The Motogenic Effects of Cyclic Mechanical Strain on Intestinal Epithelial Monolayer Wound Closure Are Matrix Dependent

JIANHU ZHANG,[*,‡] CHERI R. OWEN,[*,‡] MATTHEW A. SANDERS,[*,‡] JERROLD R. TURNER,[§] and MARC D. BASSON[*,‡]
[*]Department of Surgery, John D. Dingell VA Medical Center, Detroit, Michigan; [‡]Department of Surgery, Wayne State University, Detroit, Michigan; and [§]Department of Pathology, University of Chicago, Chicago, Illinois

Background & Aims: Complex deformation during normal digestion due to peristalsis or villous motility may be trophic for the intestinal mucosa. Because tissue fibronectin is increased in inflammatory states that may accompany mucosal injury, we evaluated the effects of cyclic mechanical strain and fibronectin on intestinal epithelial monolayer wound closure in Caco-2 and IEC-6 intestinal epithelial cells. **Methods:** Wounds created in intestinal epithelial monolayers were subjected to cyclic deformation. Wound closure was assessed by morphometry using microscopic imaging. Cell signals were assessed by Western blot and confocal microscopy. **Results:** Mechanical strain stimulated wound closure on fibronectin but inhibited closure on collagen in Caco-2 and IEC-6 cells. The effect was independent of proliferation or cell spreading. Myosin light chain (MLC) and extracellular signal–regulated kinase (ERK) were phosphorylated in response to strain in confluent monolayers on both collagen and fibronectin. Blocking MLC or ERK phosphorylation inhibited the motogenic effect of strain on fibronectin. Although phosphorylated MLC was redistributed to the leading edge of migrating cells following 6 hours of strain on collagen and fibronectin, phosphorylated ERK was redistributed to the lamellipodial edge only on fibronectin. **Conclusions:** Strain promotes intestinal epithelial wound closure by a pathway requiring ERK and MLC kinase. Fibronectin-dependent ERK redistribution in response to strain in confluent migrating cells may explain the matrix dependence of the motogenic effect. Repetitive deformation stimulates intestinal epithelial proliferation on a collagen substrate, but not fibronectin. Deformation may exert matrix-dependent effects on intestinal epithelial cells, promoting epithelial restitution in fibronectin-rich tissue and proliferation in fibronectin-poor mucosa.

The intestinal mucosa is subject to repetitive deformation from diverse physical forces under normal and pathophysiologic conditions. A variety of deformation patterns occur in the small intestine to ensure mixing and propulsion of contents during normal digestion,[1,2] including peristaltic contractions,[3] segmental contractions,[4,5] and pendular movements.[1,6] Villous motility is a distinct but important phenomenon in which intestinal villi repetitively lengthen and shorten in a fashion regulated by neural and humoral inputs and by the constituents of luminal chyme.[7–10] The luminal contents, while liquid, are largely noncompressible[11] and themselves interact repetitively with the gut mucosa in complex ways as they pass along the intestinal villi, inducing additional deformation of the villi and mucosa and even mucosal injury.[12] Such repetitive deformation stimulates intestinal epithelial proliferation and modulates intestinal epithelial differentiation in vitro[13] and activates tyrosine kinase signaling within the gut mucosa in vivo.[14] Indeed, a recent preliminary investigation suggested that a different model of repetitively increasing stretch could stimulate intestinal epithelial proliferation in pigs.[15] However, the effects of such deformation on epithelial migration and mucosal wound healing have not been investigated beyond the suggestion that villous shortening may aid restitution in guinea pig mucosal sheets.[16]

Mucosal healing is affected in many pathophysiologic states that have alterations in intestinal mucosal deformation. Sepsis, postsurgical ileus, and prolonged fasting may be associated with altered contractile rhythms, altered villous motility, and decreased mucosal deformation from luminal contents. The villi also change shape in adaptation to nutrients within the gut[17] and shorten in starvation or fasting,[18] and such changes in shape induce further deformation. Under these conditions, the mucosa atrophies,[19,20] proliferation slows,[21–23] brush border enzyme and transporter function are altered in a complex manner, [19,20,24–26] and mucosal barrier function is impaired, suggesting abnormalities in the mucosal response to injury.[27,28]

Early in response to injury, a complex healing process begins with cell migration. Restitution, independent of cell proliferation, is regulated by signals from growth factors, signaling molecules, and the extracellular matrix to which cells bind and across which they move via integrin receptors for matrix proteins.[29] Cell migration is a coordinated event of extension and retraction as integrins at the leading and trailing edges of the cell interact with matrix proteins and cause net movement across a surface.[30] Motility is more complex than simple cell spreading, which is necessary for cell migration and is also dependent on actin and myosin activation but does not result in net movement.[31,32]

The intestinal mucosa is constantly subjected to mucosal injuries that must heal to maintain normal gut function.[33] This physiologic repair system is required for recovery from pathologic mucosal injury such as chronic ulceration and inflammation in ulcerative colitis and Crohn's disease and is likely deficient when the mucosal barrier deteriorates in sepsis. Failure of mucosal healing is critical in these disease states and has even been implicated in their pathogenesis.[34] If mechanical forces in the gut contribute to intestinal homeostasis by influencing intestinal epithelial proliferation and differentiation, the ques-

Abbreviations used in this paper: DMSO, dimethyl sulfoxide; ERK, extracellular signal–regulated kinase; MLC, myosin light chain; pMLC, myosin light chain phosphorylation; pERK, extracellular signal–regulated kinase phosphorylation.

© 2006 by the American Gastroenterological Association (AGA) Institute
0016-5085/06/$32.00
doi:10.1053/j.gastro.2006.08.007

tion then arises whether alterations in such forces during disease might alter intestinal epithelial restitution, another important aspect of mucosal homeostasis.

Recent literature suggests that extracellular matrix plays a vital role in regulating cell migration and wound healing. Human colonic epithelial cells migrate rapidly on the dominant protein of the interstitial matrix, type I collagen, but more slowly on fibronectin.[35] However, blocking fibronectin can also impair corneal epithelial cell migration across a fibronectin substrate,[36,37] suggesting that fibronectin may nevertheless facilitate wound closure under some circumstances. Strain is mitogenic for intestinal epithelial cells on various matrix substrates in vitro, but the effect is inhibited by tissue fibronectin.[13] Studies in 2 animal models suggest that similar pathways may operate in vivo.[14,38] Because inflammation and injury lead to increased deposition of tissue fibronectin in chronic inflammatory states such as Crohn's disease or ulcerative colitis[39–41] and these and other disease states have altered gut motility, it is crucial to understand how fibronectin might modulate mucosal healing during altered intestinal strain states.

Precisely regulating mechanical deformation and matrix composition in vivo would be difficult without altering diverse other aspects of an organism's physiology. We therefore applied repetitive mechanical strain in vitro via standard techniques[42–44] to a common in vitro monolayer wound closure model[45–47] to examine the regulatory role of fibronectin and cyclic mechanical strain on intestinal epithelial motility during restitution. We hypothesized that mechanical strain modulates intestinal epithelial wound closure via extracellular signal–regulated kinase (ERK) phosphorylation (pERK) and myosin light chain (MLC) phosphorylation (pMLC) and that such modulation is matrix dependent.

Materials and Methods

Cell Culture

Human intestinal epithelial Caco-2 cells, an established highly differentiated human colonocyte cell line, were isolated and maintained as previously described.[48] Briefly, cells were maintained at 37°C in 5% CO_2 in Dulbecco's minimal essential medium containing 10% fetal calf serum and 10 μg/mL transferrin. All studies were performed on cells within 8 passages. The cells were grown to confluence in Petri dishes or matrix-precoated Flexwell I plates (Flexcell International Corp, Hillsborough, NC). IEC-6 normal rat intestinal epithelial cells were obtained from American Type Culture Collection (Manassas, VA) and grown in high-glucose (4.5 g/L) Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum and 0.1 U/mL bovine insulin. Cells were maintained in a 5% CO_2 in-air atmosphere and subcultured every third day. Only cells below passage 25 were used for experiments.

