Elastic fibers are responsible for the extensibility and resilience of many vertebrate tissues, and improperly assembled elastic fibers are implicated in a number of human diseases. It was recently demonstrated that in vitro, cells first secrete tropoelastin into a punctate pattern of globules. To study the dynamics of macroassembly, that is, the assembly of the secreted tropoelastin globules into elastic fibers, we utilized long-term time-lapse immunofluorescence imaging and a tropoelastin p Timer fusion protein, which shifts its fluorescence spectrum over time. Pulse-chase immunolabeling of the fibroblast-like RFL-6 cells demonstrates that tropoelastin globules aggregate in a hierarchical manner, creating progressively larger fibrillar structures. By analyzing the correlation between cell and extracellular matrix movements, we show that both the aggregation process and shaping the aggregates into fibrillar form is coupled to cell motion. We also show that the motion of non-adjacent cells becomes more coordinated as the physical size of elastin-containing aggregates increases. Our data imply that the formation of elastic fibers involves the concerted action and motility of multiple cells. J. Cell. Physiol. 207: 97–106, 2006.
coordinated motion of cells results in elastic fiber formation via connecting progressively larger segments into a composite ECM structure.

**MATERIALS AND METHODS**

**Cell culture**

Rat lung fibroblastic RFL-6 cells were obtained from ATCC (ATCC, Manassas, VA) and were grown in Hams F12 medium (Sigma, St. Louis, MO), containing 20% cosmic calf serum (HyClone, Logan, UT), 50 units/ml penicillin–streptomycin (Sigma) and 2 mM L-glutamine (Sigma). Prior to imaging, cells were plated on glass culture dishes (Delta T system, Biophtechs, Inc., Butler, PA) near confluence and were maintained in the same medium under standard tissue culture conditions (37 °C and 5% CO2/95% air atmosphere).

**Construct and antibody**

As described in Kozel et al. (in press), RFL-6 cells were transiently transfected with a construct encoding bovine tropoelastin with the pTimer reporter (BD Bio-sciences, Mountain View, CA). Transfection was continued for 6 h before imaging was begun.

The polyclonal IgG antibody (N6-17) was generated against a recombinantly produced murine tropoelastin fragment, and also recognizes rat tropoelastin (Kozel et al., 2005, 2004). Cy3 conjugated (FluoroLink, Amersham Pharmacia Biotech, Piscataway, NJ) N6-17 antibodies were added to the medium 12 h after seeding, at a concentration of 2 μl/ml. Imaging started 24 h after seeding, with fresh (antibody free) medium.

**Electron microscopy**

Post-confluent cultures of RFL-6 cells, plated in four-well Lab-Tek tissue culture chamber slides (VWR), were washed in phosphate-buffered saline (PBS) and fixed in situ with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 30 min. After fixation, the cell layers were washed with several changes of cacodylate buffer and then treated sequentially with 1% osmium tetroxide in buffer, 2% tannic acid in buffer, and 2% uranyl acetate in distilled water. Cell layers were then dehydrated in a graded series of methanol to propylene oxide, infiltrated and embedded in Epon (Canemco, Inc., St. Laurent, QC). Cross-sections of the cultures were cut using a Reichert ultracut ultramicrotome and counterstained with methanolic uranyl acetate followed by lead citrate. Sections were examined in a Philips 410 transmission electron microscope and negatives were scanned using a Nikon Super Coolscan 9000 ED negative scanner.

**Time-lapse imaging**

Cell cultures were imaged for 2–5 consecutive days with an automatized microscope system (Czirok et al., 2002). Multiple (4–12) microscopic fields were observed in each culture with the 20× (NA = 0.4) objective of an inverted, wide-field differential interference contrast/epifluorescence microscope (Leica DMI2RE2, Leica Microsystems, Wetzlar, Germany) as described in Kozel et al. (in press). For each field and microscopy mode, 3–10 images were taken in multiple focal planes, separated by 10 μm. The practical result of this technology is that no feature moves out of focus during the extended recording time.

