Microtextured substrata alter gene expression, protein localization and the shape of cardiac myocytes

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Abstract

Many of the experiments designed to understand fundamental principles in cardiac physiology are performed in vitro using myocytes isolated from adult or neonatal hearts. However, these cells have probably lost some of their original properties in culture prior to study. Our objective is to recapitulate cardiac myocyte structure and function by growing cells on microtextured silicone substrata produced by photolithography and microfabrication techniques. Myocytes are plated on nontextured, micropegged (5 \(\mu\)m high), microgrooved (parallel grooves with a depth of 5 \(\mu\)m) or combination (micropegged and grooved) substrata. Myocytes plated on microtextured surfaces display a change in cell shape with an increase in myofibrillar height and a decrease in cell area. This shape change did not affect the stoichiometry of the myofibrillar proteins but did elicit microenvironmental remodeling of proteins that mechanically attach the cell to its surroundings. Cells terminate in a sarcomeric striation on the vertical interface of the peg whereas on nontextured surfaces they end in long nonstriated cables. Vinculin, a focal adhesion protein, was found to decrease in expression on combination surfaces as compared to nontextured substrata. A three-dimensional microtextured substratum appears to reintroduce a more physiological microarchitecture for tissue culture that may have potential uses in biological research as well as in tissue engineering and diagnostic applications.

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1. Introduction

The objective of this work is to develop a new cell culture system that will provide a three-dimensional environment enabling the maintenance of the in vivo cardiac myocyte phenotype. It will then be possible to effectively study cell processes in a physiological in vitro environment. Experimental modifications of the intact heart are valuable in understanding the integrated cardiovascular physiological responses but are limited in that gross manipulations could result in compromised function or even the death of the animal. In order to study isolated pathways in a controlled manner, it is necessary to turn to a tissue culture model. Currently, primary cardiac myocytes are isolated from the newborn rat heart and plated on traditional flat, two-dimensional dishes to be used for study. In these two-dimensional culture models, cells are confined to associate laterally on the flat surface of the dish beneath a layer of medium. This results in the formation of a monolayer of cells. Unfortunately, this distribution does not resemble the original organization in the heart.

Most cell types in culture that adhere to substrata reside in an environment with flat topography. Some specialized surfaces have been designed consisting of other cells, extracellular matrix or artificial materials \cite{1}. The observation that cells respond to solid structures was made as long ago as early in the 20th century \cite{2} but went largely unnoticed until the 1970s when this phenomenon was re-explored by examining the effect of grooves on fibroblast-like cells \cite{3}.

Recently, three-dimensional models in vitro have been shown to have a variety of applications in basic and clinical studies \cite{4}. Three-dimensional cultures have
proven to be advantageous in maintaining hepatocytes in an environment that provides structural support and is functionally active [5,6]. Such a system would be particularly useful in the study of cardiac myocytes as mechanical influences such as force and cell attachment are known to be involved in their maintenance. It has been suggested that neonatal cardiac myocytes possess an innate capacity to re-establish complex, three-dimensional cardiac organization in vitro, even in the absence of an exogenous matrix [7]. A three-dimensional collagen matrix has been developed to form a spontaneously and coherently beating cardiac myocyte populated matrix that could be used to assess long-term performance in vitro [8]. Others have used a biodegradable polymer scaffold to which cardiac myocytes could attach to form contractile cell–polymer constructs [9,10] that could be later used as building steps towards the tissue engineering of cardiac patches.

In order to overcome some of the limitations in the conventional cardiac culture system, we have incorporated micropegged, microgrooved and peg-groove combination topographical features on the surface where cardiac cells are to be grown. The dimensions of the topographical features on which the cardiac myocytes grow correspond to the cell’s dimensions. We then study the effects of surface topography on cardiac myocyte morphology, myofibrillar protein stoichiometry and local remodeling in neonatal cardiac myocytes in culture. Our findings indicate that myocytes attach to a vertical structure and remodel to increase in myofibrillar height and decrease in cell area. The change in cell shape did not alter the myofibrillar protein content or myosin isoforms. The myocytes did show signs of remodeling at the vertical interface as was evidenced by the striations terminating at the peg. Vinculin, a focal adhesion protein, was found to decrease expression on peg-groove combination surfaces as compared to non-textured substrata. We have established a novel three-dimensional model for tissue culture that may be utilized in biological research as well as to serve as a template for tissue-engineering applications.