Matrix Precoating and Medium Treatments

Petri dishes and 6-well amino-coated Flexwell I plates were precoated with 12.5 μg/mL collagen I or tissue fibronectin (Sigma Chemical Co, St Louis, MO) at saturating concentrations.[13,48] Cells were seeded 300,000/well and again grown to confluence. ERK activation induced by strain was blocked by 20 μmol/L MEK antagonist PD98059 (Calbiochem, La Jolla, CA) or MLCK activation induced by strain was blocked with 10 μmol/L MLCK antagonist ML7 (Sigma Chemical Co) or 250 μmol/L specific MLCK inhibitor PIK,[49] all for 30 minutes before the application of strain to assess wound closure with inhibited signaling. Because PD98059, ML7, and PIK were dissolved in dimethyl sulfoxide (DMSO), control cells in these studies were similarly treated with 0.1% DMSO. In another study, during closure of defects made in the cell monolayers, cells were incubated in 15 μg/mL mitomycin C in phosphate-buffered saline for 2 hours before strain to block proliferation.

Strain Application

Once the cell monolayers were confluent, Flexwell plates were placed in a cell culture incubator (5% CO_2, 37°C) and the membranes were repetitively deformed, utilizing a computer-controlled vacuum manifold (FX3000; Flexcel, McKeesport, PA), by −20 kPa vacuum at 10 cycles/min, producing an average 10% strain on the adherent cells during periods of deformation. Nonuniformity of strain in the center of the flexible wells was addressed by placing a Plexiglas ring in the center and cells were plated around the periphery of the ring where strain is relatively uniform. Previous studies have shown that the cells remain adherent under these conditions and experience parallel elongation and relaxation.[50]

Motility Measurements

Caco-2 or IEC-6 monolayers on deformable membranes precoated with fibronectin or collagen I were subjected to 10% deformation at 10 cycles/min for 0–72 hours (Caco-2) or 0–24 hours (IEC-6) after induction of a small uniform circular wound. The wound model, as previously described by other investigators,[45–47] was created using a 1000-mL pipet tip (1.5-mm diameter) attached to a vacuum source, and suction was applied for approximately 1 second. At each time interval, the remaining wound area in each wound, an index of migration, was calculated after visualization on a Kodak Image Station (Perkin Elmer, Boston, MA) and compared with migration without strain.

Western Blot Analysis

Following strain, Caco-2 cells were lysed in lysis buffer (50 mmol/L Tris [pH 7.4], 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L Na3VO4, 50 mmol/L NaF, 10 mmol/L sodium pyrophosphate, 2 μg/mL aprotinin, and 2 μg/mL leupeptin [pH 7.4]) and centrifuged at 10,000*g* for 15 minutes at 4°C. Supernatant protein concentrations were determined using bicinchoninic acid reagent (Pierce Chemical, Rockford, IL). Equal amounts of protein were resolved by sodium dodecyl sulfate/polyacrylamide gel electrophoresis and electrophoretically transferred to Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were blotted with specific antibodies for phospho-ERK Thr 202, Tyr204 (Cell Signaling Technology, Beverly, MA), or phospho-MLC (Santa Cruz Biotechnology, Santa Cruz, CA), followed by incubation in appropriate secondary antibody conjugated to horseradish peroxidase. Bands were visualized using enhanced chemiluminescence (Amersham Pharmacia Biotech) and analyzed with a Kodak Image Station 440CF. Membranes were then stripped and reprobed with antibodies specific for total ERK and MLC, followed by incubation with appropriate secondary antibodies. All exposures used for densitometric analysis were within the linear range.

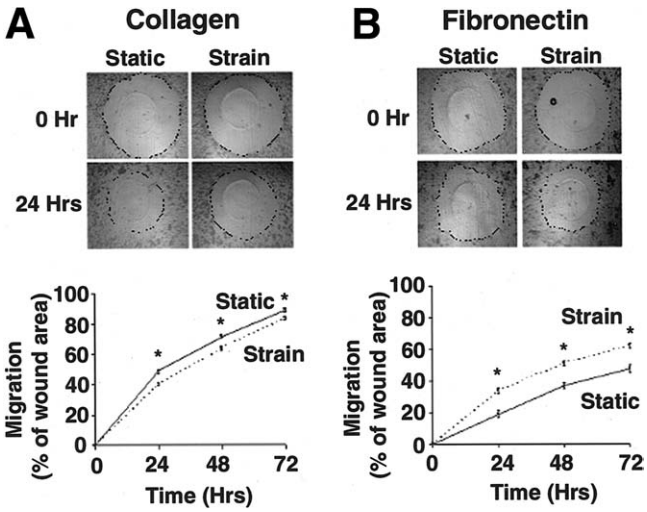


Figure 1. (*Top*) The initial size of each wound is depicted at 0 hours. After 24 hours, restitution in static cells is faster on collagen (*left*) than on fibronectin (*right*). However, strain inhibits motility on collagen but enhances it on fibronectin. Quantification showed that strain slightly inhibited motility on collagen (*A*; n = 9; $*P < .001$). Although static cells on fibronectin migrated more slowly than on collagen at all time points, strain significantly enhanced motility on fibronectin (*B*; n = 7; $*P < .001$) at all time points evaluated in this time course.

Immunocytochemistry

Following strain, cells were washed with 0.1 mol/L phosphate-buffered saline, fixed with periodate-lysine-paraformaldehyde fixative for 30 minutes on ice, and permeabilized with 0.2% Triton X-100 for 10 minutes at room temperature. Cells were then blocked in 10% heat-inactivated normal goat serum for 30 minutes at room temperature and incubated with polyclonal rabbit anti–phospho-ERK, polyclonal rabbit anti–phospho-MLC (both 1:50; Cell Signaling Technology), or integrin subunit αv (1:100; Chemicon, Temecula, CA) overnight at 4°C. Detection of proteins was achieved by incubating cells with Alexa Fluor 633–conjugated goat anti-rabbit secondary antibody for phospho-ERK, phospho-MLC, and αv, Alexa Fluor 488–conjugated phalloidin for actin (both 1:50; Molecular Probes, Eugene, OR), and Hoechst (1:20,000) stain for the nucleus. Flexwell membranes were removed from the wells and mounted on glass slides and coverslipped using Geltol mounting media (Immunon, Pittsburgh, PA); the cells were visualized on an LSM 510 confocal microscope (Carl Zeiss, Jena, Germany) equipped with a 40× objective and LSM software version 3.2 (Carl Zeiss).

Statistical Analysis

All data are expressed as mean ± SE. Statistical analysis was performed using paired or unpaired *t* tests or analysis of variance as appropriate. A *P* value of $<.05$ was considered significant.

Results

Strain Effects on Wound Closure in Caco-2 Cells

Basal migration was more rapid on collagen I than on fibronectin, consistent with previous findings.[51] Motility on collagen was slightly inhibited by repetitive strain (Figure 1*A*). In contrast, the relatively slower rate of migration observed on a fibronectin substrate was reversed by strain, which indeed significantly enhanced wound closure (Figure 1*B*). Cells preconditioned with 24 hours of mechanical strain on fibronectin showed a 34% ± 4.4% decrease in cell spreading when replated on fibronectin for 6 hours (n = 26; $P < .01$), a change in the opposite direction to the effect of strain on motility, suggesting that increased wound closure on fibronectin reflects some aspect of cell motility other than cell spreading.

We have previously reported that strain stimulates cell proliferation on collagen substrates but not on fibronectin, indicating that the effects we observed here were not due to increased proliferation on fibronectin. We further confirmed this by observing that strain increased motility across fibronectin as early as 6 hours after wounding (Figure 2*A*), too soon for substantial proliferation. Strain also promoted wound closure across fibronectin for up to 48 hours even with 2-hour pretreatment with mitomycin C to ablate proliferation (Figure 2*B* and data not shown). These results indicated that the observed effects of strain on the rates of wound closure on fibronectin are due to migration rather than proliferation.

Strain Effects on Wound Closure in IEC Cells

Although Caco-2 cells are commonly used to model diverse aspects of nonmalignant intestinal epithelial biology and signal transduction,[52–54] they are nevertheless an established cell line originally derived from a colon cancer.[55,56] We therefore exposed normal small intestinal epithelial IEC-6 cells to strain for up to 24 hours (Figure 3). Stimulation of wound closure across tissue fibronectin by strain was statistically significant at 8 and 24 hours. In contrast, strain inhibited wound closure on collagen I (n = 6, $*P < .05$). This parallel study in IEC-6 cells confirms that our observations in Caco-2 cells are not artifacts of a single established cell line.

Strain Effects on MLC

pMLC is essential in actin-myosin interaction, stress fiber and contractile ring formation, and ultimately cell motility.[57] We evaluated pMLC during strain on either collagen or fibronectin to determine if there was variation in MLC activation and localization on different matrix proteins.