**Image processing**

First, the best-focused optical plane was selected from the acquired “z-stacks,” as described in Czirok et al. (2002). Thermal camera noise was reduced in the low-intensity fluorescence images by applying a median filter (Chen et al., 1995). Each image shown in Figures 2–6 was locally normalized (Czirok et al., 2002, 2004) to compensate for uneven background and photobleaching. To preserve the ratio of red-green fluorescence intensities, images obtained from the pTimer transfections (Fig. 7) were processed differently. To correct for uneven field illumination in Figure 7, synthetic background images were calculated using a low-pass filter, and the estimated background was subtracted from each image (Chen et al., 1995). Finally images were converted from 12 bit to 8 bit intensity resolution by the same linear scaling for each frame, which transformed 1% of the total pixels to 255 (white) and 5% of the pixels to 0 (black).

**Object tracking and velocities**

Various objects, like ECM fibrils or cell organelles were traced manually in the time-lapse image sequence, using software described in Czirok et al. (2004). Briefly, the program allows the user to place various markers on the images, and keeps track of the marker displacements between consecutive images. The velocity of object i at frame t is denoted by v_i(t). The velocity, v_i(t), of object i at time t was calculated as v_i(t) = |x_i(t + Δt) − x_i(t)|/Δt with Δt = 30 min.

**Velocity correlations**

The similarity of two velocity vectors, v_i and v_j, is characterized by their scalar product, v_i.v_j, which is positive or negative if the two directions are parallel or antiparallel, respectively. The velocity correlation function, C_ij(d, t), between two sets of objects, I and J, describes how this similarity tends to change with the distance d between two objects, each from the corresponding set, at any given time point t. Thus,

\[ C_{ij}(d, t) = \frac{v_i(t) v_j(t)}{\langle v_i(t) v_i(t) \rangle} \]

where the average (….) denotes the ensemble average over all object pairs, i, j, such that their distance is close to d. That is, d − 5 μm < |x_i(t) − x_j(t)| < d + 5 μm, and i ∈ I, j ∈ J. The normalizing factors v_i and v_j in Equation (1) denote the possibly time-dependent average velocities of the objects belonging to the two sets, for example, v_i(t) = |v_i(t)|. Higher values of C indicate more similar (parallel) velocities. A null hypothesis of velocity vectors pointing in independent random directions would predict C ≈ 0. To determine the statistical significance of C > 0, that is, of correlated motion, the following Monte-Carlo randomization test was performed. A sequence of artificial samples was generated by randomizing the direction vectors, yielding the distribution F(C) of the resulting C values. P = 0.05 significance levels were then established as F^−1(0.05).

**Space-time plot of fluorescence intensities**

Temporal changes in fluorescence intensity within a long (y) and narrow (x) rectangular area (see Fig. 3D) are visualized with a spatio-temporal fluorescence map. For each frame t the fluorescence intensity I(x, y) is averaged over the rows, yielding a fluorescence profile \( I(x, y) \) along the y axis as \( \bar{I}(y) = \langle I(x, y) \rangle_y \), where the average is denoted by \( \langle \ldots \rangle \). This profile is then plotted against each frame, resulting in the space-time plot \( I(x, y) = I(x, y) \). These manipulations were performed with the “stack-Z-project” feature of ImageJ (http://rsb.info.nih.gov/ij).

**Image analysis**

A number of image analysis steps were performed using ImageJ (http://rsb.info.nih.gov/ij) functions and plugins. To compare the red- and green-channel intensities, correlation plots were calculated using the Red–Green Correlator plugin (http://www.uhnresearch.ca/facilities/wcif/imagej). Fluorescence intensity profiles were obtained using the PlotProfile function.

**RESULTS**

**RFL-6 cells secrete, organize, and assemble ECM in culture**

RFL-6 cells are a fibroblast-like cell line derived from fetal lung mesenchyme from normal Sprague–Dawley rats. In culture, RFL-6 cells display the key characteristics of the pulmonary fibroblasts in that they continue to express collagen type I, tropoelastin, and lysyl oxidase (Chen et al., 2005). At confluence, RFL-6 cells continue to divide and eventually form multiple layers interspersed with extracellular matrix (ECM) (Fig. 1A). Between the cell layers, and between the culture dish and the bottom layer of cells, deposits of elastin and elastic fibrils can
be seen. At higher magnification, elastin globules of various sizes can be seen adjacent to the cell surface (Fig. 1B, arrows). The globules appear to coalesce to form small fibrils (Fig. 1C) and larger fibers (Fig. 1D) in between the cells.