2. Methods and materials

2.1. Membrane production

The process for fabricating microtextured membranes with both upward projecting pegs and downward projecting grooves is schematically diagrammed in Figs. 1 and 2. Microchem SU8-10 photoresist spun on a clean dry silicon wafer at 3000 RPM for 30 s creates the peg pattern. The photoresist is 10 μm thick when spun at this speed and is next soft baked at 65°C for 2 min initially then at 95°C for 5 min. The wafer is then lithographically patterned by exposing the photoresist to UV light (for 15 s at 20 mW) through a mask placed on a Karl Suss mask aligner, followed by a hard bake at 65°C for 2 min followed by 95°C for 5 min. The wafer is then lithographically patterned by exposing the photoresist to UV light (for 15 s at 20 mW) through a mask placed on a Karl Suss mask aligner, followed by a hard bake at 65°C for 2 min followed by 95°C for 5 min. The pegs are finally developed for 5 min in photoresist developer.

![Micropegged Surface + Microgrooved Surface = Combination Surface](image-url)
To create the combined pattern, Shipley 1818 photoresist is spun on top of the peg patterned wafer at 500 RPM for 3 min. The 1818 photoresist is baked for 2 min at 90°C and patterned with grooves by exposing the 1818 photoresist to UV light at 20 mW for 13 s through a patterned mask. The 1818 photoresist is then developed in Shipley 351 developer. The peg and groove patterns can be positioned with respect to one another by aligning the pegs on the wafer with the windows on the “groove” mask before exposing the 1818 photoresist to UV light. As opposed to creating only a single repeating pattern which uses only one photomask, this technique uses a two-mask process to get multiscale and multidimensional features which can be arranged precisely into a 3D microtopography.

After wafer patterning, a 25 μm thick layer of parylene is deposited on the patterned surfaces by placing them in a parylene vapor deposition chamber (Labcoater 1 PDS 2010). All surfaces are coated with a release solution to allow easy parylene removal from both the wafer surfaces and the surface of the deposition chamber. Parylene dimer (Specialty Coating Systems) is placed in the vaporizer chamber. The parylene is heated to vaporization (175°C) and monomerization temperature (690°C). It cools as it enters the deposition chamber, with the wafers, and polymerizes, coating every surface. The run is complete when the parylene dimer supply is exhausted. This parylene layer can then be cut away and peeled making a template. A variety of curable polymers such as PDMS can be patterned using this parylene template.

The silicone (PDMS) membrane is made by mixing elastomer and catalyst (A103 Factor II, Inc.) in a 10:1 ratio and then gently spreading the gel over the parylene template. It is cured at room temperature for 24–48 h before peeling the silicone membrane off from the parylene. In the final micropitted silicone substrata, the micropogs are 10 μm in diameter, placed every 30 μm within a row and 100 μm between rows. In the combination substrata, micropogs are placed every 100 μm inside of a 10 μm wide groove (with a depth of 5 μm below the ridge). The 10 μm high pegs then project 5 μm out above the height of the ridge (see schematic illustration in Fig. 1).

2.2. Neonatal rat primary culture

The neonatal myocyte cultures were carried out as previously described [11]. Myocytes were maintained in complete media containing 5% FBS for 48 h after plating but were switched to serum-free media 24 h prior to drug treatment. Treatments were performed by the addition of pharmacological agents to the extracellular media. L-phenylephrine (15 μM) was used as a positive control to induce hypertrophy.

2.3. Immunocytochemistry

Myocytes were grown on nontextured and textured silicone membranes coated with laminin. The fixation and subsequent processing was carried out as previously described [11]. Paxillin (BD Transduction Laboratories, San Diego, CA) and vinculin (Chemicon International, Temecula, CA) were detected with monoclonal antibodies diluted at 1:1000 and 1:500, respectively. Myosin (Hybridoma laboratories, Iowa City, IA) and α-sarcomerotic actin (Sigma, St. Louis, MO) were each diluted at 1:500. A goat-anti-mouse secondary conjugated with
rhodamine was used at 1:500 for each of the above primary antibodies. The counter staining of the actin filaments was carried out using phalloidin conjugated with rhodamine to produce red filaments or ALEXA 488 for green filaments (Molecular Probes, Eugene, OR) at a dilution of 1:1000. The nuclei were stained with DAPI in the mounting medium (Vector Laboratories, Burlingame, CA).