Effects of strain on pMLC. Caco-2 cells grown to high confluence were subjected to 1 hour of strain on either collagen or fibronectin, and levels of pMLC were assessed by Western blot analysis (Figure 4). Strain was associated with a

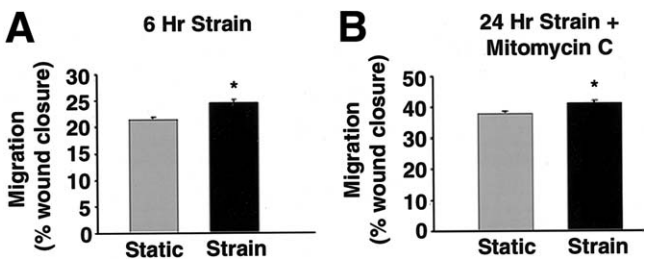


Figure 2. (*A*) Strain enhances motility on fibronectin as early as 6 hours after wounding (n = 13; $*P < .001$). (*B*) At 24 hours, strain stimulates motility across fibronectin even if proliferation is prevented using mitomycin C (n = 10; $*P < .05$).

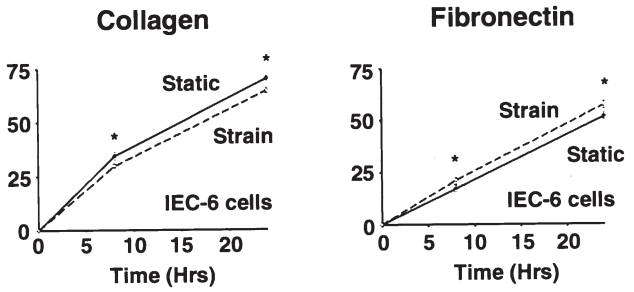


Figure 3. IEC-6 cells were cultured on membranes coated with (*A*) collagen I or (*B*) fibronectin. Migration of IEC-6 cells is different in response to strain on collagen I compared with fibronectin (n = 6; $*P < .05$). As with Caco-2 cells, although static IEC-6 cells on fibronectin migrated more slowly than on collagen at all time points, strain significantly enhanced motility on fibronectin.

42% increase in pMLC on collagen (n = 5; $*P < .05$) and a 35% increase in pMLC on fibronectin (n = 3; $*P = .01$). This suggests that MLC is phosphorylated in response to strain in a matrix-independent manner.

Strain and matrix effects on phosphorylated MLC localization. To assess the effects of strain and matrix substrate on MLC localization, Caco-2 cells grown to high confluence were subjected to 1 hour of strain on either collagen or fibronectin and localization of pMLC was determined by immunofluorescence confocal microscopy (Figure 5). Immunoreactivity for pMLC is generally increased in both static cells on fibronectin substrates and cells subjected to repetitive strain on fibronectin compared with cells grown on collagen under the same conditions and appear to colocalize with actin filaments (Figure 5, column 1, rows 3 and 4; actin: column 3, rows 3 and 4). pMLC is redirected to the lamellipodial edge in cells cultured on collagen and fibronectin substrates only after being subjected to 6 hours of strain (Figure 5, column 1, rows 2 and 4). Distribution of pMLC, nuclei, and actin are shown in single-channel and overlay images. Thus, the presence of fibronectin increased the amount of phosphorylated myosin immunoreactivity, but redirection to the lamellipodial edge occurred only with strain.

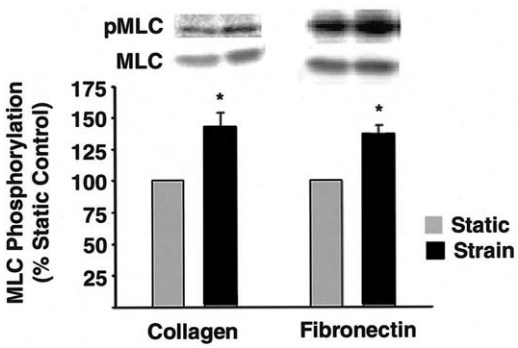


Figure 4. pMLC in confluent Caco-2 cells increased 42% on collagen and 35% on fibronectin following 1 hour of strain (n = 5 and n = 3, $*P < .05$). There was no change in total MLC.

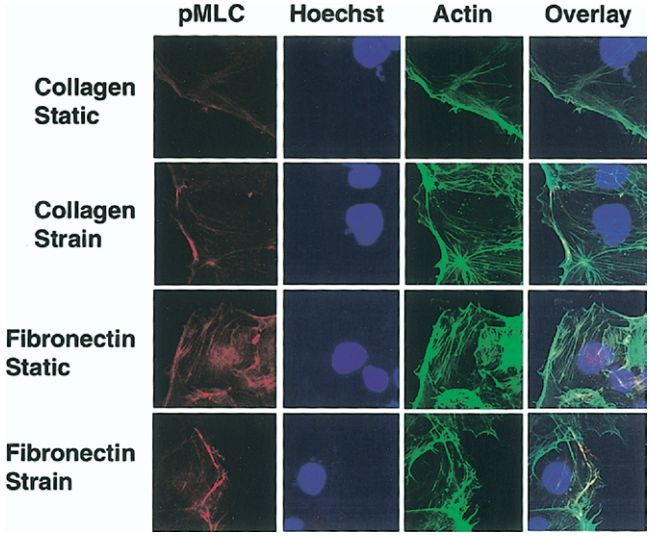


Figure 5. Confocal fluorescent microscopy of phosphorylated MLC relocalization following strain. Immunoreactivity of Alexa Fluor 633–stained pMLC is increased in cells plated on fibronectin, particularly along actin fibers. Strain is associated with redistribution of pMLC to the lamellipodial edge in cells plated on either collagen or fibronectin. Nuclei were stained with Hoechst and actin stained with Alexa Fluor 488 phalloidin. Distribution of pMLC, nuclei, and actin is shown in single-channel and overlay images.

Strain Effects on ERK

Signaling diversity between subconfluent and confluent cells on fibronectin. We previously reported the absence of pERK[13] and the apparent decrease in cell number in response to strain on a fibronectin substrate. In these previous studies of strain effects on cell proliferation, cells were seeded at low density and subjected to strain at very low confluence to avoid contact inhibition that might inhibit cell proliferation. However, subsequent work conducted in our laboratory suggested different results when confluent monolayers were studied and that the confluence of Caco-2 cell monolayers on fibronectin might alter the way the cells respond to strain. For this reason, we investigated the strain effect on Caco-2 monolayers that were confluent at the time of wound induction and cyclic mechanical strain. We observed activation of ERK 1 and 2 when confluent cell monolayers were subjected to strain on tissue fibronectin substrates (Figure 6). In contrast, little stimulation of ERK activation by strain was observed in subconfluent cells cultured on fibronectin, consistent with our previous report.

Matrix dependence of ERK phosphorylation following strain in confluent Caco-2 cells. Caco-2 cells grown to high confluence were subjected to 1 hour of strain on either collagen or fibronectin, and levels of pERK were assessed by Western blot analysis (Figure 7). We observed a 53% increase in pERK on collagen (n = 4; $*P = .04$) and a 67% increase in pERK on fibronectin (n = 4; $*P = .001$) following strain. This indicates that strain induces phosphorylation of ERK in cells plated on both collagen and fibronectin substrates in confluent Caco-2 monolayers.

Phosphorylated ERK relocalization following strain. Phosphorylated ERK is faintly present at the lamellipodial edge in static cells plated on collagen but not cells plated

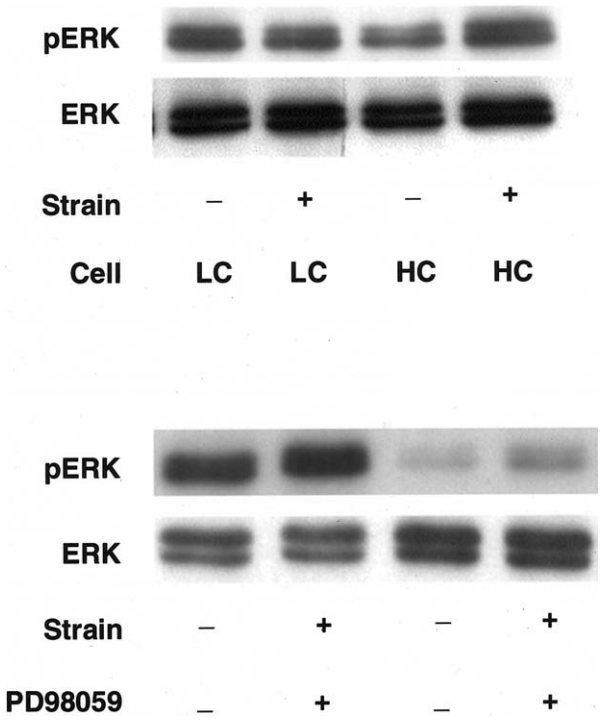


Figure 6. Caco-2 cells grown on fibronectin and subjected to 1 hour of cyclic strain show increased phosphorylation of ERK 1 and 2 in high-confluence cells but not in low-confluence cells. pERK was inhibited by PD98059. Thus, the level of confluence affects pERK in cells subjected to strain on fibronectin. A representative blot from an independent experiment is shown.

on fibronectin (Figure 8). Interestingly, this coincides with our observation that wound closure occurs more rapidly in static cells plated on collagen and more slowly on fibronectin (Figure 1). Strain results in the translocation of phosphorylated ERK to the nucleus slightly on collagen but more substantially on fibronectin (Figure 8, column 1, rows 2 and 4). However, phosphorylated ERK translocates to the lamellipodial edge only on fibronectin following 6 hours of strain (Figure 8, column 1, row 4). Thus, phosphorylated ERK is redirected to the lamellipodial edge by strain only on fibronectin and not when cells were plated on collagen.

