Mathematical modeling of airway epithelial wound closure during cyclic mechanical strain

Ushma Savla, Lars E. Olson, and Christopher M. Waters

Mathematical modeling of airway epithelial wound closure during cyclic mechanical strain. J Appl Physiol 96: 566–574, 2004; 10.1152/japplphysiol.00510.2003.—The repair of airway epithelium after injury is crucial in restoring epithelial barrier integrity. Because the airways are stretched and compressed due to changes in both circumferential and longitudinal dimensions during respiration and may be overdistended during mechanical ventilation, we investigated the effect of cyclic strain on the repair of epithelial wounds. Both cyclic elongation and compression significantly slowed repair, with compression having the greatest effect. We developed a mathematical model of the mechanisms involved in airway epithelial cell wound closure. The model focuses on the differences in spreading, migration, and proliferation with cyclic strain by using separate parameters for each process and incorporating a time delay for the mitotic component. Numerical solutions of model equations determine the shape of each process and incorporating a time delay for the mitotic component. Information on experimental results, we developed a mathematical model of the wound closure process.

Several mathematical models of wound closure have been developed that contain nonlinear reaction and diffusion terms, with the simplest and most cited model developed by Fisher

\[
\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} + u(1-u), \quad 0 \leq u(x,t) \leq 1
\]

where \( \partial \) represents a partial derivative, \( u \) is cell density, \( t \) is time, and \( x \) is position. This partial differential equation describes the evolution of a cell density function \( u(x,t) \). The change in the density \( (\partial u/\partial t) \) is influenced by the second-order influx of particles or cells represented by the \( \partial^2 u/\partial x^2 \) term and by a generation of particles \( [u(1-u)] \). This equation has also been used with constants added to define a diffusion term \( (D) \) and a growth term

\[
\frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} + ku(1-u)
\]

where \( k \) is a coefficient for cell growth. The diffusion term is analogous to the diffusion term in Fick’s law, with \( D \) representing a “cellular diffusion coefficient.” The mitosis term is expressed as logistic growth, which results in a reduced growth rate as the cell population is increased and limited population growth at confluence. Fisher’s equation has two steady states:

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The airways undergo cyclic deformation at a frequency dependent on the respiration rate, and we have recently shown that these forces impact cellular motility, spreading, and proliferation (17, 29). Others have demonstrated that mechanical deformation of alveolar epithelial cells causes increased cell death (25, 26) and plasma membrane stress failure (27, 28). We used an in vitro model to study the repair mechanisms of wounded monolayers of human and cat AEC cultured on elastic membranes and subjected to cyclic strain. Owing to the radially dependent strain profile in the older Flexercell plates, we were able to compare the effects of cyclic elongation and compression in the same culture well (17), and we verified these findings in a custom-built stretching device that applied uniform biaxial strain or compression (29). We have shown that both cyclic elongation and compression significantly attenuate wound closure in a frequency-dependent manner, in part by limiting spreading and migration (17). To gain more insight into these mechanisms and to develop tools to aid in our interpretation of experimental results, we developed a mathematical model of the wound closure process.

INJURY TO THE AIRWAY EPITHELIUM can occur in diseases such as asthma (11), in response to different insults such as cigarette smoke (1) and bacterial or viral infections (14, 16), or after endotracheal intubation and mechanical ventilation (4, 13, 23). High stresses induced by surface tension during airway reopening (2) or during mucosal wall folding (31) may also cause epithelial injury in the airways. The rapid regeneration of a continuous epithelium is crucial for maintaining barrier function (4) and in limiting airway hyperreactivity (15). The reepithelialization process involves several steps, including cell spreading, migration, and eventually proliferation of the cells surrounding the wound (33–35). Zahm et al. (33, 35) showed rapid closure of small in vitro wounds in airway epithelial cells (AEC) due to progressive elongation of cells adjacent to the wound and rapid migration rates of cells at the wound edge; however, in vivo, closure of large AEC wounds is also dependent on proliferation after 15–30 h (5).
MODELING AEC WOUND CLOSURE

u = 0 (spatially empty population) and u = 1 (spatially full population). The model supports solutions of locally saturated populations spreading into empty regions; these solutions develop into fronts that connect solutions at u = 1 to u = 0. The interaction of diffusive and growth terms yields steady profile solutions termed traveling waves (3, 32) or diffusive waves, in which the front propagates at a constant shape and velocity, the magnitude of which depends on the size of D and k. Fisher’s equation or adaptations thereof are often used in mathematical biology for population dynamics, gene expression (6), or chemical and polymer diffusion (32), as well as in other applications. This approach has been used to model wound healing of endothelial (22), epidermal (20, 24), and corneal epithelial cells (3).