2.4. Nonstriated fiber length

Myocytes from neonatal rats were plated on textured or nontextured surfaces for 4 days, fixed, and stained for actin filaments and viewed with confocal microscopy (Zeiss LSM 510). The distance was measured (in μm) from the end of the cell to the first striation on nontextured, micropegged, microgrooved or combination surfaces. On textured surfaces, the measurement was taken from the vertical surface of the peg to the first sarcomere.

2.5. Height of myofibrillar mass

Confocal microscopy obtained optical sections of myocytes in a Z-stack near the nucleus from the bottom to the top of the cell. The sections were then reconstituted into three-dimensions to reveal the vertical plane. The height of the myofibrillar mass is in μm.

2.6. Attachment of myocytes to pegs

Micrographs were taken of myocytes in culture on day 4 using a Nikon TMS phase-contrast microscope. All fields were selected randomly and images taken at the same magnification using a 20X objective. Approximately a 100 × 175 μm² area in the center of each micrograph was used for analysis. Each cell in the allotted area was examined for attachment to a peg. Cells that terminated on a peg were counted (C) in each micrograph. In order to determine the percent attachment, the number of cells terminating on a peg was divided by the total number of cells in the field (F).

\[
\text{% attachment} = \frac{C}{F} \times 100.7
\]

Images of pegs were superimposed over the micrograph of myocytes grown on nontextured surfaces at the location where they would have been found on micropegged surfaces. These “pseudopegs” were used to assess the percent of attachment on nontextured surfaces.

2.7. Myocyte cell area

Phase-contrast fields were selected randomly and images taken at the same magnification using a 20X objective. A square grid with 45 μm spacing was placed over a micrograph area of about 200 × 325 μm². A count, P, was taken of the number of points made on a cell at the cross hairs of the grid lines. Another count, N, was taken of all the cells in the field and d is designated as the grid spacing in microns. The cell area was then calculated by the following formula:

\[
\text{Average cell area} = \frac{P d^2}{N}.
\]

The area is that projected from the xy plane of the micrograph.

2.8. Protein and DNA of cells

Whole cell protein extracts were prepared from 60 mm dishes with 28 mm silicone substrata inserts seeded with myocytes at 1000–2000 cells/mm². Cells were washed twice in Ca²⁺, Mg²⁺ free phosphate buffered saline. The inserts were then placed in clean dishes where the cell membranes were dissolved in 250 μl of cell lysis buffer [89% H₂O, 1% sodium dodecyl sulfate (SDS) and 10% protease inhibitor cocktail (Sigma, Saint Louis, MO)]. The cell extracts were scraped into an eppendorf tube with a rubber policeman. The Bradford method [12] was utilized to quantify proteins for each sample. Briefly, 10 μl of each sample was added to 90 μl of molecular grade water. The Bio-Rad protein dye (Bio-Rad Laboratories, Hercules, CA) was diluted 1:10 in molecular grade water and 5 ml was added to each sample and standard. Each solution was vortexed and incubated at room temperature for 5 min. The standard curve was derived by plotting absorbance at 595 nm versus the known bovine serum albumin (BSA) concentration in the standards. The absorbance of each sample was then compared to the standard curve to determine the protein concentration.

The DNeasy kit (Qiagen, Valencia, CA) was used to determine the DNA content of each sample. Briefly, 200 μl of each sample was loaded onto the DNeasy membrane and then rinsed through a series of steps to remove any impurities. The DNA was eluted into a clean eppendorf tube and the absorbance was read at 260 nm. The total yield was determined by the following formula:

\[
\text{Total DNA} = 50 \text{ (μg/ml) } \times \frac{A_{260} \times \text{dilution factor}}{\text{volume of sample (ml)}}.
\]