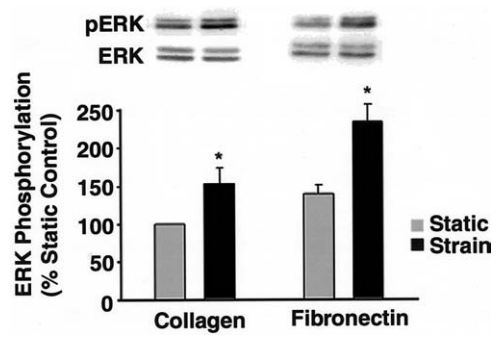


Figure 7. Phosphorylation of ERK in confluent Caco-2 cells increased 53% on collagen and 67% on fibronectin following 1 hour of strain (n = 4; *$P < .05$). There was no change in total ERK.

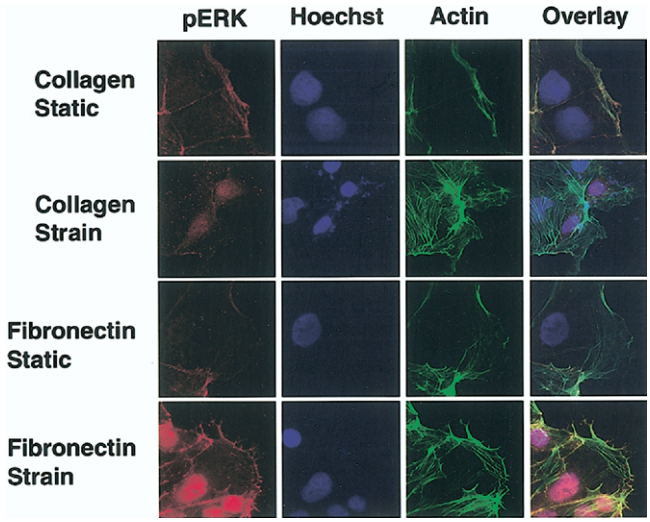


Figure 8. Confocal fluorescent microscopy of phosphorylated ERK relocalization following strain. Immunoreactivity of Alexa Fluor 633–stained pERK is increased in the nucleus following 6 hours of strain slightly on collagen and substantially on fibronectin. On fibronectin, however, activated ERK is also redistributed to the lamellipodial edge. Nuclei were stained with Hoechst and actin stained with Alexa Fluor 488 phalloidin. Distribution of pERK, nuclei, and actin is shown in single-channel and overlay images.

Role of MLC and ERK Following Strain on Fibronectin

To determine the role of MLCK and the ERK kinase, MEK, in pMLC following strain on fibronectin, we inhibited MLCK (ML-7 and the peptide, PIK) and MEK (PD98059) for 30 minutes before strain and assessed the effect on pMLC by Western blot. Inhibition with PD98059 had no effect on pMLC following strain on fibronectin. Treatment with ML-7 reduced the level of pMLC in static cells, and both ML-7 and PIK eliminated the strain effect on fibronectin (Figure 9*A*).

To determine the role of MLCK and the ERK kinase, MEK, in pERK following strain on fibronectin, we inhibited MLCK (ML-7 and PIK) and MEK (PD98059) for 30 minutes before strain and assessed the effect on pERK by Western blot (Figure 9*B*). Inhibition with PD98059 greatly reduced the levels of pERK but did not eliminate the strain effect on fibronectin. Treatment with ML-7 had no effect on pERK following strain on fibronectin. Treatment with PIK substantially reduced basal pERK in static cells. However, PIK did not eliminate the strain effect. Indeed, we demonstrated a 49% increase in pERK with no inhibitor treatment following strain and a very similar 39% increase in pERK following treatment with PIK during strain.

The effect of MEK inhibition and MLCK inhibition on wound closure was determined by plating Caco-2 cells on collagen or fibronectin and treating for 30 minutes with PD98059 or ML-7, respectively, before strain for 24 hours (Figure 10). Photomicrographs were taken at times 0 and 24 hours to assess wound closure (Figure 10*A*). Quantification of motility indicated that neither MEK or MLCK inhibition eliminated the strain effect on collagen (Figure 10*B*, left), with a 27% decrease in restitution under all experimental conditions on collagen (DMSO: n = 10, *$P = .007$; ML7:

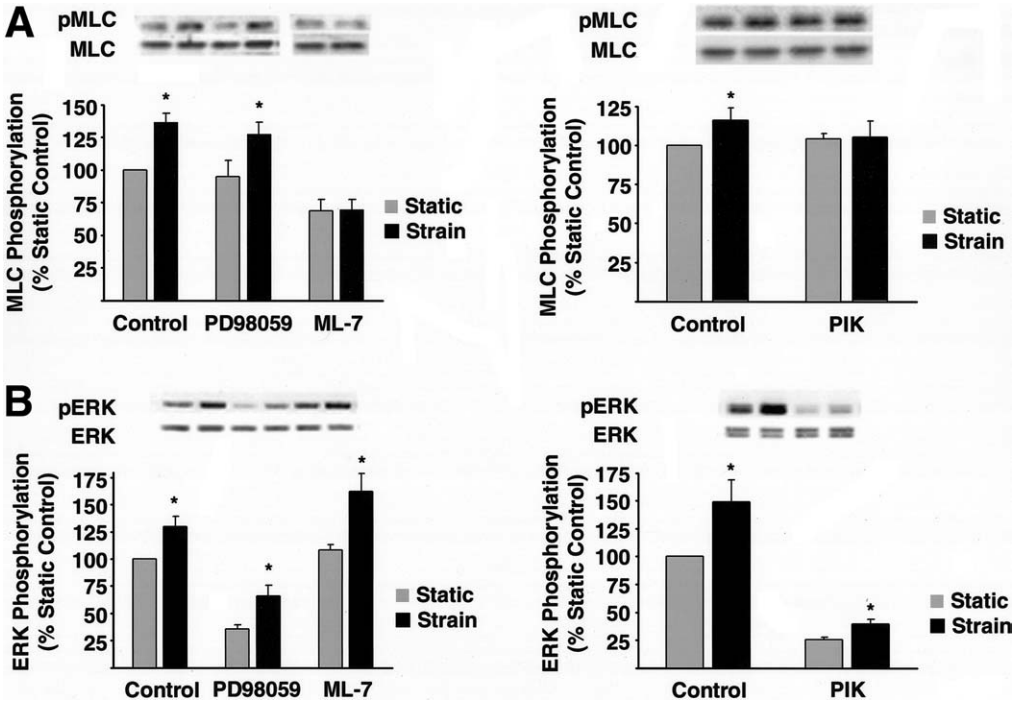


Figure 9. (*A*) pMLC on fibronectin following strain was not affected by MEK inhibition with PD98059 (n = 5; $P < .05$). However, MLCK inhibition with ML-7 eliminated strain-induced increases in pMLC on fibronectin. Inhibition of MLCK with the specific inhibitor PIK[49] also eliminated the strain effect on pMLC on fibronectin. (*B*) pERK following MEK (PD98059) or MLCK (ML-7) inhibition in Caco-2 cells strained for 1 hour on fibronectin. MEK inhibition reduced basal levels of pERK but did not eliminate the strain effect on fibronectin (n = 6; DMSO: $^*P = .03$; PD: $^*P = .00949$). MLCK inhibition had no effect on pERK following strain when inhibited with ML-7. There was a significant decrease in overall pERK when PIK was used, but this did not eliminate the strain effect (n = 6; $^*P = .01$).

n = 10, $^*P = .02$), although the amount of wound closure under static conditions was significantly less with MEK inhibition and even more so with MLCK inhibition (Figure 10*A*; n = 8, $^{\#}P < .05$). However, MEK inhibition on fibronectin eliminated the strain effect of a 15% increase in wound closure, and MLCK inhibition significantly reversed the strain effect on fibronectin (Figure 10*B*, right; DMSO: n = 6, $^*P < .001$; ML7: n = 9, $^*P = .007$).