Most previous models of wound healing were developed with the contributions of proliferation and migration alone and provide a framework for understanding the relationship between wound healing and chemical stimulation or inhibition. None of the previous models has specifically addressed the contribution of cellular spreading or the influence of cyclic mechanical strain. In addition, very few studies have compared model predictions with experimental measurements of cell density and wound closure rate. In this paper, we describe a new modification of Fisher’s equations to describe wound healing of AECs. New modifications include the addition of a term that describes spreading and the incorporation of a time delay into the mitotic term. Model parameters were estimated by comparing model predictions with experimental data from several AEC types.

MATERIALS AND METHODS

Materials. PBS, MEM, Ham’s F-12 medium, FBS, gentamycin, trypsin/EDTA, and nonessential amino acids were obtained from GIBCO (Grand Island, NY). Bronchial epithelial medium was obtained from Clonetics (San Diego, CA). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Cell culture. Cat tracheal epithelial cells (CTE) were isolated as described previously (17). Isolated cells were seeded on six-well Flex-I plates, 2.5–3 × 10^5 cells/well, and cultured in CTE media with 10% FBS (Ham’s F-12 medium with 10 μg/ml insulin, 7.5 μg/ml endothelial cell growth supplement, 0.5 μg/ml transferrin, 0.4 μg/ml hydrocortisone, 2 μg/ml triiodothyronine, 1% antibiotic/antimycotic solution, 2% sodium bicarbonate). The medium was changed every 2 days, and the cells were used in experiments on days 6 or 7 of culture. Calu 3 cells (American Type Culture Collection, Rockville, MD) are derived from a human lung adenocarcinoma and resemble tracheal epithelial cells (18). Cells were maintained in T-150 culture flasks in Calu 3 media (MEM, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 0.1% gentamycin, and 10% FBS) before experiments. Cells between passages 18 and 26 were used in experiments 4–5 days after plating into Flex-I six-well plates or manufactured stretch membranes at 3–3.5 × 10^5 cells/well (~350,000 cells at confluence). These cells retain constant properties over repeated passages. The medium was changed every 2 days.

AECs transformed with the SV40 virus (16HBE14o^-) were obtained from Dr. D. Grunert and have been characterized by his laboratory (9). Cells were grown in MEM containing 10% FBS, 2 mM l-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin G. Cells were seeded on six-well Flex-I plates at 2–3 × 10^5 cells/well and used on days 4 or 5 of culture (~350,000 cells/well at confluence).

Normal human bronchial epithelial (NHBE) cells were obtained from Clonetics (San Diego, CA) and maintained in bronchial epithelial medium (0.5 μg/ml hydrocortisone, 0.5 ng/ml human recombinant epidermal growth factor, 0.5 μg/ml epinephrine, 10 μg/ml transferrin, 5 μg/ml insulin, 0.1 ng/ml retinoic acid, 6.5 ng/ml triiodothyronine, 50 μg/ml gentamycin, 50 ng/ml ammonium B, and bovine pituitary extract). Passage 2–4 NHBE cells were seeded onto six-well Flex-I plates at 1.75 × 10^4 cells/well. The medium was changed every 2 days, and the cells were used in experiments on days 4 or 5 of culture.

