography overcomes the limitations of homogeneous unconfined adhesive substrates on which cells display various shapes even though they belong to the same cell type, which may partially alter their normal functions [Thery et al., 2006]. Using soft lithography, previous studies showed that the degree of cell spreading influences DNA synthesis [Singhvi et al., 1994], proliferation and apoptosis [Chen et al., 1997, 1998], and focal adhesion assembly [Chen et al., 2003] in human and bovine capillary endothelial cells; differentiation in mesenchymal stem cells [McBeath et al., 2004]; and gene expression and protein synthesis in primary rat bone cells [Thomas et al., 2002]. However, for a fixed spreading area, studies on cell shape regulation are mainly limited to cell motility, i.e., lamellipodia extension and direction of cell migration. For example, cells preferentially extended lamellipodia from their corners regardless of their geometric shapes [Parker et al., 2002; Brock et al., 2003]. During migration, narrow teardrop-shaped or triangular cells moved predominantly toward the blunt end, while wide cells did not; rectangular cells migrated outward towards their two ends without bias for either one, but circular or square cells moved in random directions [Jiang et al., 2005].

In this study, we aimed to determine the effect of cell shape on collagen expression in human tendon fibroblasts (HTFs). In vivo, tendon fibroblasts are highly elongated and are aligned along the longitudinal direction of the intact tendon, a well-organized fibrous connective tissue that is mainly composed of parallel collagen fibers [Wang et al., 2003]. By producing extracellular matrix (ECM) proteins, especially collagen type I, tendon fibroblasts serve to maintain, repair, and remodel the tendon matrix [Wang, 2006]. As such, synthesis of collagen type I is one major function of tendon fibroblasts. Therefore, the first aim of this study was to determine how this particular elongated cell shape is related to collagen type I expression in HTFs.

It is known that cells generate mechanical tension in their actin cytoskeleton and exert forces on cell-cell or cell-ECM contacts [Harris et al., 1980]. Tension in the actin cytoskeleton transmits to ECM via focal adhesions, and this force is termed as cell traction. The alterations in cell traction forces (CTFs) can result in change of cell shape [Maniotis et al., 1997; Chicurel et al., 1998; Wang and Lin, 2007]. Moreover, the physical connections between focal adhesions, actin cytoskeleton, and nuclear scaffold is thought to provide a pathway for mechanical signal transfer from cell surface to nucleus [Maniotis et al., 1997]. Therefore, the second aim of this study was to test the hypothesis that change of collagen type I expression in HTFs with various shapes is associated with alterations in the actin cytoskeletal structure and tension distribution. Specifically, we examined the distribution of actin cytoskeleton, focal adhesions, and CTFs in HTFs of various shapes and correlated them to the expression of collagen type I in these tendon cells.

MATERIALS AND METHODS

Fabrication of Micropatterned Substrates

Micropatterned substrates were fabricated by microcontact printing method according to the following procedures (Fig. 1). First, silicon masters were fabricated by using standard photolithography technologies. Negative photoresist SU-8 was spun on cleaned silicon wafer (Fig. 1A). The photoresist-coated silicon wafer was then exposed under UV light through the patterned photomask on Suss MA 6 mask aligner for 20 s (SUSS MicroTec, Santa Clara, CA) (Fig. 1B). The features were then developed, and the silicon master with the desired pattern was generated (Fig. 1C). In order to facilitate subsequent release of Poly(dimethylsiloxane) (PDMS) from the silicon master, the template was oxidized in air plasma for 1 min and silanized with (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane vapor overnight under vacuum. PDMS was then prepared by thoroughly mixing two silicone components, 601A and 601B (Wacker Chemical, Adrian, MI), in a weight ratio of 10:1, then slowly poured onto the silicon master (Fig. 1D). After curing at room temperature for 24 h, the PDMS was removed and cut to the desired size (Fig. 1E). The substrate was prepared by the following procedures. The glass slides were cleaned and coated with 10-nm thick chromium and 20-nm thick gold (Fig. 1F). The PDMS stamps were cleaned with ethanol and dried with a stream of pressurized air. A 2 mM ethanol solution of tris(ethylene glycol)-terminated alkanethiol ($HS(CH_2)_{11}(OCH_2CH_2)_3OH$, ProChimia, Poland) was put onto the stamp surface and incubated for 10 min, and the solution was then removed and the stamp dried. The PDMS stamp was then gently pressed onto the slide (Fig. 1G), and after 1 min, the stamp was removed from the glass substrate. A self-assembled monolayer was formed at the region where tris(ethylene glycol)-terminated alkanethiol was stamped (Fig. 1H). The substrate was then rinsed with ethanol and dried with a steam of air. The self-assembled monolayer of tris(ethylene glycol)-terminated alkanethiol can resist the protein adsorption. Therefore, cells only adhere to the non-tris(ethylene glycol)-terminated alkanethiol-stamped region.