To study the time-course of elastic fiber assembly, cultures of RFL-6 cells were incubated for 12 h with medium supplemented with fluorochrome-conjugated N6-17 tropoelastin antibody. The presence of N6-17 antibodies did not result in detectable alteration of elastic fiber immunostaining pattern, when compared to fixed, unexposed cultures (data not shown). Likewise, addition of antibodies at 48 h did not disrupt the elastic fibers (data not shown).

![Fig. 1. Electron micrographs of post-confluent RFL-6 cell cultures viewed in cross-section. a: Multiple layers of RFL-6 cells are present in post-confluent cultures. Elastin and other ECM components can be seen between the cells (arrows) and between the cells and the surface of the culture dish (arrowheads). b: At higher magnification, individual elastin globules can be seen adjacent to the cell surface (arrows). Both short (c) and longer (d) assemblies of elastin globules, forming elastin fibrils, are also seen amongst the cells. Bars: 2.5 μm (a); 0.5 μm (b); 1.0 μm (c, d).](image)

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![Fig. 2. Elastin-containing ECM maturation in cultures of RFL-6 cells. Elastin immunofluorescence within the same area is displayed at 24, 28, 29, and 105 h in vitro (a–d). Insets show corresponding images of RFL-6 cells, obtained with DIC optics in the area marked with a rectangle in Part a. In 24 h, the pattern of elastin immunolocalization changes dramatically: linear structures emerge from apparently unconnected patches. Movie 1: Maturation of secreted tropo-elastin during a time course of four days. Elastin is visualized by a pulse of Cy3-conjugated N6-17 anti-tropoelastin antibodies. The pattern of elastin immunolocalization changes dramatically: linear structures emerge from apparently unconnected globules. A second pulse of antibodies was added between the 71st and 79th h of recording. Number of frames: 242, total length 95 h, area shown: 400 μm × 320 μm, resolution: 0.67 μm/pixel.](image)
Following a 12-h incubation with antibodies (24 h after plating in the imaging chamber) RFL-6 cells were recorded with scanning time-lapse microscopy for 1–3 days. As Figure 2 and Movie 1 demonstrate, the immunofluorescence pattern changed substantially during the second day in vitro (the first 24 h of imaging) as a result of cell activity. A punctate staining pattern of secreted elastin was observed at the beginning of the recordings (Part A, 24 h), followed by the localization of elastin to discrete fibrils 4 h later (Part B, 28 h). By 39 h, large elastin-containing fibrils were observed, which remain present throughout the remainder of the recordings (Part D). Antibodies added after 72 h to the cultures revealed no substantially different staining pattern from that already observed following the initial exposure to the tropoelastin antibody (Movie 1). These data indicate that although the majority of the initial immunofluorescent particles remain distinguishable throughout the recordings, their spatial distribution changes remarkably, in a process that generates fiber-like, elongated structures.

Elastin-containing ECM assembly is a hierarchical process

Tracing the pattern of elastin immunofluorescence in time-lapse image sequences revealed hierarchical aggregation dynamics. During the process, increasingly large segments are progressively connected (Fig. 3, Movie 2). As an example, the areas marked by the cyan and yellow ellipses in Figure 3B approach and merge to become parts of one larger immunofluorescent aggregate (green). The observed dynamics create ECM structures that span a few hundred micrometers (Fig. 3D) and remain stable. Disassembly is never visible in the form of an increasing gap between proximal elastin particles, or localized loss of fluorescence signal. This stability is not due to trivial loss of cell viability, as cells continue to move and proliferate.

Tracks of fluorescent foci that are ECM aggregate components reveal the aggregation dynamics in more detail, and help to better define an aggregate. The trajectories shown in Figure 3E represent the motion of two immunofluorescent particles from a representative intermediate elastic fiber aggregate. The similar trajectories indicate that the aggregate is mobile, and moves with a certain degree of rigidity. In contrast, non-aggregated particles present at early culture stages, such as those in Parts A, B, show uncoordinated, erratic movements with frequent direction changes (Fig. 3F). The switch from independent to coordinated motion, indicating the merger of previously unconnected ECM aggregates, was always found to be preceded by the
approach of the subunits within a close (less than 10 μm) range (Fig. 3F). Such a close proximity between elastin-containing ECM particles usually becomes persistent over the subsequent culturing period.