Experimental protocol. Cells were grown to confluence on Flex-I six-well plates, and a metal spatula was used to create linear wounds of ~500 μm width across the diameter of the well. Before the initiation of the experiment, the cells were rinsed twice with PBS to remove cellular debris. Two milliliters of complete growth medium were added to the wells; this medium was not removed during the course of the experiment, unless otherwise indicated. Cytochalasin D (CYP, 1 μg/ml) was dissolved in DMSO before dilution (wound healing was not affected by low concentrations of DMSO, data not shown). CYP was applied for 2 h before wounding and was replaced with unsupplemented growth medium at the time of wounding. CYD was not kept in the media during wound closure because preliminary studies showed that cell detachment began to occur after several hours. Mitomycin C (MMC, 10 μg/ml) was dissolved in growth medium. Images were obtained at the initial time of wounding and at various times up to 72 h postwounding (etopic strain). The original Flexcell strain unit was used in these studies and has been described in detail (8). The system utilizes vacuum pressure regulated by a solenoid valve to deform a silicone rubber substrate on which the cells have been cultured. When the vacuum is applied, the culture plate bottom deforms downward to a known percentage elongation, which is translated to the cells. On release of the vacuum, the Silastic substrate returns to its original conformation. The frequency, duration, and magnitude of applied strain can be varied in this system. When cells were stretched at 30 cycles per min, the membrane was stretched for 1 s and relaxed for 1 s. Finite-element analysis of the membrane strain has shown that, on deformation at ~15 kPa, the Flex-I plate yields a nonhomogeneous radial strain profile that is maximum at the periphery (~20%) and minimal at the center, with 1–2% compression seen in the center of the stretched wells (8). The newer versions of this device have been designed to reduce the nonuniform strain distribution, but we utilized the older system in these studies to examine both elongation and compression within the same culture well. There was no appreciable cell death over the 48-h time course in any cell type as measured by lactate dehydrogenase release or Trypan blue uptake (data not shown).

Imaging the wound. Images of the wounds were collected at specified times with a Nikon Diaphot 300 inverted microscope equipped with a Hamamatsu integrating charge-coupled device camera, an Argus-20 real-time digital image processor, and a Pentium DataStor computer with a frame grabber (Fryer, Huntley, IL). Images were analyzed by using the Metamorph image analysis program (Universal Imaging, West Chester, PA). After the image was acquired, it was converted from pixels to micrometers by using a calibration image. Using Metamorph, an outline of the wound was manually drawn, and mean wound width w was tabulated by a Metamorph defined parameter. The w is given by

\[ w = 0.25[P - \sqrt{(P^2 - 16A)}] \]  

where P and A are the perimeter and area, respectively, of the wound in the image. This equation is derived from the relations between length and width and area and perimeter. Data are expressed as a percentage of the time 0 wound width to normalize variability in wounding from well to well and experiment to experiment, although similar-size initial wounds were consistently formed.

5-Bromo-2′-deoxyuridine staining. 5-Bromo-2′-deoxyuridine (BrdU) labeling was performed to measure new DNA synthesis as an indication of cell proliferation. For the BrdU studies, cells were grown to confluence, treated, and wounded as described. BrdU (10 μM) was added for 60 min at the end of the incubation period. The cells were
washed with a PBS-based wash buffer, fixed in 70% ethanol (in glycine buffer, pH 2.0) for 20 min at −20°C, and washed again. The cells were subsequently incubated with anti-BrdU antibodies (1:10 dilution, Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C. The cells were rinsed, incubated with anti-mouse-IgG-alkaline phosphatase solution for 30 min at 37°C, and processed according to the manufacturer’s instructions. The number of stained cells was determined by visual examination in at least eight high-power fields.

Cell spreading studies. As a measure of cell spreading and motility, the area of cells was measured in images taken of the center and periphery of stretched and unstretched wells over time. Cells were outlined, and the area was calculated with Metamorph imaging software. The accuracy of the cell size measurements was confirmed by measuring cell size of the same field at higher magnifications. For each experimental condition, at least four sets of cells at the leading edge of the wound were traced in each experiment.

Cell density was measured by counting the number of cells contained within consecutive 20 × 100 μm boxes, starting within the wound (0 cells) up to 200 μm from the edge. Density was normalized by the cell number in a box far from the wound edge and was measured in two different images from six different experiments.

Model development. We considered the simplest case of epithelial cell movement controlled mainly by spreading, migration, and later by proliferation. The governing equation takes the following form: rate of increase of cell density = cell migration + cell spreading + mitotic generation.