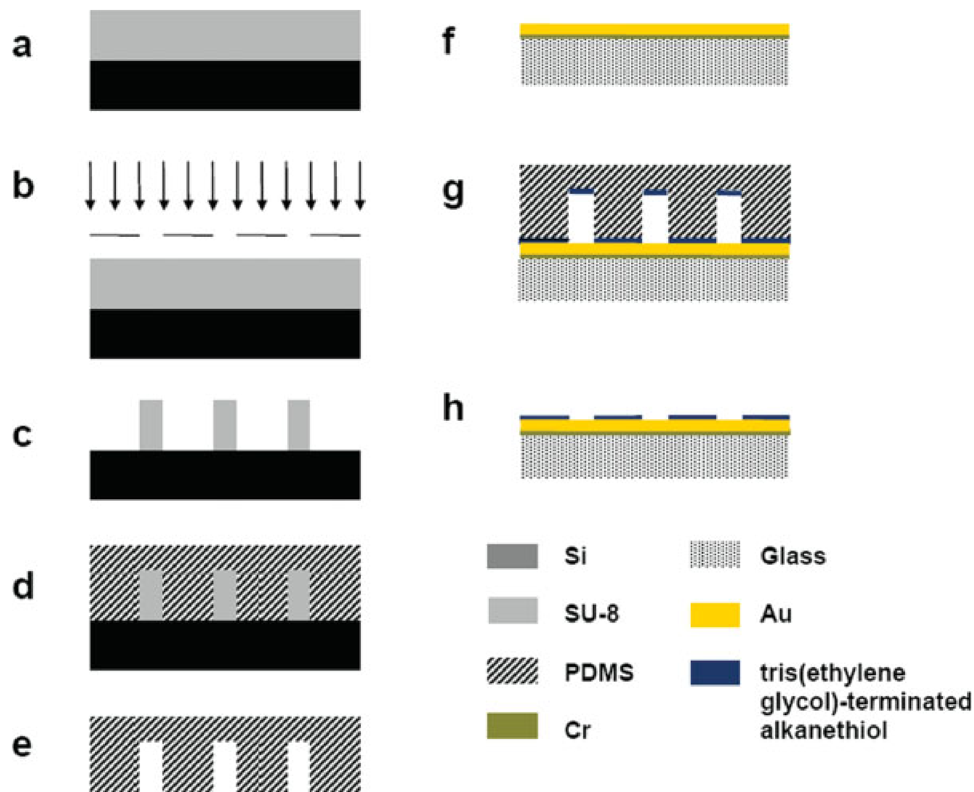

Fig. 1. A schematic of microcontact printing method used for micropatterning gold surfaces in this study. **A**. Negative photoresist SU-8 was spun on silicon wafer. **B**. The photoresist-coated silicon wafer was exposed under UV light through a photomask. **C**. The features were then developed, and the silicon master with desired patterns was generated. **D**. PDMS prepolymer was poured onto the silicon master. **E**. After curing, the PDMS was removed and cut to stamps of desired size. **F**. Glass slide was cleaned and coated with chromium and gold. **G**. PDMS stamp was inked with ethanol solution of tris(ethylene glycol)-terminated alkanethiol ($HS(CH_2)_{11}(OCH_2CH_2)_3OH$) and gently pressed onto gold-coated slide. **H**. self-assembled monolayer was formed at the region where tris(ethylene glycol)-terminated alkanethiol was stamped.

Microcontact printing method was also been used for fabrication of adhesive islands on polyacrylamide (PA) gel according to a method modified from the literature [Engler et al., 2004]. Briefly, after the PA gel was fully cross-linked, Sulfo-SANPAH (Pierce, Rockford, IL), a heterobifunctional cross-linker, was applied onto the gel surface and linked to the gel by exposing it to UV light (Fig. 2A). The PDMS stamps were treated with oxygen plasma and then dipped in 100 μg/ml collagen type I solution (Angiotech Biomaterials, Palo Alto, CA). After carefully scraping the solution off the top surface of stamp, the stamp with the solution of collagen filled in the holes was gently pressed onto the PA gel overnight to allow conjugation of collagen type I to PA gel surface (Fig. 2B). The stamp was removed from gel surface and the gel was then rinsed with PBS prior to cell seeding (Fig. 2C).