2.9. α-sarcomeric actin, myosin and vinculin protein expression

Equal amounts of protein from cells grown on nontextured, micropegged and combination surfaces were loaded onto and resolved by a 7.5% SDS-polyacrylamide gel, then transferred to a nitrocellulose membrane. The membrane was probed for α-sarcomeric actin (Sigma, St. Louis, MO) at a dilution of 1:5000 then
followed with a goat-anti-mouse IgM conjugated with horseradish peroxidase (BD Transduction Laboratories, San Diego, CA) secondary (1:10,000) for 1 h at room temperature. The monoclonal antibodies vinculin (Chemicon International, Temecula, CA) and myosin (Hybridoma laboratories, Iowa City, IA) were used to probe the membrane at a dilution of 1:4000 and 1:1000, respectively. The membranes were then incubated with a goat-anti-mouse IgG with horseradish peroxidase conjugated secondary antibody diluted at 1:10,000 (BD Transduction Laboratories, San Diego, CA). The proteins were visualized using electrogenerated chemiluminescence (ECL, Amersham, Piscataway, NJ) and exposed to Hyperfilm (Amersham, Piscataway, NJ). Membranes were stripped with 1% SDS, 15 mM Tris buffer pH 6.8, 0.02% α-mercaptoethanol at 55°C for 30 min and stained with Amido Black staining solution (Sigma, St. Louis, MO). Both stained membranes and ECL films were scanned and analyzed for optical density with Gel-Pro Analyzer 3.0 (Media Cybernetics, Des Moines, IA). All data have been expressed as the densitometric ratio of ECL signal to total protein staining.

3. Results

3.1. Evaluation of textured surfaces

Using the microfabrication process outlined in Fig. 2, multiple microtextures in various polymers can be reliably produced. The silicon wafer with dual patterned photore sist is made and a template is produced by the parylene deposition process. The purpose of parylene is twofold: (1) to create a flexible and reusable template (2) to allow for easy separation of the polymer gel from the patterned template. The final micropeg and microgrooved combination texture made from a polydimethyl siloxane (silicone) membrane is shown in Fig. 3a. The peg heights were uniform (±5%) as determined by the profilometer (data not shown). The micropegs are spaced 30 µm center to center along the row (z-direction), and 100 µm center to

![Fig. 3. (A) Phase micrograph of a combination silicone membrane showing the micropegs within the microgrooves. (B) The schematic drawing showing dimensions of these structures.](image-url)
center between the rows (x-direction) as shown in Fig. 3b. The grooves are 10 μm wide, with a spacing of 10 μm between each groove.

The alignment of the grooves and pegs can be adjusted as necessary. Spacings can be controlled by the mask pattern used and by careful alignment of the two patterns. For instance, one can have pegs line up so that they lie inside a groove or between individual grooves. Moreover, pegs can be spaced to be either in every groove or in every other groove, depending on desired application. Peg and groove height and depth, respectively, can be tailored by controlling photoresist layer thickness through spinning speed during deposition.

3.2. Myocyte attachment to vertical surface of the pegs

In order to study the effect of the surface topography on the myocytes, we first examined the percentage of cell attachment to the vertical pegs. Micrographs were taken of myocytes plated on nontextured, micropoged and combination substrata (Figs. 4a–c, respectively) after 4 days in culture. Only a small fraction of cells attach to pseudo-pegs on nontextured surfaces (15.4 ± 3.0%, n = 12 images) as seen in Fig. 4d. In contrast, the vast majority of myocytes on micropoged (89.3 ± 1.2%) and combination (91.0 ± 0.9%) surfaces terminated on an actual peg.

3.3. Height of myofibrillar mass

The thickness of the myofibrillar layers was examined (Fig. 5a) near to the peg in textured surfaces or near the nucleus (in nontextured surfaces). As a point of reference, myocytes on nontextured surfaces were 6.5 ± 0.6 μm thick (n = 60 cells from 6 cultures).
myocytes on micropegged surfaces were significantly thicker (9.8 ± 0.5 µm, p < 0.01) than those on nontextured surfaces. The myofibrils on combination membranes were also thicker than those on nontextured surfaces (11.2 ± 0.6 µm, p < 0.001) but not significantly thicker than those on micropegged surfaces (p > 0.05).

3.4. Protein and DNA of cells

The ratio of protein-to-DNA is a tool used to assess the average protein mass per nucleus of myocytes in each sample. Because the DNA content of myocytes is thought to stay constant, this offers the opportunity to test out the possible occurrence of hypertrophy. We examined the protein-to-DNA ratio (Fig. 5b) in order to examine the total protein accumulated per nucleus. We found that on nontextured surfaces, the protein-to-DNA ratio was 54.4 ± 5.5 (n = 8 cultures). The ratio was not significantly different in myocytes plated on micropegged (43.6 ± 9.5) or combination membranes (61.2 ± 7.1, p > 0.05). A series of myocytes plated on nontextured surfaces were treated for 48 h with at 15 µm l-phenylephrine (a known hypertrophic agent) to ensure that our system could indeed detect hypertrophy. As expected, the samples treated with L-phenylephrine had significantly higher protein-to-DNA ratios (87.9 ± 9.0, p < 0.05) than the samples from either the nontextured or textured surfaces.