We did not include a term for cell loss, because it is unlikely that there is appreciable cell death during the time course of examination (~24 h). Cell movement was modeled as Fickian diffusion to capture cells moving down a gradient in cell density due to the sudden loss in contact inhibition. In addition to migration and proliferation terms, we included a term for cellular spreading, because the spreading of cells at the wound edge also contributes to wound closure, separately from proliferation. In the model, parameters are based solely on the existing mechanical environment and not on the production or inhibition of production of autocrine factors or the presence of growth factors, because most experiments were done in basal media (with serum). We also modeled each of the component to have maximal effect at the wound edge, decreasing to a minimal contribution far back from the wound edge. After previous studies, we used a logistic growth form for the mitosis time (20, 22, 24, 32). However, we also incorporated a time delay, because proliferation does not begin until several hours after wounding (5, 12).

The thickness of the monolayer is much smaller than the wound length and width, so we can treat the wound as two-dimensional, and, by considering only a linear wound geometry, we can simplify the spatial domain to only one dimension, x. We solved the equations on the semi-infinite domain 0 ≤ x < ∞, with 0 ≤ x ≤ w₀, representing one-half of the original wound, where 0 is the center of the wound and w₀ is one-half of the initial wound width. Because the monolayer did not contain fibroblasts, we can assume that 0 is a fixed boundary and that the wound does not contract due to force generated by the cells. Our mathematical adaptation of the diffusion equation for cell density n(x,t) at position x and at time t is initially

\[
\frac{\partial n}{\partial t} = D \frac{\partial^2 n}{\partial x^2} + k_s \left( 1 - \frac{n}{n_w} \right) n, \quad t < t_d
\]

(4)

and after a time delay (t_d)

\[
\frac{\partial n}{\partial t} = D \frac{\partial^2 n}{\partial x^2} + (k_s + k_p) \left( 1 - \frac{n}{n_w} \right) n, \quad t \geq t_d
\]

(5)

where n₀ is the unwounded level of cell density, kₙ is the spreading coefficient, and kₚ is a coefficient representing proliferation.

Boundary conditions. The biologically relevant initial conditions considered in this model are

\[n(x, 0) = 0 \text{ for } 0 \leq x \leq w₀ \text{ and } n(x, 0) = n_w \text{ for } w₀ < x < \infty \]

(6)

Initially, there are no cells in the wound, and the density is initially constant in the unwounded area. Boundary conditions include the following

\[
\frac{\partial n}{\partial x} (0, t) = 0 \text{ and } n(\infty, t) = n_w \text{ for all } t \geq 0
\]

(7)

By symmetry, the solution must be continuous at both ends, hence the derivatives at x = 0 and x = ∞ are zero.

The model results for cell density evolve as diffusive wave fronts to assume the same shape of a front of cells spreading into the denuded area, and, to achieve this set of curves, we solved Eqs. 4 and 5 numerically. Using nondimensionalized parameters, n is normalized by n_w. n* = n/n_w, t is normalized by the final time (t_final); t* = ut_final; position x is normalized by w₀; x* = x/w₀, kₙ and kₚ are normalized by t_final; k*ₙ = kₙt_final; and the D is normalized by t_final and w₀. D* = Dt_final/w₀².

Substituting into Eqs. 4 and 5 and dropping asterisks for convenience, we have the dimensionless model for 0 ≤ t ≤ 1.

Initially

\[
\frac{\partial n}{\partial t} = D \frac{\partial^2 n}{\partial x^2} + k_s (1 - n)n, \quad t < t_d
\]

(8)

and after a time delay

\[
\frac{\partial n}{\partial t} = D \frac{\partial^2 n}{\partial x^2} + (k_s + k_p)(1 - n)n, \quad t \geq t_d
\]

(9)