**Fibril formation: Orientational ordering and stretching**

The above described aggregation process does not only create large structures, but it also results in a fiber-like, linear distribution of the constituents. Two underlying mechanisms seem to be operational. First, during aggregation, the components align. This is clearly visible in Figure 3, where the merger of particles 6 and 7 (Part B), or 9 and 10 (Part C) takes place in a linear arrangement. Second, as Figure 4 demonstrates, a fibrillar aggregate can substantially elongate. The end-to-end distance within an aggregate, an approximate measure of fiber length, can exhibit both reversible and permanent changes. In the case of reversible length fluctuations, the original state (length, thickness) of the fiber is restored within a few hours, even after a relative...
extension of 50% (see bracket in Figure 4C). The elongation of the fibers always appears to be coupled to a decrease in fiber diameter (Fig. 4C, inserts).

**Cell and elastic fiber movements are correlated**

To clarify the connection between elastic fiber organization and cell motility, we systematically determined both cell (Fig. 5, red markers) and elastin (Fig. 5, green markers) displacements in three independent cultures after 1 and 2 days (25 h and 51 h) in vitro. These time points represent early and mature phases of elastic fiber assembly in our model system (see Figs. 2 and 3). Cell movements were established by tracking organelles and cell boundaries visible on DIC images. The traced DIC structures did not coincide with immunolabeled epitopes, thus there was no crosstalk between the two microscopy modes and we did not follow the same ECM structures in both modes. Irrespective of the assembly phase, the direction of adjacent elastic fibers and cell displacement vectors were similar. In the late assembly phase, the ECM motion vectors are similar throughout the entire field (Fig. 5c,d), yielding additional support to the observation that mature elastin-containing ECM aggregates move as a unit.

To statistically quantify the observed similarities between cell–cell, cell–ECM, and ECM–ECM velocities, distance-dependent correlations, $C_{\text{cell-cell}}(d)$, $C_{\text{cell-ECM}}(d)$, $C_{\text{ECM-ECM}}(d)$, were calculated from the corresponding velocity fields. The value of the correlation, $C$, gives the average similarity between concurrent velocities of two objects, which are separated by a distance of $d$. $C = 1$ indicates identical directions, $C = -1$ indicates antiparallel directions, and independent (random) velocity pairs result in $C = 0$. Figure 6 shows the $C(d)$ correlation functions, determined as an average over three independent microscopic fields, from two independent cultures. In the early assembly phase, the cell organelle and elastin-containing ECM velocities are equally similar to each other (Fig. 6A). No significant differences were found between cell-cell, ECM–ECM, or cell–ECM velocity correlations. Thus, the similarity between cell organelle and ECM displacements is the same as the similarity between the displacements of two cell organelles in the same region. Moreover, velocities become uncorrelated ($C = 0$) for distances larger than 50 μm, which is approximately the diameter of a cell. In contrast, after 2 days in vitro (51 h), the correlation length (the distance where $C(d)$ diminishes), increases substantially and exceeds 120 μm. The long correlation length indicates an ordered motion of both cells and ECM fibers, extending over multiple cell diameters. Although the correlation values are positive for all three sample pairs, elastin-containing ECM exhibits a more correlated motion than the cells do in the late phases of assembly (Fig. 6B).

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**Fig. 6.** Velocity correlation ($C$) versus distance ($d$) in the early and late phases of elastic fiber assembly. $C(d)$ measures the average similarity between the velocity of two objects, separated by a distance $d$. $C(d) = 1$ and $C(d) = -1$ indicates parallel and antiparallel motion, respectively. Each curve represents an average obtained from three independent microscopic fields. In the early phase of assembly (Part a) the correlation between the motion of objects separated by more than 40 μm becomes statistically non-significant ($P > 0.05$). In the late phase of assembly (Part b) even distant objects tend to move in similar directions as the positive correlation values demonstrate. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