αv Integrin Relocalization in Response to Mechanical Deformation on a Fibronectin Substrate

Cell motility across matrix proteins requires the binding and interaction of matrix-specific integrin heterodimers. Integrins containing the αv subunit bind specifically to fibronectin but not to collagens. We therefore evaluated αv subunit immunoreactivity by confocal microscopy in Caco-2 cells at the edge of monolayer wounds on a fibronectin substrate under static conditions or after 1 hour of repetitive mechanical deformation. Immunoreactivity for αv was predominantly perinuclear in cells under static conditions, with only modest immunoreactivity at the migrating lamellipodial edge. However, under conditions of repetitive mechanical deformation, we observed increased αv immunoreactivity at the lamellipodial edge (Figure 11).

Discussion

Basal Caco-2 motility was more rapid on collagen I than on fibronectin in a wound closure assay, consistent with previous observations of Caco-2 radial sheet migration.[48] However, in Caco-2 monolayers subjected to repetitive deformation, wound closure was accelerated on fibronectin but inhibited on collagen I. Neither proliferation nor cell spreading contributed to this increase in wound closure on fibronectin, and nonmalignant intestinal epithelial IEC-6 cells behaved similarly. Repetitive deformation stimulated the phosphorylation of 2 proteins that influence epithelial migration in response to other stimuli, MLC and ERK, and these increases were matrix independent. MEK inhibition to block pERK eliminated the motogenic effects of strain across fibronectin. MLCK inhibition to block pMLC not only blocked the motogenic effect of strain across fibronectin, but reversed it. Blocking MEK or MLCK also slowed basal wound closure on collagen but did not eliminate the further decrease in wound closure on collagen associated with mechanical strain. Thus, pERK by MEK and pMLC by MLCK may be required for strain-induced promotion of wound closure across fibronectin, while the deformation-induced inhibition of wound closure on collagen appears independent of MEK and MLCK and awaits further study.

The magnitude of wound closure in these studies appears relatively small, but the effect is highly statistically significant. We[13,58] and others[59–62] have described changes in migration, proliferation, or cell signaling of similar magnitude due to various other stimuli in intestinal epithelial cells. In a previous study, Caco-2 cells plated on various matrix proteins were subjected to mechanical strain and quantification of cell proliferation showed an average 22% change.[13] Here, wound closure decreased an average 12% on collagen and increased an average 35% on fibronectin compared with cells not subjected to mechanical strain. Such apparently modest alterations may well be biologically important in determining whether a mucosal wound heals or fails to heal in vivo.

Abnormalities in mucosal healing may be critical in intestinal disease states such as Crohn's disease, ulcerative colitis, and the loss of intestinal mucosal barrier function during chronic critical illness with ileus. Physical forces generated under normal conditions of digestion by intestinal contraction, villous motility, shear stress, and wall stretch due to luminal contents

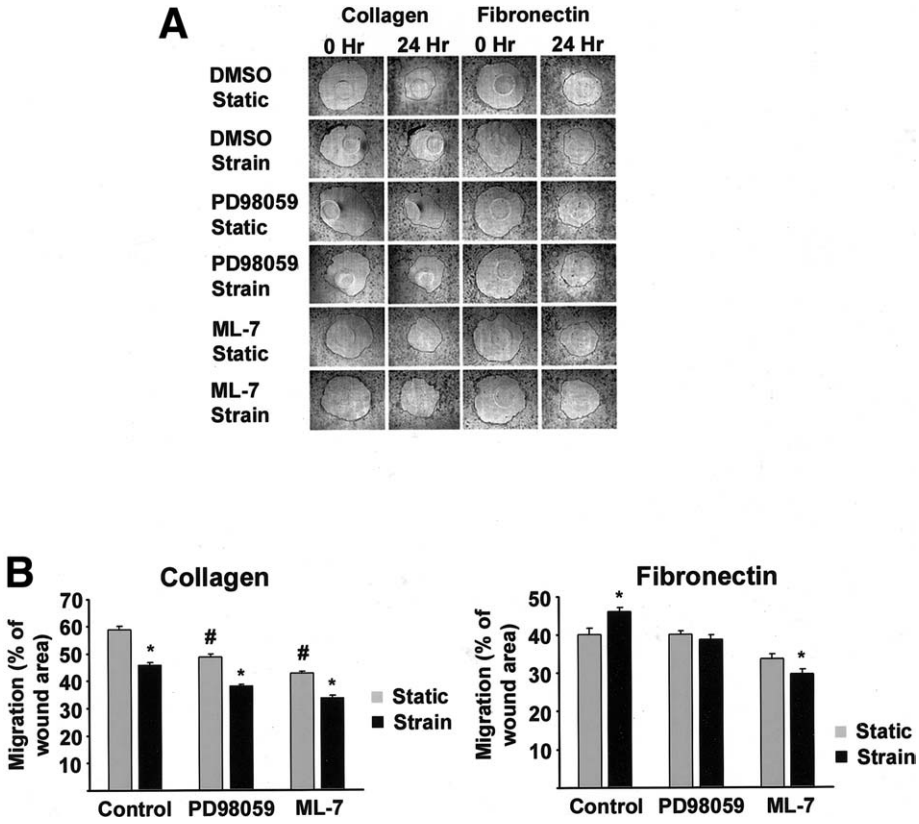


Figure 10. (*A*) Photomicrographs of initial size of each wound is depicted at 0 hours. After 24 hours, restitution in static cells is faster on collagen (*left*) than on fibronectin (*right*). Treatment with MEK inhibitor PD98059 for 30 minutes before strain showed inhibition of wound closure on collagen but not fibronectin with cyclic strain. Treatment with MLCK inhibitor ML-7 showed inhibition of wound closure on both fibronectin and collagen with cyclic strain. (*B*) Quantification of wound images demonstrates a 27% decrease in restitution in vehicle-, PD98059-, and ML-7–treated cells on collagen (collagen: DMSO, n = 10, $^{*}P$ = .007; ML-7, n = 10, $^{*}P$ = .02; PD and ML-7, n = 8, $^{\#}P < .05$). Cells treated with vehicle on fibronectin demonstrated a 15% increase in restitution following cyclic strain, with no significant change when treated with PD98059. ML-7 treatment resulted in a 14% decrease of restitution following strain (fibronectin: DMSO, n = 6, $^{*}P < .001$; ML-7, n = 9, $^{*}P$ = .007).

may affect cell signaling, and changes in these rhythms may alter mucosal healing. Our previous work,[13,14,50,58,63–66] consistent with studies of other organs and tissues,[67–70] suggests that physical forces affect intestinal epithelial cells by flexing the matrix, altering integrin binding, and initiating matrix-dependent signals that regulate enterocyte proliferation and function. However, mucosal healing requires epithelial sheet migration as well as proliferation. We therefore sought to investigate the influence of repetitive deformation and extracellular matrix proteins on intestinal epithelial migration.

The 10-cycles/min frequency of cyclic mechanical strain studied here was consistent with normal peristaltic frequencies. One recent magnetic resonance imaging study clocked average peristaltic frequency at 10.96 waves/min,[71] although the frequency and pattern of peristalsis in vivo is actually highly variable. To address the relevance of the amplitude of the deformation studied here, we measured the percentage deviation from average luminal diameter over 30 seconds at 0.5-second intervals at 5 random points in the small bowel in 5 patients who had normal small bowel follow-through studies after upper gastrointestinal contrast studies. The average deformation over this time interval for each of these 25 series ranged from 7.2% to 27.4% (data not shown), consistent with the 10% deformation studied here.

Villous motility similarly varies along the gut axis and with neurohumoral and environmental factors, but it is of generally similar frequencies to the 10 cycles/min that we studied here.[8–10] We are not aware of measurements of the magnitude of villus deformation during villous shortening, but published images suggest that the amplitude of such deformation is likely even greater than the 10% average strain that we studied in the present work.[72]

Although we have previously reported that mechanical strain is mitogenic, inducing intestinal epithelial proliferation on collagen substrates, our present data suggest that it is also motogenic. Extant literature on the potential motogenic effects of cyclic mechanical strain has previously seemed in conflict. Cyclic mechanical strain inhibits respiratory epithelial wound closure on collagen[44,73] but increases the migration of endothelial cells and vascular myocytes on fibronectin.[59,74] These differences have previously been attributed to cell-specific response patterns to deformation. However, the motogenic effects of repetitive deformation on different matrix substrates had not previously been compared in these cell types. Although these previous observations appear disparate, they are actually all consistent with our results suggesting that strain promotes migration on fibronectin but inhibits it on collagen.