Cell Culture Experiments

HTF culture was established according to the published protocol [Yang et al., 2004]. For immunofluorescence assays, HTFs were seeded onto patterned gold-coated glass slides in cell growth medium (DMEM, 10% FBS and 1% penicillin/streptomycin). After HTFs (passage 5 or 6) were cultured on the patterned surface for 36 h, cells were fixed with 4% paraformaldehyde and then proceeded for immunostaining. For measuring CTFs, after collagen type I was patterned onto the PAG using stencil micropatterning method, 3000 HTFs were plated onto each gel disk in DMEM with 1% FBS and 1% penicillin/ streptomycin. Non-adhered cells were removed 1 h later, and fresh cell growth medium was added. Cells were then allowed to spread on PAG for 6 h prior to image acquisition for CTF measurement. During cell culture, cells were maintained at 37°C in humidified atmosphere with 95% air and 5% carbon dioxide.

Immunofluorescence Assays for Collagen Type I, GAPDH, Actin Filaments, and Vinculin

Collagen type I, actin filaments, vinculin, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were stained using the following protocols. Cells were fixed in 4% paraformaldehyde (FD NeuroTechnologies, Ellicott City, MD) for 15 min and then blocked with a Blotto buffer (4% dry milk and 0.4% Triton X-100 in PBS) for

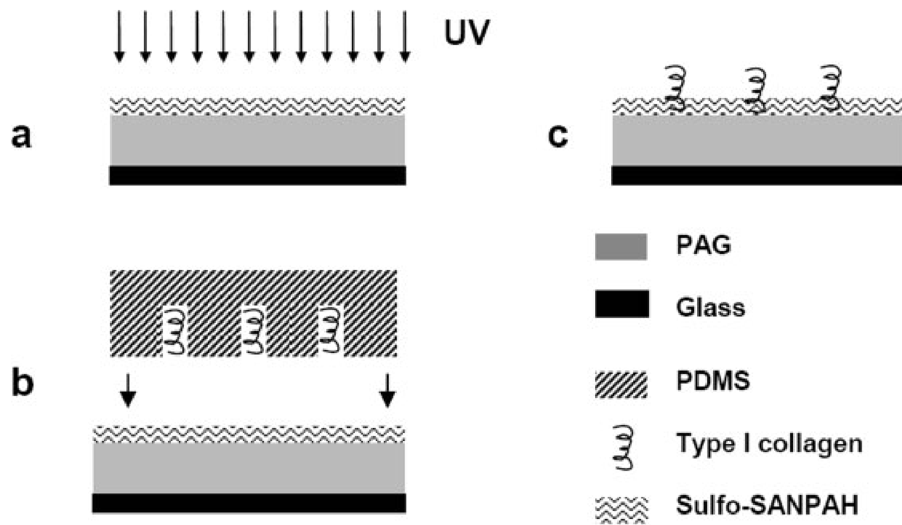

Fig. 2. A schematic of microcontact printing method used for micropatterning PA gel in this study. **A**. PA gel was subjected to treatment with sulfo-SANPAH and UV exposure. **B**. a stamp with collagen type I filled in the holes was applied on the gel. **C**. The stamp was removed from PA gel, and micropatterned islands coated with collagen type I on the gel was formed.

45 min. For staining collagen type I and GAPDH, the sample was incubated with goat polyclonal anti-type I collagen antibody (Santa Cruz Biotechnology, Santa Cruz, CA) overnight or monoclonal antibody for GAPDH (Biogenesis, UK) for 1 h with a dilution ratio 1:100 and 1:300, respectively, and then washed with block solutions. FITC-conjugated donkey anti-goat antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were then applied at a dilution ratio of 1:250 for 1 h. The sample was washed extensively with block solutions and PBS to remove excess antibody. A FAK100 kit (Chemicon International, Temecula, CA) was used to stain actin filaments and vinculin. Samples were incubated with anti-vinculin with a dilution ratio 1:100 for 1 h after cells were fixed and blocked with a Blotto buffer. After samples were washed with block solutions, secondary antibody (goat anti-mouse, FITC-conjugated, dilution 1:200) and TRITC-conjugated phalloidin (dilution 1:200) were applied simultaneously for 1 h at room temperature. Cell nuclei were then stained with DAPI. Afterward, the sample was mounted and examined using fluorescence microscope or laser confocal microscope.