Model equations were solved numerically by using MATLAB software. We used an explicit scheme and replaced the derivatives with their finite (forward and central) difference approximations. The x dimension was discretized into 101 points. Model parameters were estimated by comparing either cell density or wound width data with model predictions. All fits were optimized by varying parameters using a nonlinear least squares algorithm, the Levenberg-Marquardt optimization routine. The algorithm summed the squared difference between model and experimental data at each point and altered parameters to determine whether the sum squared difference would be decreased. The initial parameter estimates used in the optimization routine were based on order of magnitude estimates of each process.
from experimental data. For example, $D$ was initially estimated at $1 \times 10^{-13}$ cm$^2$/s, and $k_s$ and $k_p$ were estimated to be $1$/h. To account for the possibility of localized minima in the solution space, we often changed the starting point of the parameters. In the case of experimental treatments intended to knock out a specific process (such as treatment with MMC to block proliferation), we selected initial values of 0.0, but allowed the parameter to vary with the optimization. Also, in cases in which we were comparing experimental conditions such as control vs. elongated or compressed, the initial parameters were set at the same values for all groups. If solutions were constrained to a local minima, then it is unlikely that we would have observed changes in the parameter values. In the optimizations involving transformed human bronchial epithelial (16HBE) cells, the time delay parameter was allowed to vary. In the optimizations involving other cell types, there were fewer data points, and the time delay parameter was fixed to reduce the number of parameters to be fit.

Parameter sensitivity. Parameter sensitivity was evaluated to determine whether our model predictions were sensitive to small changes in parameters. Because we wished to evaluate wound-healing parameters by comparing model predictions to the experimental data, we determined the sensitivity functions for each parameter. We calculated Bode’s sensitivity function (7) over time using wound width model simulations with nominal parameter values. For example, the sensitivity ($S$) of predictions of wound width ($W$) to changes in the $D$ would be given by

$$S_D^W = \bar{D} \frac{\partial W}{\partial D}$$

where the overbar indicates the nominal value for $D$ or $W$. Parameters were increased by 20% separately to determine the sensitivity to that parameter alone over the time course of wound closure. The partial differential term $\partial W/\partial D$ represents the change in predicted wound width when the parameter $D$ is changed from a nominal value to one increased by 20%. Similar sensitivity functions were generated for the other parameters. In general, a nonzero function indicates sensitivity of the model prediction to the parameter, with larger magnitudes indicating more sensitivity. As shown in Fig. 1, there is sensitivity for the measurement of $D$ and $k_s$ that increases over the entire time course. In the case of $k_p$ and time delay, an increase in sensitivity is seen only after the time delay. These plots indicate that parameter estimations from experimental wound width data will be sensitive to each of the four parameters.

RESULTS

Cell density measurements and model predictions. To compare our model predictions of cell density with experimental
measurements, wounds were induced in monolayer cultures of 16HBE cells, and cell density was measured as a function of time and position. Model predictions were compared with measured cell densities during wound closure for static 16HBE cells (Fig. 2A) and cells that were cyclically elongated (Fig. 2B) or compressed (Fig. 2C). The curves in Fig. 2 show the model simulations for these experiments. Note that the model yields a solution of cell density with time that is similar to the experimental measurements. Whereas the measured cell density at the wound edge decreased over time in the static cells, indicating cell spreading, cell density remained relatively unchanged in the elongated cells and actually resulted in increased cell density (decreased cell area) in compressed cells.

Wound widths. Based on the predictions of cell density, we also predicted the wound width, defined as the position where there are no cells. In other experiments, we measured the wound width (not cell density), and Fig. 3 shows experimental wound closure data from four different types of AEC compared with model predictions. We optimized the fits by varying parameters using a nonlinear least squares algorithm, the Levenberg-Marquardt method. The best fit parameters used are shown in Table 1. The time course of wound closure differed for each cell type. For example, wounds in Calu3 cells closed much more slowly than in other cells. In all cases, both cyclic elongation and cyclic compression inhibited wound closure compared with static cells, and these changes are reflected in the parameters (Table 1).

Effect of cyclic strain. We previously showed that cyclic elongation and compression (30 cycles/min, 20% maximum elongation, -2% compression) significantly inhibited cellular spreading and migration in AEC monolayers (17). As indicated in Table 1, parameters for cells undergoing cyclic strain were altered relative to static cells. For each cell type, the $D$, indicative of migration velocity, was decreased by cyclic strain. This is consistent with previous measurements of cell migration velocity during wound healing (30). For each cell type, the $k_s$ in Table 1 was either unchanged or decreased by cyclic strain. To test this prediction, we measured cell spreading at the wound edge as shown in Fig. 4. The decrease in $k_s$ in Table 1 correlates with experimental measurements indicating that elongated cells do not spread compared with static cells, and that compressed cells actually decrease in size. The decrease in size and the corresponding increase in cell density were not predicted well by the model simulations in Fig. 2. Table 1 also suggests that cyclic strain increased cellular proliferation in 16HBE, CTE, and NHBE cells, whereas Calu3 cells exhibited negligible proliferation. To verify this predic-