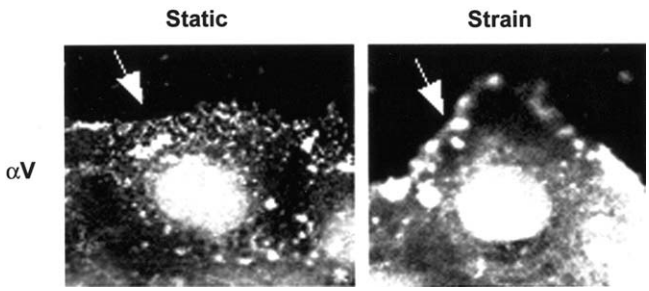


Figure 11. Lamellipodial staining for the integrin subunit αv is more punctate and intense at the edge of the healing wound in response to strain on fibronectin (*right*) than in static cells on the same matrix (*left*). *Arrows* indicate the migrating lamellipodial edges of the cells.

The mechanisms by which deformation elicits any cellular response are poorly understood, and the mechanisms by which matrix modulates the motogenic effects of strain thus await further study. However, several possibilities exist. First, the response to strain seems likely to depend critically on cell shape. Matrix proteins regulate cell shape,[13,48,75,76] which critically influences static cell biology,[77] and the response to mechanical strain may also be so influenced. Second, some investigators postulate that the cellular response to physical forces involves deformation of the cytoskeletal tensegrity structure[78] against the constraints of anchorage to the extracellular matrix through focal adhesion complexes and clusters of matrix receptors, including integrins. Different matrix proteins elicit different sorts of matrix receptor localization and would be expected to constrain cytoskeletal architecture differently. A third important concept is that cell-matrix adhesion involves specific integrins or nonintegrin receptors, and different receptors associate with focal adhesion complex proteins and initiate intracellular signals differently.[79,80] Varying the matrix alters the type of matrix receptor expressed on the intestinal epithelial cell surface[48] as well as the conformation of these receptors and might be expected to therefore alter the signaling associated with receptor-associated focal adhesion complexes after cytoskeletal deformation. We have previously reported that the mitogenic effects of strain on intestinal epithelial cells are similarly not only matrix dependent but also integrin dependent.[13] Finally, cell migration is itself receptor dependent as well as matrix dependent. If strain alters the surface expression and localization of integrin heterodimers or other matrix receptors that bind to collagen differently from those that bind to fibronectin, then it would not be surprising if motility across one matrix was affected differently by strain than motility across another. Indeed, preliminary studies suggest that mechanical strain reorganizes integrins containing the αv integrin subunit, which are specific for fibronectin, and would be expected to modulate motility across fibronectin but not collagen.

Fibronectin regulates cell migration, adhesion, and wound healing[81,82] through receptor-associated focal adhesion complex signaling. Tissue and plasma fibronectin may increase substantially in chronic inflammatory states or following injury.[39,40,70] Thus, a fibronectin-regulated switch in deformation-induced responsiveness from a mitogenic response (maintaining normal tissue homeostasis) to a motogenic response (dedicated to wound healing) might be teleologically appropriate in the setting of fibronectin deposition during inflammation.

In this study, strain induced both pERK and pMLC on fibronectin, and each was independently required for the motogenic effect of strain. pMLC by MLCK is the first step in activating the myosin motor that moves cells. ERK promotes intestinal epithelial cell motility in vitro[83] and activation of ERK, which appears to mediate the motogenic effect of mechanical strain in our model, has been implicated in the mucosal response to injury in various settings in vivo.[84–87] The difference between the motogenic effect of ERK activation by mechanical strain on fibronectin and the mitogenic effect of ERK activation by mechanical strain on collagen[13] may reflect the differences in the subcellular distribution of ERK on each substrate that we observed here. Studies of ERK activation in whole cell lysates may not effectively distinguish between the actions of ERK at the lamellipodial migrating edge of an intestinal epithelial cell, to which ERK is localized in response to strain on fibronectin, and ERK actions in other parts of the cell.

We have previously reported that ERK is phosphorylated in Caco-2 cells in response to repetitive mechanical strain on collagen I but not fibronectin.[13] However, these previous studies were performed in sparse subconfluent cell populations to model the effects of repetitive mechanical strain on proliferating intestinal epithelial cells, while the present studies were performed in confluent cell monolayers to model wound closure in the epithelial sheets more characteristic of intestinal mucosal restitution. In these confluent monolayers, we observed ERK activation on both substrates in response to mechanical strain. Differences in cell-cell contact and confluence have previously been reported to alter the expression and activation of various signal proteins, including ERK, in a matrix-dependent fashion in intestinal epithelial cells in the absence of mechanical strain.[83,88–90] Because such signals interact and activate or suppress each other, it may not be surprising that confluence also may affect signal responsiveness to mechanical deformation. Changes in cell signaling in response to levels of confluence have also been observed in other cells.[83,91,92]

Intestinal mucosal healing is a complex process involving lamellipodial extension of epithelial cells, migration, and proliferation. Physical forces due to intestinal contraction as in peristalsis, villous motility, and interactions with luminal contents likely interact with the effects of cell-matrix interactions and soluble factor signaling to influence these processes. Cyclic mechanical strain applied to cells plated on fibronectin activates cell signals necessary for cell movement, modulating cell motility and monolayer wound closure in a matrix-dependent fashion. Because fibronectin would be expected to increase in settings of chronic injury or inflammation, the fibronectin-dependent nature of this trigger may be teleologically appropriate. These results suggest that mechanical forces in the gut may influence epithelial wound healing in intestinal disease or injury.

References

1. Sarna SK, Otterson MF. Small intestinal physiology and pathophysiology. Gastroenterol Clin North Am 1989;18:375–404.
2. Costa M, Brookes SJ, Hennig GW. Anatomy and physiology of the enteric nervous system. Gut 2000;47(suppl 4):iv15–iv19; discussion iv26.
3. Hennig GW, Costa M, Chen BN, Brookes SJ. Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps. J Physiol 1999;517:575–590.
4. Grivel ML, Ruckebusch Y. The propagation of segmental contractions along the small intestine. J Physiol 1972;227:611–625.
5. Gwynne RM, Thomas EA, Goh SM, Sjovall H, Bornstein JC. Segmentation induced by intraluminal fatty acid in isolated guinea-pig duodenum and jejunum. J Physiol 2004;556:557–569.
6. Melville J, Macagno E, Christensen J. Longitudinal contractions in the duodenum: their fluid-mechanical function. Am J Physiol 1975;228:1887–1892.
7. Mailman D, Womack WA, Kvietys PR, Granger DN. Villous motility and unstirred water layers in canine intestine. Am J Physiol 1990;258:G238–G246.
8. Womack WA, Mailman D, Kvietys PR, Granger DN. Neurohumoral control of villous motility. Am J Physiol 1988;255:G162–G167.
9. Womack WA, Tygart PK, Mailman D, Kvietys PR, Granger DN. Villous motility: relationship to lymph flow and blood flow in the dog jejunum. Gastroenterology 1988;94:977–983.