Cell Traction Force Microscopy

The determination of CTFs by CTFM was described previously [Butler et al., 2002]. It is based on the deformations in the surface of a thin elastic substrate induced by cells cultured on it. The fluorescent beads embedded in a thin PAG serve as markers for determining substrate displacements by image analysis, which are then used to compute CTFs based on continuum mechanics [Wang and Lin, 2007].

The PAG embedded with 0.5 μm red fluorescent micro-beads (Molecular Probes, Eugene, OR) was prepared first. The gel disk was 120 μm in thickness, 10 mm in diameter, and attached to the bottom of a 35-mm dish (MatTek, Ashland, MA), which had a circular glass area with a diameter of 14 mm. The gel stiffness was controlled by changing the relative concentration of acrylamide (Sigma, St. Louis, MO) and *N*,*N*′-methylene-bisacrylamide (bis-acrylamide, Sigma, St. Louis, MO). In this study, gel containing 5% acrylamide and 0.1% bis-acrylamide with a Young's modulus of 3 kPa and a Poisson's ratio of 0.48 was used [Chen et al., 2007]. After collagen type I was patterned onto the PAG using microcontact printing method and HTFs were cultured on those gels for 6 h, phase contrast images of individual cells that were fully spread on single collagen-coated islands and fluorescence images of the embedded fluorescent beads were taken. After cells on the gel disk were trypsinized, fluorescence images of the fluorescent beads in the same view were taken. The CTFs were then computed using a MATLAB program based on a published method [Butler et al., 2002].

Quantification of Collagen Type I Expression Levels

The total collagen type I expression level per cell was quantified as follows. The immunofluorescence microscopy images of cells with different shapes were taken under the same conditions, including the same exposure time and gain factor. Cells that were fully spread on entire islands, as confirmed by phase contrast images, were selected for analysis. Using an image analysis

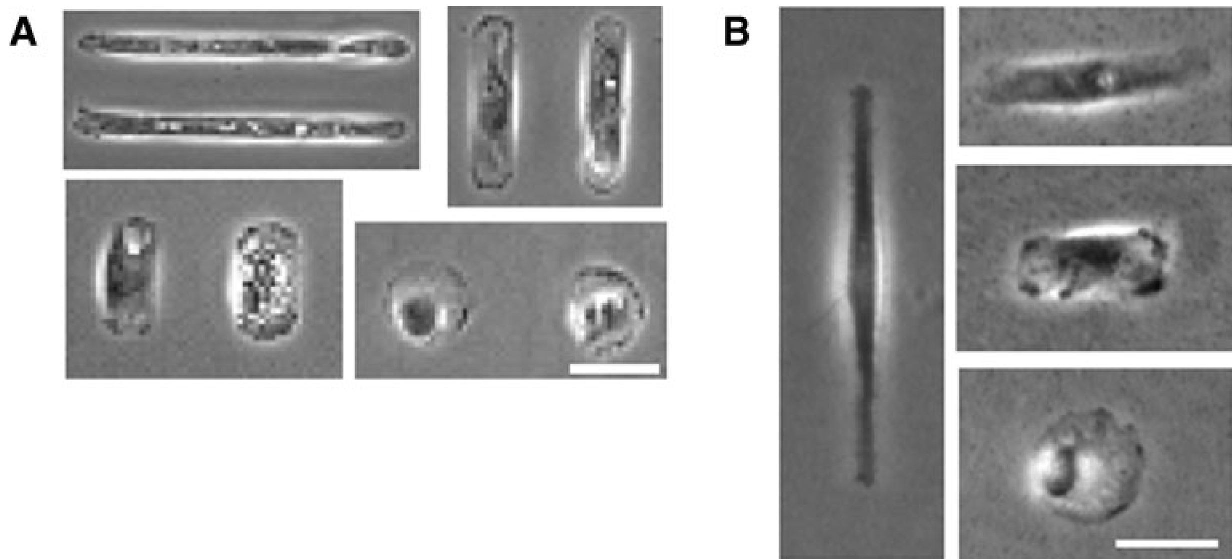

Fig. 3. HTFs on micropatterned surfaces. **A**. Micropatterned gold-coated substrates fabricated by microcontact printing method. **B**. micropatterned polyacrylamide gel fabricated using stencil micropatterning method. As seen, the shapes of these tendon cells were close to those of the adhesive islands. (Scale bars: 50 μm).

software, (MetaMorph software. Molecular Devices, Sunnyvale, CA), the boundary of the chosen cell was defined manually first. The intensities per pixel in fluorescence microscopy image were automatically measured and the average intensity over the defined cell area was computed. To subtract the background noise, we defined four small areas near cell boundary. By subtracting the average intensity over these the four small areas, we obtained the immunofluorescence intensity level for each chosen cell. A total of 20 cells from each group were chosen for measurement. To verify that cell shape affected the collagen type I synthesis, we also performed immunocytochemistry for GAPDH and measured its fluorescence intensity of each cell for four different shapes.