![Fig. 3. Model fit to wound width data from static, cyclically elongated, and cyclically compressed cells (30 cycles/min, 20% maximum elongation, 2% maximum compression). A: transformed human bronchial epithelial (16HBE) cells. B: normal human bronchial epithelial (NHBE) cells. C: Calu 3 cells. D: cat tracheal epithelial (CTE) cells. Symbols represent experimental data points for static (○), elongated (●), and compressed (□) cells, and curves represent model predictions. Values are means ± SE; $n = 6$. Fits were determined by fixing the $t_{wedge}$ at 9.6 h for CTE and NHBE and 33.6 h for Calu3 cells (note the difference in time scale). The 3 other parameters were varied; parameters are listed in Table 1. For 16HBE cells, the $t_{wedge}$ was varied along with the 3 other parameters, and the $t_{wedge}$ is given in Table 2 for untreated cells.](image)

<table>
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<th>Cell Type</th>
<th>$D$, $10^{-11}$ cm$^2$/s</th>
<th>$k_s$, h$^{-1}$</th>
<th>$k_p$, h$^{-1}$</th>
<th>CV</th>
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<tr>
<td>16HBE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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Summary of best fit model parameters for 4 different cell types based on wound width data. The time delay was fixed for the parameter estimation at 9.6 h for cat tracheal epithelial (CTE) and normal human bronchial epithelial (NHBE) cells and at 33.6 h for Calu3 cells. The time delay for transformed human bronchial epithelial (16HBE) cells was allowed to vary and is given in Table 2. The coefficient of variation (CV) is given for each fit. $D$, diffusion coefficient; $k_s$, spreading coefficient; $k_p$, proliferation coefficient.
tion, we measured the incorporation of BrdU into 16HBE cells at the wound edge. As shown in Fig. 5A, incorporation of BrdU occurred earlier and more extensively in cells undergoing cyclic strain, in agreement with the model predictions.

Effects of CYD and MMC. We also determined model parameters for wound-healing data from cells treated with CYD (1 μg/ml), which prevents actin polymerization. CYD disrupts the actin cytoskeleton, which is necessary for locomotion and spreading and, in this manner, can be used to decrease or eliminate the contributions of spreading and migration to wound closure. Because cells detached with prolonged exposure to CYD, we pretreated the cells for 2 h before wounding and then removed CYD. Wound healing was substantially inhibited by CYD treatment, as shown in Fig. 6A, and the model parameters in Table 2 show that the $k_s$ was essentially zero. The $k_p$ did not change dramatically with cyclic strain, although experimental BrdU incorporation was increased with mechanical strain with CYD treatment (Fig. 5B). The increased BrdU labeling could, however, be due to incorporation of DNA during strand repair without proliferation. Because there was little closure in CYD-treated wells and model fits to these data resulted in very low values for the $k_s$, this supports our earlier hypothesis that closure in untreated AEC is dominated by spreading and migration.

We also determined parameters for the experiments in which we used the DNA cross-linking inhibitor MMC (10 μg/ml) on 16HBE cells to eliminate the contribution of proliferation. We verified that MMC blocked DNA synthesis by demonstrating negligible BrdU incorporation in 16HBE cells (Fig. 5C). MMC treatment resulted in some decrease in wound closure at later times (Fig. 6B), particularly in the compressed and elongated cells, and this was reflected by the changes in parameters in Table 2. MMC effectively lowered the $k_s$ to negligible values in all three groups. The effect of MMC on $D$ and $k_s$ was small, except in the case of elongated cells, where $k_s$ increased twofold. This again supports our findings that wound closure in AEC is dominated by spreading and migration and that the model fits utilize parameters that reflect this.