3.5. Area of myocytes

We measured the average area per myocyte when plated on each surface (Fig. 5c). Myocytes plated on nontextured membranes occupied an average area of
3.6. Formation of nonstriated cables

Myocytes plated on nontextured, microgrooved, micropegged and combination (Figs. 6a-d) substrata are seen by confocal microscopy. Myocytes on nontextured and microgrooved surfaces had striations in the center of the cell but had long nonstriated cables at the ends (Figs. 6a and b). However, in myocytes that terminate on a vertical peg, the striations extend all the way to the end (Figs. 6c and d). The length of the nonstriated cables was measured from the various surfaces studied as shown in Fig. 6e. Myocytes plated on nontextured surfaces had nonstriated cables that were 26.0 ± 1.1 μm long. The lengths of these cables on micropegged surfaces were significantly shorter at 3.1 ± 0.3 μm and 3.1 ± 0.5 μm on combination surfaces (p < 0.001).

3.7. Protein expression of α-sarcomeric actin and myosin

Western blot analysis was used to quantify the amount of α-sarcomeric actin (Fig. 7b) and myosin (Fig. 7d) in myocytes plated on nontextured and textured surfaces. The data was normalized to total protein then expressed as a percentage of control (nontextured which was set at 100%). There were no significant changes in α-sarcomeric actin expression on micropegged (111.5 ± 11.4%) or combination (115.7 ± 14.3%) compared to nontextured surfaces (p > 0.05, n = 6 cultures). Similarly, there were no significant changes in myosin expression on micropegged (107.3 ± 10.8%) or combination (101.8 ± 17.3) compared to nontextured surfaces (p > 0.05, n = 8 cultures).

3.8. Localization and distribution of actin and myosin in the myocyte

The localization and distribution of the sarcomeric proteins, α-actin and myosin, were examined in myocytes plated on nontextured and textured surfaces by immunocytochemistry and visualized through the use of confocal microscopy. As seen in Fig. 7a, α-sarcomeric actin is present in a striated pattern inside the myocyte. However, despite the absence of striations near the cell edge where the myocyte contacts the bottom surface of the silicone membrane, the α-actin is still present. The same pattern holds true for myosin distribution (Fig. 7c) in the sarcomere and near the cell’s edge. A schematic drawing depicting the assembled and unassembled sarcomeric proteins throughout the myocyte is seen in Figs. 8a–c. A myocyte plated on a nontextured surface (Fig. 8a) has striations in the center of the cell (indicative of assembled sarcomeric proteins) but not in the periphery. In contrast, myocytes plated on micropegged or combination substrata (Figs. 8b and c) have assembled sarcomeres that extend to the end of the cell when attached to a vertical peg.

3.9. Localization of paxillin

Myocytes form focal adhesion contacts with the matrix as schematically depicted in Fig. 9. The localization and distribution of the focal adhesion protein, paxillin (Figs. 10a–d), was examined in myocytes plated on nontextured and textured surfaces. Paxillin is concentrated in punctate clusters at points where the myocyte attaches to the substrate. The distribution of paxillin on the bottom surface of traditional, nontextured surfaces is illustrated in Fig. 10a. However, on textured surfaces where a third dimension was introduced (Figs. 10b,d), attachment was no longer limited to the bottom of the dish. Paxillin localized around the peg as well as the floor of the membrane on micropegged surfaces (Fig. 10b). Paxillin is localized on the sides of the grooves as well as around the peg in combination surfaces seen in Fig. 10d, taken a few microns above the bottom surface of the dish. It is clear from the images taken close to the bottom of the dish that paxillin is distributed near the pegs in the groove as well as on the floor of the membrane.

3.10. Protein expression of vinculin

The localization pattern of vinculin is similar to that of paxillin (data not shown). Western blot analysis was used to quantify the amount of vinculin in myocytes plated on nontextured and textured surfaces. There was no difference in vinculin expression (Fig. 10e) in myocytes plated on nontextured and micropegged membranes (p > 0.05, n = 5 cultures). However, there was a significant decrease in vinculin expression on combination surfaces as compared to nontextured membranes (p < 0.05).