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to analyze the effect of cell shape on collagen type I expression level and CTF magnitude, followed by Duncan's test for multiple comparisons with the significance level set at $\alpha = 0.05$.

RESULTS

Micropatterning Human Tendon Fibroblasts With Different Shapes

Micropatterned substrates containing an array of cell-adhesive islands surrounded by PEG-covered non-adhesive regions were fabricated using microcontact printing technique. The adhesive islands were designed to be of the same area (1.96×10^3 μm^2) but in different geometric forms (rectangular with aspect ratios of 19.6, 4.9, and 2.2, respectively, and circular). This area is comparable with typical spreading area of HTFs on a smooth surface. To prevent cells from spreading across multiple islands, the non-adhesive spacing between islands was designed to be 50 μm. When HTFs were seeded onto the patterned substrates, they preferentially attached to adhesive islands and were confined within the boundaries of these islands. Therefore, the shape of cells was as precisely controlled as that of adhesive islands (Fig. 3A). In order to measure traction forces of micropatterned cells, type I collagen-coated adhesive islands on PAGs were fabricated using stencil micropatterning method. The shape of cells was seen to be well controlled on PAG surface (Fig. 3B).

Expression Levels of Collagen Type I in Micropatterned HTFs

Expression of collagen type I in HTFs with different shapes was determined by immunofluorescence assay. The fluorescence microscopy images of collagen type I for cells with different shapes (Fig. 4) showed that fluorescence intensity decreased with decreasing aspect ratio of cells. This observation was confirmed by quantification using image analysis (Fig. 5). Compared to rectangular cells with an aspect ratio of 19.6 (i.e. the most elongated cells), cells with an aspect ratio of 4.9 expressed about 10% lower collagen type I, while cells with an aspect ratio of 2.2 expressed more than 20%

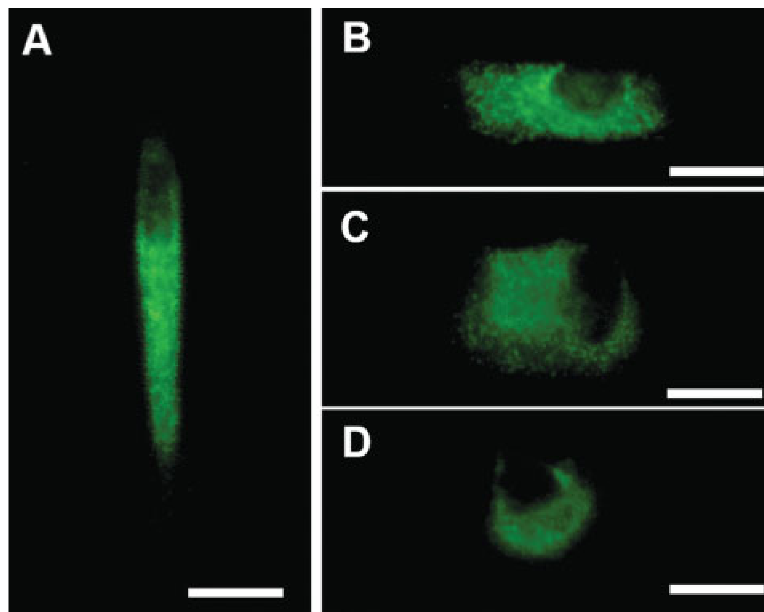

Fig. 4. Immunofluorescence microscopy images of collagen type I expression in rectangular HTFs with aspect ratios of 19.6 (**A**), 4.9 (**B**), and 2.2 (**C**), respectively, and circular HTF (**D**). (Scale bars: 20 μm).