**DISCUSSION**

We made improvements on existing models of wound closure based on the Fisher equation and Fickian diffusion to

![Fig. 5. S-Bromo-2'-deoxyuridine (BrdU) labeling at the wound edge in 16HBE14o- cells as an indication of cell proliferation. Cells were either untreated (A), treated with CYD (B), or treated with mitomycin C (C). Labeled cells were counted in 8 high-power fields (hpf) at the wound edge for static (Œ), elongated (I), and compressed (F) cells. There were ~150–200 cells per hpf. Labeling in untreated static cells began to increase after 10 h, but this increase was seen in elongated and compressed cells after only 4 h. Values are means ± SE. *Significant difference from static or between treated and untreated, $P < 0.05$.](attachment:fig5.png)
better fit experimental data of AEC wound closure. While existing models may be appropriate for other cell types (19–21), the additions made in this model are consistent with biological findings in static and cyclically strained AECs and produce predictions that are consistent with experimental data. Our new model adequately describes changes in the wound closure process in AEC with cyclic stretch and has directed us toward new hypotheses about the effects of elongation, compression, and chemical mediators on these cells.

Because spreading and migration are the primary mechanisms of closure in AEC wounds, we added a parameter to the basic wound closure equation to account for spreading, $k_s$. The spreading term contributes to wound closure immediately and has its maximal effect at the wound edge and minimal contribution elsewhere. Although there is a mechanistic difference between spreading and motility (10), no previous models have accounted for this phenomenon. During the initial stages of wound healing, cells flatten and spread, and migration begins. These processes may be indistinguishable initially, but, at later times, cell spreading can be determined by changes in cell area (Fig. 4) and cell migration can be determined by measuring cell velocity (30). Experimental wound closure data suggest that we use a representation of the migration term that is highest at the wound edge and rapidly decays behind the wound edge. An increase in cell density resulting from cell proliferation and wound closure is accompanied by a decrease in average cell speed (34); there should be little spreading or migration once the wound is closed, so all of the contributions (from spreading, migration, and proliferation) should decline, even at the wound edge and back from it when the density approaches $n_o$.

We fit the curves to the experimental data by using a least squares routine to optimize the parameters. Parameter optimization yielded biologically relevant parameters, which reflected changes in wound closure processes after application of cyclic strain. For example, in most AEC types, strain induced an early increase in proliferation and decreased spreading and migration. This was reflected by increased $k_p$. The spreading/migration term ($D$) for cells in static wounds was often twice as large as $D$ in the compressed case and more than triple that in the elongated case. There were also decreases in $k_s$ with strain compared with $k_s$ in the static case. This indicates that our model is sensitive to alterations in strain and that it can recognize changes in wound width and density data. One potential complication of our experimental approach is that we examined both elongation and compression in the same culture well. Because cellular responses to stretch and compression may differ, the presence of both factors in the same well may limit the ability to distinguish the effects of each. However, we demonstrated nearly identical inhibition of wound closure, cell spreading, and cell migration when we compared cells stretched at different locations in the old Flex-I system with cells strained with the corresponding magnitude of uniform stretch or compression using our custom-made biaxial stretching device (29). Also, we were careful to obtain measurements at the same radial location within the wells so that the strain amplitude would be consistent. For example, maximal compression (2%) occurred at the center of the wells, and we consistently measured wound widths at the center location. One limitation of our model is that it did not predict the increase in cell density in the compressed cells; however, the overall wound closure was predicted by the diffusive wave solution.

These changes were also reflected when proliferation and spreading and migration were isolated pharmacologically. The agent CYD was used experimentally to isolate the contribution of spreading and migration. Wounds treated with CYD closed only slightly, and the parameters reflected these changes. $D$ and