4. Discussion

Topographical cues have significant effects on cellular behavior. This study indicates that the three-dimensional topography of the surface significantly affects cardiac myocyte architecture and attachment, altering the cell’s microenvironment and eliciting local remodeling. We have shown that when myocytes attach to pegs, there is (1) an increased myofibrillar layer, (2) a decrease in cell area, (3) a decrease in the formation of nonstriated cables, (4) an alteration in the distribution
Fig. 6. Actin filaments (red) are stained on (A) nontextured, (B) microgrooved, (C) micropegged and (D) combination surfaces. The striations in myocytes that terminate on a peg (P) extend all the way to the peg (arrow heads in C and D). However, when the myocytes terminate on the nontextured, two-dimensional surface, there are nonstriated cables (arrows in A, B and C). (E) The length of the nonstriated cables. Myocytes that terminate on a vertical structure (micropegged or combination substrata) have sarcomeric striations much closer to a peg than in a cell on a nontextured surface (p<0.001). Scale bar = 10 µm.
of paxillin and vinculin and (5) a decrease in the expression of vinculin on combination surfaces. Reactions to topography include cell orientation, changes in cell motility, cell adhesion and cell shape [13]. Some cell types react to topographical impressions as shallow as 11 nm [14]. Surfaces with microtopography have been shown to enhance the attachment of fibroblasts to the substratum [15]. Here, nontextured, micropegged and combination surfaces were used to examine the effect on cardiac myocyte morphology, myofibrillar protein stoichiometry and local remodeling.

We show here that myocytes attach to micropegged surfaces, perhaps permitting force transmission as in vivo. In fact, our data suggests that myocytes attached preferentially to the pegs on such surfaces. Early studies have shown that myocytes grow in more physiological arrangements when attached to a perpendicular surface, such as one created by a pin impaled in a soft dish [16]. Myocytes terminating on a peg demonstrated a subsequent increase in the thickness of the myofibrillar layers. This layering was correlated with a concomitant decrease in cell area of myocytes plated on micropegged and combination surfaces. We have interpreted the increase in the myofibrillar layer to be associated with the change in cell shape that we have reported. The fact that there were no significant changes in the protein-to-DNA ratios strengthens our argument that these differences were due to an alteration in cell shape. This is not surprising since the mechanics of the cell’s microenvironment has been shown to determine cell shape [17,18].

The texture that we have added to the culture system is similar to some of the features naturally found in the myocyte’s in vivo environment. In fact, the basement membrane is a complex meshwork consisting of pores, fibers, ridges and other features from dimensions ranging in the nano to micrometer scale. This sub-stratum becomes the topography and chemistry upon

Fig. 7. (A) α-Sarcomeric actin (red) and actin filaments (green) are stained using immunofluorescence and found throughout the cell, including the nonstriated portions (arrow). (B) Levels of α-sarcomeric actin are measured by Western blot analysis and normalized to total protein, and expressed as a percentage of cells from nontextured substrata. There are no differences in α-sarcomeric actin protein levels in myocytes plated on nontextured, micropegged or combination surfaces (p > 0.05, n = 6 cultures). (C) Myosin (red), actin filaments (green) and nuclei (blue). Both myosin and actin filaments are found throughout the cell, including the nonstriated portions (arrow). (D) Western blot analysis shows myosin expression as a percentage of that found in cells from nontextured substrata. There is no difference in myosin protein expression in myocytes plated on nontextured, micropegged or combination surfaces (p > 0.05, n = 8 cultures). Scale bar = 20 μm.
Fig. 8. Schematic of assembled and unassembled sarcomeric proteins in myocytes plated on nontextured (A), micropegged (B) and combination (C) substrata.

Fig. 9. Schematic of myocytes forming cell–cell and cell–matrix contacts showing localization of connexin43, N-cadherin and the focal adhesion complex including paxillin and vinculin.
which the overlying cells and their structures are supported in vivo [19]. The topography of the basement membrane has been mimicked by synthetic surfaces and shown to influence the behavior of cells such as fibroblasts, hepatocytes and osteocytes to name a few [13,14,19]. Technologies utilizing microtopography are critical in understanding how cell adhesion and shape regulate different cellular responses [20] as well as providing a means to control these responses in vitro. By using such a tissue culture environment, we are better equipped to address questions about cellular processes.