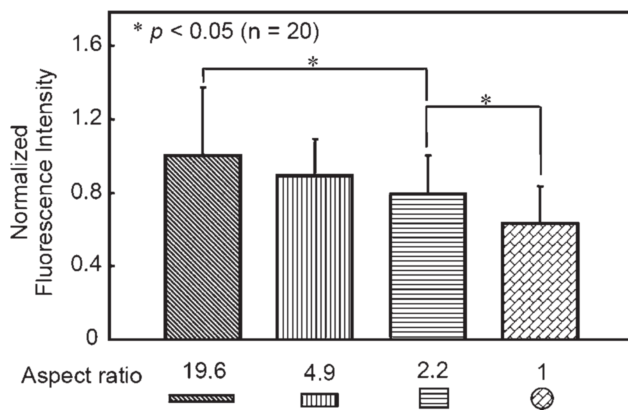

Fig. 5. Effect of cell shape on collagen type I expression level. Twenty cells from each group were examined for fluorescence intensity of collagen type I signal. The fluorescence intensities were normalized to the value of rectangular cells with an aspect ratio of 19.6. Expression of collagen type I decreased with decreasing aspect ratio of cells.

lower. For circular cells, whose aspect ratio is 1, the collagen expression was 40% lower than that of the most elongated cells with an aspect ratio of 19.6. However, we found that GAPDH expression levels were not significantly different among cells with the four shapes (data not shown).

Organization of Actin Filaments and Focal Adhesions in Micropatterned HTFs

Long and parallel actin filament bundles were formed in elongated cells, with aspect ratios of 19.6 and 4.9, respectively, along the longitudinal direction, while in short cells with an aspect ratio of 2.2 and circular cells, they were along the circumference of cells, and several bundles were formed from the nuclear area to the edge of cells (Fig. 6). Focal adhesions, revealed by staining vinculin, were concentrated at the two ends of elongated cells or corners of short cells. In circular cells, however, focal adhesions were uniformly distributed along the edge of cell. Merged images of F-actin and vinculin showed that F-actin filaments (or stress fibers) at their ends linked with focal adhesions, where cells anchor to underlying substrate and hence exert traction forces.

Cell Traction Forces in Micropatterned HTFs

For all cells examined in this study, the largest CTFs were located at the two ends of elongated cells, at the corner regions of short cells, and along the outer edge of the circular cells. The traction force directions appeared to be parallel to stress fibers. For example, the directions of the largest CTFs were in parallel to the long axis in elongated cells, along the diagonal in short cells, and along the radial in circular cells (Fig. 6). The magnitude of CTFs decreased with increasing the aspect ratio of cell shape (Fig. 7).

DISCUSSION

Micropatterned substrates allow us to control the shape and projection area of cells precisely. As shown in this study, human tendon cells can be confined on