Table 2. Summary of model parameters from cells treated with cytochalasin D or mitomycin C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$D \times 10^{-11}$ cm$^2$/s</th>
<th>$k_s$, h$^{-1}$</th>
<th>$k_p$, h$^{-1}$</th>
<th>Time Delay, h</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>Static</td>
<td>0.96</td>
<td>1.85</td>
<td>0.01</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Compressed</td>
<td>0.15</td>
<td>1.78</td>
<td>3.98</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>Elongated</td>
<td>0.22</td>
<td>1.48</td>
<td>3.59</td>
<td>6.3</td>
</tr>
<tr>
<td>Cytochalasin D</td>
<td>Static</td>
<td>0.15</td>
<td>0.0004</td>
<td>2.07</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Compressed</td>
<td>0.11</td>
<td>0.0002</td>
<td>1.48</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Elongated</td>
<td>0.22</td>
<td>0.0004</td>
<td>1.52</td>
<td>0.4</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>Static</td>
<td>0.68</td>
<td>2.15</td>
<td>0.004</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Compressed</td>
<td>0.22</td>
<td>1.81</td>
<td>0.004</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>Elongated</td>
<td>0.13</td>
<td>3.60</td>
<td>0.004</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Wound-healing model parameters for cytochalasin D-treated (1 μg/ml) and mitomycin C-treated (10 μg/ml) static, compressed, and elongated 16HBE14o$^-$ cells (30 cycles/min, 20% maximal elongation).
were significantly decreased, and, to account for the healing that did occur in these wells, \( k_p \) was increased and the time delay was decreased (Table 2). However, experimental measurements of BrdU incorporation in Fig. 5B do not suggest that proliferation was increased substantially, and an increase in proliferation within 1 h is unlikely. The reduced time delay in the parameter estimation results in \( k_s \) and \( k_p \) being indistinguishable. Because there was little closure in CYD-treated wells and model optimization, using these data resulted in lower values for the \( k_s \). This supports our hypothesis that closure in these cells is dominated by spreading and migration. Because prolonged exposure to CYD caused detachment of cells, we treated the cells for only 2 h before wounding. It is likely that CYD diffused out of the cells during the time course of wound healing, and actin repolymerization may have occurred during the later stages of the time course. Thus, because proliferation is unlikely to occur in <10 h, the lower time delay more likely reflects the recovery of the cells after CYD diffusion out of the cells. However, even this pretreatment profoundly affected cell spreading and migration during wound closure, and the parameters reflect this. Although the \( D \) for compressed and elongated cells was unaffected by CYD, in the static cells CYD treatment substantially decreased \( D \).

Significant cell division as a mechanism of wound healing is not likely to occur in cells before 15–24 h (5), but may contribute to wound closure after this time. We observed in Fig. 5A (BrdU data) both elongation and compression stimulated early (~4 h) and increased DNA synthesis while BrdU staining was not apparent in static wells until 10 h postwounding. BrdU staining was more abundant in strained wells than in static wells. Despite this increase, only ~20% of the total cells were ever labeled. Although there may be basal proliferation occurring before the monolayer is wounded, it is unlikely that substantial proliferation occurs immediately. To account for this, our model incorporates a time delay before which spreading and migration are solely responsible for closure. After the time delay, the proliferation term is defined similarly to spreading; the term has the largest contribution at the wound edge and falls off to zero rapidly behind the edge. This scheme is more biologically consistent for AECs. Zahm et al. (34) showed that maximal proliferation occurs at the wound edge and at an intermediate distance behind it; in examining BrdU-labeled wounded monolayers, there was little to no staining observed more than 100 \( \mu \)m back from the edge. In the absence of significant spreading and migration, these cells increased proliferation as a mechanism to promote wound closure. The model reflected this with larger parameters for proliferation with mechanical strain. The contribution of proliferation was similarly investigated with MMC. The largest differences from the untreated case are shown with a lower \( k_p \) compared with untreated (Table 2). This is consistent with the effect that MMC has on these cells; they should not proliferate in the presence of MMC, hence \( k_p \) should be minimal. These data also demonstrate that model fits to the data are consistent with independent biological data (Figs. 4 and 5). Although the time delay parameter increased with MMC treatment, there is probably little sensitivity to the estimation of this parameter when \( k_p \) is very low.

Previous models of wound closure have not incorporated a time delay, nor a separate parameter for spreading, although studies have noted that migratory and proliferative behavior are highly dependent on time and location (34). Moreover, very few models of wound healing have been directly compared with experimental data. While spreading and migration are two separate mechanisms, previous models have not accounted for this fact. The time delay for the proliferation term is important because we have shown that proliferation does not begin for several hours in AEC; our representation of proliferation accounts for this. We were able to make several improvements over the most sophisticated existing model (3) with the use of biologically relevant boundary conditions and parameters. Our model serves as a first step toward defining parameters that can be used to characterize wound closure under different conditions and to determine parameters that are affected. Furthermore, our model predicted changes in parameters from wound-healing data that were then used to design experiments to verify these changes independently.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grant HL-64981.

**REFERENCES**