10. Womack WA, Barrowman JA, Graham WH, Benoit JN, Kvietys PR, Granger DN. Quantitative assessment of villous motility. Am J Physiol 1987;252:G250–G256.
11. Miftakhov R, Wingate D. Numerical simulation of the peristaltic reflex of the small bowel. Biorheology 1994;31:309–325.
12. McNeil PL, Ito S. Gastrointestinal cell plasma membrane wounding and resealing in vivo. Gastroenterology 1989;96: 1238–1248.
13. Zhang J, Li W, Sanders MA, Sumpio BE, Panja A, Basson MD. Regulation of the intestinal epithelial response to cyclic strain by extracellular matrix proteins. FASEB J 2003;17:926–928.
14. Basson MD, Coppola CP. Repetitive deformation and pressure activate small bowel and colonic mucosal tyrosine kinase activity in vivo. Metabolism 2002;51:1525–1527.
15. Spencer A, Sun X, El-Sawaf M, Haxhija E, Yang H, Brei D, Luntz J, Teitelbaum D. Enterogenesis using an implantable mechanotransduction device. J Surg Res 2006;130:189–190.
16. Moore R, Carlson S, Madara JL. Villus contraction aids repair of intestinal epithelium after injury. Am J Physiol 1989;257:G274–G283.
17. Gallois M, Gidenne T, Fortun-Lamothe L, Le Huerou-Luron I, Lalles JP. An early stimulation of solid feed intake slightly influences the morphological gut maturation in the rabbit. Reprod Nutr Dev 2005;45:109–122.
18. Martins MJ, Hipolito-Reis C, Azevedo I. Effect of fasting on rat duodenal and jejunal microvilli. Clin Nutr 2001;20:325–331.
19. Gorostiza E, Poullain MG, Marche C, Gobert JG, Broyart JP, Macry J, Cezard JP. Effect of fasting and refeeding on the adaptation of the small intestine in rats. A model for physiopathologic studies. Gastroenterol Clin Biol 1985;9:790–796.
20. Inoue Y, Grant JP, Snyder PJ. Effect of glutamine-supplemented total parenteral nutrition on recovery of the small intestine after starvation atrophy. JPEN J Parenter Enteral Nutr 1993;17:165–170.
21. Brown HO, Levine ML, Lipkin M. Inhibition of intestinal epithelial cell renewal and migration induced by starvation. Am J Physiol 1963;205:868–872.
22. Al-Jurf AS, Younoszai MK, Chapman-Furr F. Effect of nutritional method on adaptation of the intestinal remnant after massive bowel resection. J Pediatr Gastroenterol Nutr 1985;4:245–252.
23. Mayhew TM. Striated brush border of intestinal absorptive epithelial cells: stereological studies on microvillous morphology in different adaptive states. J Electron Microsc Tech 1990;16: 45–55.
24. Galluser M, Belkhou R, Freund JN, Duluc I, Torp N, Danielsen M, Raul F. Adaptation of intestinal hydrolases to starvation in rats: effect of thyroid function. J Comp Physiol [B] 1991;161:357–361.
25. Hodin RA, Graham JR, Meng S, Upton MP. Temporal pattern of rat small intestinal gene expression with refeeding. Am J Physiol 1994;266:G83–G89.
26. Hodin RA, Chamberlain SM, Meng S. Pattern of rat intestinal brush-border enzyme gene expression changes with epithelial growth state. Am J Physiol 1995;269:C385–C391.
27. Zittel TT, Meile T, Jehle EC, Becker HD. Intraperitoneal capsaicin treatment reduces postoperative gastric ileus in awake rats. Langenbecks Arch Surg 2001;386:204–211.
28. Woosley KP. The problem of gastric atony. Clin Tech Small Anim Pract 2004;19:43–48.
29. Mammen JM, Matthews JB. Mucosal repair in the gastrointestinal tract. Crit Care Med 2003;31:S532–S537.
30. Dunn GA, Zicha D, Fraylich PE. Rapid, microtubule-dependent fluctuations of the cell margin. J Cell Sci 1997;110:3091–3098.
31. Cunningham CC, Vegners R, Bucki R, Funaki M, Korde N, Hartwig JH, Stossel TP, Janmey PA. Cell permeant polyphosphoinositide-binding peptides that block cell motility and actin assembly. J Biol Chem 2001;276:43390–43399.
32. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell 2003;112:453–465.
33. McNeil PL, Ito S. Molecular traffic through plasma membrane disruptions of cells in vivo. J Cell Sci 1990;96:549–556.
34. Kirsner JB, Shorter RG. Recent developments in "nonspecific" inflammatory bowel disease (first of two parts). N Engl J Med 1982;306:775–785.
35. Basson MD. In vitro evidence for matrix regulation of intestinal epithelial biology during mucosal healing. Life Sci 2001;69: 3005–3018.
36. Nishida T, Nakagawa S, Awata T, Ohashi Y, Watanabe K, Manabe R. Fibronectin promotes epithelial migration of cultured rabbit cornea in situ. J Cell Biol 1983;97:1653–1657.
37. Nishida T, Nakagawa S, Ohashi Y, Awata T, Manabe R. Fibronectin in corneal wound healing: appearance in cultured rabbit cornea. Jpn J Ophthalmol 1982;26:410–415.
38. Spencer A, Sun X, El-Sawaf M, Haxhija E, Yang H, Brei D, Luntz J, Teitelbaum D. Enterogenesis using an implantable mechanotransduction device. J Surg Res 2006;130:189–190.
39. Verspaget HW, Biemond I, Allaart CF, van Weede H, Weterman IT, Gooszen HG, Pena AS, Lamers CB. Assessment of plasma fibronectin in Crohn's disease. Hepatogastroenterology 1991;38: 231–234.
40. Fallingborg J, Nielsen D, Pedersen JO. Decreased gelatin-binding fibronectin in patients with chronic inflammatory bowel diseases. Scand J Gastroenterol 1985;20:1062–1064.
41. Allan A, Wyke J, Allan RN, Morel P, Robinson M, Scott DL, Alexander-Williams J. Plasma fibronectin in Crohn's disease. Gut 1989;30:627–633.
42. Lee T, Kim SJ, Sumpio BE. Role of PP2A in the regulation of p38 MAPK activation in bovine aortic endothelial cells exposed to cyclic strain. J Cell Physiol 2003;194:349–355.
43. Osada T, Watanabe S, Tanaka H, Hirose M, Miyazaki A, Sato N. Effect of mechanical strain on gastric cellular migration and proliferation during mucosal healing: role of Rho dependent and Rac dependent cytoskeletal reorganisation. Gut 1999;45:508–515.
44. Savla U, Waters CM. Mechanical strain inhibits repair of airway epithelium in vitro. Am J Physiol 1998;274:L883–L892.
45. Providence KM, White LA, Tang J, Gonclaves J, Staiano-Coico L, Higgins PJ. Epithelial monolayer wounding stimulates binding of USF-1 to an E-box motif in the plasminogen activator inhibitor type 1 gene. J Cell Sci 2002;115:3767–3777.
46. Lotz MM, Rabinovitz I, Mercurio AM. Intestinal restitution: progression of actin cytoskeleton rearrangements and integrin function in a model of epithelial wound healing. Am J Pathol 2000; 156:985–996.
47. Nusrat A, Delp C, Madara JL. Intestinal epithelial restitution. Characterization of a cell culture model and mapping of cytoskeletal elements in migrating cells. J Clin Invest 1992;89:1501–1511.
48. Basson MD, Modlin IM, Madri JA. Human enterocyte (Caco-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. J Clin Invest 1992;90:15–23.
49. Zolotarevsky Y, Hecht G, Koutsouris A, Gonzalez DE, Quan C, Tom J, Mrsny RJ, Turner JR. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in in vitro models of intestinal disease. Gastroenterology 2002;123:163–172.
50. Basson MD, Li GD, Hong F, Han O, Sumpio BE. Amplitude-dependent modulation of brush border enzymes and proliferation by cyclic strain in human intestinal Caco-2 monolayers. J Cell Physiol 1996;168:476–488.
51. Duzgun SA, Rasque H, Kito H, Azuma N, Li W, Basson MD, Gahtan V, Dudrick SJ, Sumpio BE. Mitogen-activated protein phosphorylation in endothelial cells exposed to hyperosmolar conditions. J Cell Biochem 2000;76:567–571.