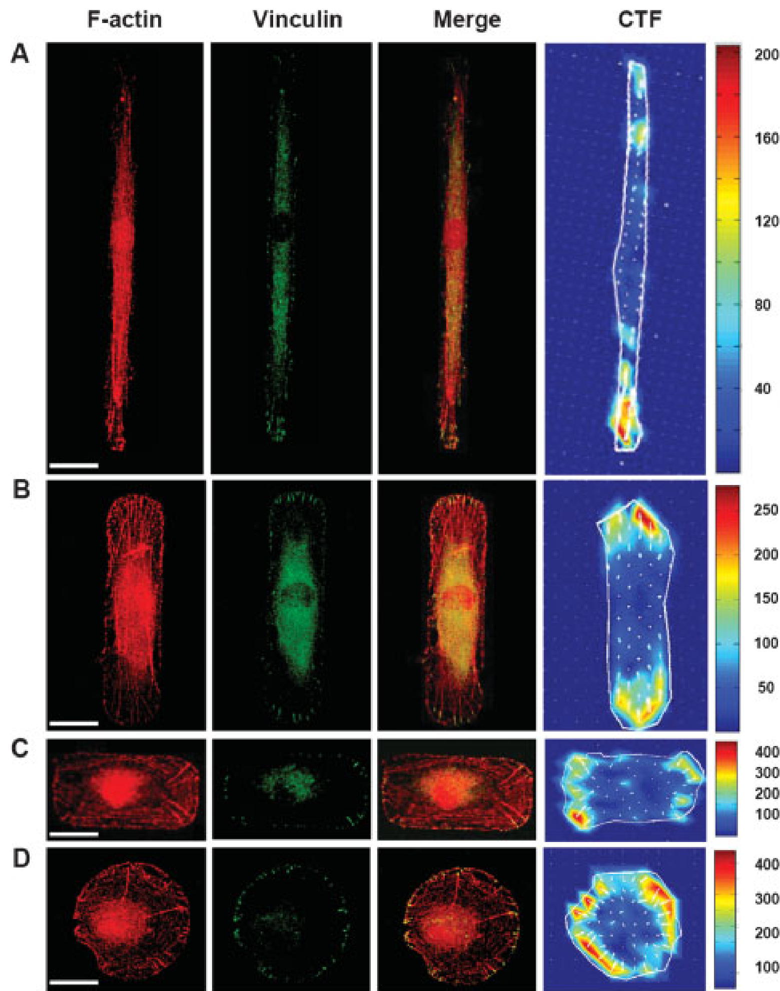

Fig. 6. Immunofluorescence microscopy images of F-actin, vinculin, and the overlay of them, as well as CTFs in micropatterned HTFs. **A**–**C**. Rectangular HTFs with aspect ratios of 19.6, 4.9, and 2.2, respectively. **D**. circular HTF. (Scale bars: 20 μm).

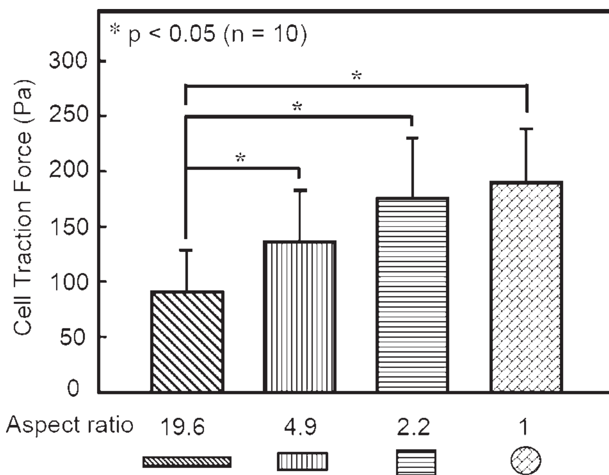

Fig. 7. The effect of cell shape on CTF magnitude. It increased with decreasing aspect ratio of the cell.

micropatterned adhesive islands of any shape and size as desired. Therefore, micropatterned cell-adhesive surface provides an ideal experimental platform for studying functions of cells that have specific shapes in vivo at whole cell and sub-cellular levels [Lehnert et al., 2004]. Moreover, precisely controlling cell shape reduces cell variability as opposed to a cell population such that the biological response of single cells can be investigated, instead of an "averaged response" of a heterogeneous cell population. For example, micropatterned substrates have been used to create cell-based sensors in which cells exhibit a more reproducible and defined phenotype [Park and Shuler, 2003; Fujimoto et al., 2006]. It should be noted that, however, while micropatterning technology can control cell shape precisely and therefore creates a more uniform cell group compared to cells on non-micropatterned surfaces, it cannot control cell cycle; in

other words, these micropatterned cells may be in different cell cycles; as a result, these micropatterned cells can still differ in their functions.

In vivo, tendon cells, which are aligned with the collagen fibers in the longitudinal direction of the tendons, are highly elongated in shape [Wang et al., 2003]. By producing collagen type I and other matrix proteins, tendon cells in vivo function to maintain, repair, and remodel tendon matrix [Yang et al., 2004]. Using micropatterned HTFs, this study was able to show collagen type I expression levels at single cell level. Furthermore, collagen type I expression was the highest in the most elongated cells, which have shape most similar to those tendon cells in vivo, suggesting that normal tendon fibroblasts in vivo have an ''optimal shape'' for maximizing synthesis of type I collagen, a major function of these cells. For those HTFs assuming circular shape, their collagen type I expression levels were, on average, only ~65% compared to that of those elongated cells. The reduction in collagen expression in tendon cells of circular shape may contribute to the development of tendinopathy (Wang et al., 2006). Based on these data, it is also tempting to suggest that these circular cells may behave like fibrocartilage cells, as they are round in vivo and are known to produce low level of collagen type I but high level of collagen type II instead [Hall, 1983; Benjamin and Ralphs, 2004].

An immediate question is: How does cell shape link to the biochemical process of protein synthesis in a cell? It is known that mechanical stresses generated by cells within cytoskeleton play important roles in the control of cell shape as well as cell function [Maniotis et al., 1997; Chicurel et al., 1998]. Many signaling molecules bind to the cytoskeleton and/or link with integrins at focal adhesion complexes, both of which are highly mechanical deformation-sensitive [Ingber, 1997; Zhu et al., 2000; Geiger and Bershadsky, 2002; McBeath et al., 2004]. Therefore, deformation in cytoskeleton, regulated by the mechanical stresses in it, provides a physical basis for transducing mechanical signals into biochemical signals [Alenghat and Ingber, 2002]. In addition, because of the physical linkage between cytoskeleton (e.g. intermediate filaments) and nuclear matrix, deformation of nuclear matrix caused by cytoskeleton reorganization will alter DNA topography and transcription and consequently affect cell functions, such as gene expression and protein synthesis [Ingber, 1997].