We found no changes in the ratios or total amount of α-sarcomeric actin and myosin in cultures grown on nontextured and textured surfaces. This is not surprising given what is known about the control of muscle structures. Assembly of subcellular elements depends on the equilibrium and rate constants of the contractile proteins and their associated assembly scaffolds. Therefore, these molecules impose thermodynamic and kinetic constraints on assembly mechanisms that act in vivo [21,22]. The assembly of thin filaments has long been thought to occur independent of thick filaments [23]. The delicate balance in the stoichiometry of myofibrillar proteins under normal physiological circumstances is mostly maintained by selective degradation of excess monomeric proteins that are unincorporated into the fibril. Degradation rates increase rapidly upon disassembly from the fibril [24].

We found no notable differences between myocytes in the living heart and those that have been isolated and

Fig. 10. Cardiac myocytes are stained for paxillin (red) and nuclei (blue) on (A) nontextured, (B) microppegged and (D) combination surfaces. (C) Confocal optical section taken close to the bottom of the dish demonstrating the attachment around the peg (nucleus, N and micropegs, P). Note the punctate distribution of paxillin on nontextured substrata as marked by an asterisk (A and B). (E) Levels of vinculin (a focal adhesion protein) are measured using Western blot analysis and normalized to total protein. There is a significant decrease in vinculin protein expression in myocytes plated on combination substrata as compared to nontextured (p<0.05, n = 5 cultures).
grown in tissue culture. However, prior to our studies, cultured myocytes had myofibrils that were only striated in the center of the cell with nonstriated extensions into the cell’s periphery. These nonstriated structures have never been seen in the whole heart where the striations end at the intercalated disc abutting the adjacent myocyte. The sarcomeres we see terminate in a fashion similar to their ending at the intercalated disc of an adjacent myocyte in vivo. We have also observed these nonstriated regions in myocytes plated on flat, non-textured surfaces. In agreement with other findings, we found myosin [25] and α-sarcomeric actin [26] staining throughout the myocytes, including the nonstriated cables in the periphery of the cell. We conclude that the sarcomeric proteins are present in the nonstriated cables; however, they are not registered into regular striated patterns. Interestingly, the nonstriated cables were virtually absent in myocytes that terminate on a peg. Although the mechanism for the change in striation pattern is not known, it is possible that the peg provides intracellular mechanical stability that is otherwise lacking in standard two-dimensional cultures.

We found that terminal focal adhesions (specialized regions of the cell membrane that mediate adhesion to the extracellular matrix) were formed at the vertical and longitudinal surfaces in addition to the ones traditionally formed at the bottom surface. Cell–matrix interactions play an important role in the assembly of myofibrils as well as other biological processes [27]. Actin filaments are anchored to transmembrane receptors from the integrin family at these focal adhesion sites through junctional proteins. Some of the proteins associated with focal adhesions function as structural links between membrane receptors and the actin cytoskeleton, others are signaling molecules, and some act in both capacities [28]. In muscle, we find focal adhesions both at the ends and the circumferential surfaces of the myocytes.

The term costamere has been used to define the rib-like plaques at the circumference, containing many proteins, such as vinculin and paxillin, that are located between the cell membrane and Z-disks in cardiac myocytes [29]. Costameres are thought to be sites of lateral force transmission between the cell’s internal contractile machinery and the extracellular matrix [30] by providing a mechanical linkage. Most of the information that we have available to date on focal adhesions is derived from studies done in vitro. Due to the nature of traditional two-dimensional culture dishes, isolated cardiac myocytes can only re-attach at the bottom surface of the dish in a manner analogous to costamere formation. Relatively little is known about the cell–matrix adhesive structures formed in three-dimensional matrices of living tissue, particularly in the natural in vivo environments, spurring innovative research in this field [31,32]. However, the textured culture system that we have produced overcomes this limitation for three-dimensional attachment, allowing for the ability to study these processes in an environment that more closely resembles their natural milieu at both the ends (intercalated discs) and sides (costameres).

In conclusion, we have shown that alterations in surface topography have an impact on shape, gene expression, and protein distribution in surface micro-environment regions of the cardiac myocyte. Myocytes plated on textured surfaces display a change in cell shape stemming from the attachment to the vertical interfaces of the pegs and groove sides, which resulted in an increase in myofibrillar height and a decrease in cell area. The change in cell shape did not affect the stoichiometry of the myofibrillar proteins but did elicit microenvironmental remodeling of proteins that mechanically attach the cell to its surroundings.

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