52. Walia B, Wang L, Merlin D, Sitaraman SV. TGF-beta down-regulates IL-6 signaling in intestinal epithelial cells: critical role of SMAD-2. FASEB J 2003;17:2130–2132.
53. Kruidenier L, MacDonald TT, Collins JE, Pender SL, Sanderson IR. Myofibroblast matrix metalloproteinases activate the neutrophil chemoattractant CXCL7 from intestinal epithelial cells. Gastroenterology 2006;130:127–136.
54. Tieng V, Le Bouguenec C, du Merle L, Bertheau P, Desreumaux P, Janin A, Charron D, Toubert A. Binding of Escherichia coli adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. Proc Natl Acad Sci U S A 2002;99: 2977–2982.
55. Fogh J, Fogh JM, Orfeo T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J Natl Cancer Inst 1977;59:221–226.
56. de Vries JE, Dinjens WN, De Bruyne GK, Verspaget HW, van der Linden EP, de Bruine AP, Mareel MM, Bosman FT, ten Kate J. In vivo and in vitro invasion in relation to phenotypic characteristics of human colorectal carcinoma cells. Br J Cancer 1995;71:271–277.
57. Kamm KE, Stull JT. The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. Annu Rev Pharmacol Toxicol 1985;25:593–620.
58. Han O, Li GD, Sumpio BE, Basson MD. Strain induces Caco-2 intestinal epithelial proliferation and differentiation via PKC and tyrosine kinase signals. Am J Physiol 1998;275:G534–G541.
59. Von Offenberg Sweeney N, Cummins PM, Cotter EJ, Fitzpatrick PA, Birney YA, Redmond EM, Cahill PA. Cyclic strain-mediated regulation of vascular endothelial cell migration and tube formation. Biochem Biophys Res Commun 2005;329:573–582.
60. Kobayashi M, Nishita M, Mishima T, Ohashi K, Mizuno K. MAPKAPK-2-mediated LIM-kinase activation is critical for VEGF-induced actin remodeling and cell migration. EMBO J 2006;25: 713–726.
61. Antico G, Lingen MW, Sassano A, Melby J, Welch RW, Fiore S, Pilon AL, Miele L. Recombinant human uteroglobin/CC10 inhibits the adhesion and migration of primary human endothelial cells via specific and saturable binding to fibronectin. J Cell Physiol 2006;207:553–561.
62. Zhong J, Cornelsen Gencay MM, Bubendorf L, Burgess JK, Parson H, Robinson BW, Tamm M, Black JL, Roth M. ERK1/2 and p38 MAP kinase control MMP-2, MT1-MMP, and TIMP action and affect cell migration: a comparison between mesothelioma and mesothelial cells. J Cell Physiol 2006;207:540–552.
63. Han O, Sumpio BE, Basson MD. Mechanical strain rapidly redistributes tyrosine phosphorylated proteins in human intestinal Caco-2 cells. Biochem Biophys Res Commun 1998;250:668–673.
64. Murnin M, Kumar A, Li GD, Brown M, Sumpio BE, Basson MD. Effects of glutamine isomers on human (Caco-2) intestinal epithelial proliferation, strain-responsiveness, and differentiation. J Gastrointest Surg 2000;4:435–442.
65. Zhang J, Li W, Sumpio BE, Basson MD. Fibronectin blocks p38 and jnk activation by cyclic strain in Caco-2 cells. Biochem Biophys Res Commun 2003;306:746–749.
66. Li W, Duzgun A, Sumpio BE, Basson MD. Integrin and FAK-mediated MAPK activation is required for cyclic strain mitogenic effects in Caco-2 cells. Am J Physiol Gastrointest Liver Physiol 2001;280:G75–G87.
67. Kumar A, Murphy R, Robinson P, Wei L, Boriek AM. Cyclic mechanical strain inhibits skeletal myogenesis through activation of focal adhesion kinase, Rac-1 GTPase, and NF-kappaB transcription factor. FASEB J 2004;18:1524–1535.
68. Li C, Hu Y, Mayr M, Xu Q. Cyclic strain stress-induced mitogen-activated protein kinase (MAPK) phosphatase 1 expression in vascular smooth muscle cells is regulated by Ras/Rac-MAPK pathways. J Biol Chem 1999;274:25273–25280.
69. Jessop HL, Rawlinson SC, Pitsillides AA, Lanyon LE. Mechanical strain and fluid movement both activate extracellular regulated kinase (ERK) in osteoblast-like cells but via different signaling pathways. Bone 2002;31:186–194.
70. Evans L, Frenkel L, Brophy CM, Rosales O, Sudhaker CB, Li G, Du W, Sumpio BE. Activation of diacylglycerol in cultured endothelial cells exposed to cyclic strain. Am J Physiol 1997;272:C650–C656.
71. Froehlich JM, Patak MA, von Weymarn C, Juli CF, Zollikofer CL, Wentz KU. Small bowel motility assessment with magnetic resonance imaging. J Magn Reson Imaging 2005;21:370–375.
72. Allen D Jr, White NA II, Tyler DE. Morphologic effects of experimental distention of equine small intestine. Vet Surg 1988;17: 10–14.
73. Savla U, Olson LE, Waters CM. Mathematical modeling of airway epithelial wound closure during cyclic mechanical strain. J Appl Physiol 2004;96:566–574.
74. Li L, Chaikof EL. Mechanical stress regulates syndecan-4 expression and redistribution in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2002;22:61–68.
75. Moore R, Madara JL, MacLeod RJ. Enterocytes adhere preferentially to collagen IV in a differentially regulated divalent cation-dependent manner. Am J Physiol 1994;266:G1099–G1107.
76. Basson MD, Turowski G, Emenaker NJ. Regulation of human (Caco-2) intestinal epithelial cell differentiation by extracellular matrix proteins. Exp Cell Res 1996;225:301–305.
77. Folkman J, Moscona A. Role of cell shape in growth control. Nature 1978;273:345–349.
78. Ingber DE. Tensegrity II. How structural networks influence cellular information processing networks. J Cell Sci 2003;116:1397–1408.
79. Rozzo C, Chiesa V, Caridi G, Pagnan G, Ponzoni M. Induction of apoptosis in human neuroblastoma cells by abrogation of integrin-mediated cell adhesion. Int J Cancer 1997;70:688–698.
80. Basson MD, Emenaker NJ, Sanders MA. Alpha integrin subunits regulate human (Caco-2) intestinal epithelial proliferation and phenotype. Cell Physiol Biochem 2000;10:27–36.
81. Miyamoto S, Katz BZ, Lafrenie RM, Yamada KM. Fibronectin and integrins in cell adhesion, signaling, and morphogenesis. Ann N Y Acad Sci 1998;857:119–129.
82. Humphries MJ. Fibronectin and cancer: rationales for the use of antiadhesives in cancer treatment. Semin Cancer Biol 1993;4: 293–299.
83. Yu CF, Sanders MA, Basson MD. Human caco-2 motility redistributes FAK and paxillin and activates p38 MAPK in a matrix-dependent manner. Am J Physiol Gastrointest Liver Physiol 2000;278:G952–G966.
84. Souza RF, Shewmake KL, Shen Y, Ramirez RD, Bullock JS, Hladik CL, Lee EL, Terada LS, Spechler SJ. Differences in ERK activation in squamous mucosa in patients who have gastroesophageal reflux disease with and without Barrett's esophagus. Am J Gastroenterol 2005;100:551–559.
85. Blikslager AT, Rhoads JM, Bristol DG, Roberts MC, Argenzio RA. Glutamine and transforming growth factor-alpha stimulate extracellular regulated kinases and enhance recovery of villous surface area in porcine ischemic-injured intestine. Surgery 1999; 125:186–194.
86. Waetzig GH, Seegert D, Rosenstiel P, Nikolaus S, Schreiber S. p38 mitogen-activated protein kinase is activated and linked to TNF-alpha signaling in inflammatory bowel disease. J Immunol 2002;168:5342–5351.
87. Slomiany BL, Slomiany A. Role of ERK and p38 mitogen-activated protein kinase cascades in gastric mucosal inflammatory responses to Helicobacter pylori lipopolysaccharide. IUBMB Life 2001;51:315–320.
88. Dieckgraefe BK, Weems DM, Santoro SA, Alpers DH. ERK and p38 MAP kinase pathways are mediators of intestinal epithelial

wound-induced signal transduction. Biochem Biophys Res Commun 1997;233:389–394.
89. Levy P, Loreal O, Munier A, Yamada Y, Picard J, Cherqui G, Clement B, Capeau J. Enterocytic differentiation of the human Caco-2 cell line is correlated with down-regulation of fibronectin and laminin. FEBS Lett 1994;338:272–276.
90. Goke M, Zuk A, Podolsky DK. Regulation and function of extracellular matrix intestinal epithelial restitution in vitro. Am J Physiol 1996;271:G729–G740.
91. Jiang H, Weyrich AS, Zimmerman GA, McIntyre TM. Endothelial cell confluence regulates cyclooxygenase-2 and prostaglandin E2 production that modulate motility. J Biol Chem 2004;279: 55905–55913.
92. Glover S, Nathaniel R, Shakir L, Perrault C, Anderson RK, Tran-Son-Tay R, Benya RV. Transient upregulation of GRP and its receptor critically regulate colon cancer cell motility during remodeling. Am J Physiol Gastrointest Liver Physiol 2005;288:G1274–G1282.

Received March 17, 2006. Accepted June 28, 2006.

Address requests for reprints to: Marc D. Basson, MD, PhD, Surgical Service (11S), John D. Dingell VAMC, 4646 John R. Street, Detroit, Michigan 48301. e-mail: marc.basson@va.gov; fax: (313) 576-1002.

Supported in part by a VA Merit Award (to M.D.B.).