Besides the cytoskeleton, cell-generated cytoskeletal tension influences cell fate and functions [McBeath et al., 2004; Polte et al., 2007]. The changes in cell-generated forces have been shown to be responsible for the effect of cell spreading area on cell proliferation [Singhvi et al., 1994; Chen et al., 1997; Wang et al., 2002]. Cells with larger spreading area generate stronger tension and have an enhanced ability to proliferate [Singhvi et al., 1994; Chen et al., 1997; Parker et al., 2002]. Tension in the cytoskeleton has also been shown to be critical in the commitment of human mesenchymal stem cells into adipocyte or osteoblast lineages [McBeath et al., 2004]. Using cells populated in collagen gels, Agarwal et al. showed that higher contraction was related to higher levels of collagen production in fibroblasts [Agarwal et al., 2006]. In this study, we found that changes in cell shape resulted in different organizations in cytoskeletal structures and altered the direction and spatial CTF distribution. This may be responsible for our finding that tendon cells with various aspect ratios expressed different levels of collagen type I. In addition, given the fact that cells can use traction forces to reorganize collagen fibers [Huang et al., 1993; Eastwood et al., 1998] and elongated cells produced traction forces along their long axis, we speculate that this may be one reason why these elongated tendon cells produce aligned collagen matrix in vivo and in vitro [Wang et al., 2003].

In this study, we have also demonstrated that cell shape, controlled by micropatterned adhesive islands on substrate, influenced spatial distribution of actin cytoskeleton, focal adhesions, and direction and spatial CTF distribution. The mechanisms by which cell shape alters actin filament organization and spatial arrangement of focal adhesions and traction forces might be due to the regulation of mechanical signals from the ECM-coated islands with various shapes during cell spreading. As shown in this study, the largest traction stresses occurred at the two ends of highly elongated rectangular cells, at corners of shortened rectangular cells, or around the edge of circular cells. These mechanical stresses may promote integrin clustering and focal adhesion complex formation, resulting in cytoskeletal realignment in the formation of bundles of contractile stress fibers along the directions of stresses stretching from focal adhesions, as well as recruitment of myosin to generate cell contraction [Chicurel et al., 1998; Thery et al., 2006]. Finally, cells establish a stabilized structure of cytoskeleton and focal adhesions against ECM and achieve force balance between cells and ECM. Physical connections between integrins, cytoskeletal filaments, and nuclear scaffolds, which have been observed in our immunostaining results and also demonstrated in previous studies [Maniotis et al., 1997; Chicurel et al., 1998], may provide a pathway for the mechanical signal transfer throughout cells, thereby producing integrated changes in cellular structure in response to changes in cell-ECM adhesion [Ingber, 1997]. However, the exact molecular mechanisms that are responsible for cell shape-regulated cellular function seen in this study are not known. It could be that alterations in CTFs and other physical effects resulting from the imposed shape serve as the actual signals

that trigger a cascade of molecular events leading to changes of cellular function. Further studies are required to control cell cycle and probe those molecular signals that account for alteration of cellular function with different shapes.

In summary, using micropatterning techniques, we have investigated the effect of cell shape on the level of collagen type I expression as well as on the changes of actin cytoskeleton, focal adhesions, and CTFs. We have shown that elongated tendon cells expressed higher collagen type I than those shorter cells even though cell spreading area was constant, indicating tendon cells in vivo, which assume an elongated shape in normal tendons, has an ''optimal shape'' to perform their major function, that is, to synthesize collagen type I. We have also shown that changes in cell shape induced alterations in actin cytoskeletal structure, spatial arrangement of focal adhesions, direction and spatial distribution of traction forces in tendon cells, which may collectively affect the observed differential collagen type I expression in tendon cells with different shapes. Future studies are necessary to investigate the molecular signaling events that are responsible for cell shape-regulated cellular function.

ACKNOWLEDGMENTS

The authors thank the Center for Biologic Imaging (CBI) at the University of Pittsburgh for technical assistance with fluorescence microscopy.

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