**Fig. 7.** bTE-Timer incorporation into ECM fibers. Parts A–F represent six time points from a time-lapse sequence. The lower case letters in each part label the following: a—red channel; b—green channel, c—their superimposition with a contour marking areas selected for fluorescence correlation analysis, d—overlay on the corresponding DIC image of the cells, e—red-green correlation plot of the fluorescence emitted from the area marked by the blue contour in c. For each pixel within the marked areas, the intensity of the red/green component is used as the $x$-$y$-coordinate of the scatter-plot point, respectively. The blue arrowhead in A points to pixels of the background, which emit neither in the red nor in the green channel. Newly synthesized bTE-Timer results in a perinuclear label, visible in green (Part A). The intensity of light emitted from fresh (green) bTE-Timer (scatter-plot points within the green rectangle) increases during the first 10 h. Older elastin aggregates gradually appear in the red channel (Part B) but the label remains diffuse, that is, non-fibrillar. In this process, the pure green component diminishes as it becomes colocalized with the red signal of older bTE-Timer proteins (yellow asterisk in Ce). The formation of the fibrillar structure involves mostly old (red) bTE-Timer, and it coincides with the dislocation of an adjacent cell, marked by cyan arrowheads (Parts C, D). Further elongation and displacement of the fibers are also coupled to the motion of adjacent cells, for example, the one labeled by the cyan asterisk (Parts E, F). The forming fibrillar structure collects mostly older (red) bTE-Timer proteins as indicated by the reappearance of non-colocalized (green) bTE-Timer signal (see boxes in De, Fe). Scale bar: 20 μm. Movie 3: Elastic fibers are assembled by cell movements. RFL-6 cells were transfected with bTE-Timer, a construct, which changes fluorescence color in time. The top left and bottom left part show the fluorescence intensity in the red and green channels, respectively. Their combination is shown in the central part. In the right part, DIC images are also superimposed which allows the observation of cell movements. The blue ellipse in the central part marks an area, where green globules are turning into red filaments. These filaments are pulled into a long fiber by the motion of adjacent cells (small ellipses on the right part). Number of frames: 68, total length 25 h, area shown: 100 μm × 100 μm, resolution: 0.33 μm/pixel. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
Direct visualization of cell–ECM interactions

The active interaction of cells and elastin-containing ECM was directly visualized by transfecting RFL-6 cells with the bTE-Timer construct (Kozel et al., in press). The commercially available pTimer is a prokaryotic expression vector that encodes DsRed1-E5, a mutant of the red fluorescent protein, DsRed1 (Terskikh et al., 2000). When synthesized, DsRed1-E5 fluorescence is green (excitation and emission maxima are at 483 nm.
and 500 nm, respectively). After synthesis, the fluorophore undergoes additional changes that shift its fluorescence to longer wavelengths (excitation and emission maxima are at 558 nm and 583 nm, respectively). The green-to-red transition happens about 6 h after the protein first becomes fluorescent. While the fluorophore exists in either of two distinct states (green and red emission), an optically colocalized population of DsRed1-E5 proteins gradually changes the color of fluorescence emission from green through yellow to red.

As demonstrated in Figure 7 and Movie 3, newly synthesized proteins (green channel) contour the nuclei of transfected cells (Parts A, B). By the time tropoelastin is secreted, a shift towards red has occurred, and the tropoelastin appears as small, usually yellow globules (Part C) over the cell surface (Fig. 1). Older proteins (red channel) are always observed as part of filamentous structures (Parts D–F). Formation of these filaments involves the collection of secreted globules from the cell surface by the motility of adjacent cells (Parts C–F).

DISCUSSION

Elastic fibers are typically large, multi-component ECM structures, spanning multiple cell diameters. Therefore, elastic fibers assembly requires the concomitant secretion and organization of a number of other ECM molecules and involves multiple cells. RFL-6 cells, like other cultured cells that assemble elastic fibers (Robb et al., 1999), are post-confluent and multi-layered by the time substantial insoluble ECM can be detected. The complexity of elastic fibers is also reflected in the time course of assembly as revealed by classic electron microscopic studies of primary vascular smooth muscle cell cultures (Hinek and Thyberg, 1977; Toselli et al., 1981). These studies identified three stages of elastic fiber formation. First, microfibrils are assembled. Then, immature elastic fibers appear, which consist of small conglomerates of amorphous material, distributed among the bundles of microfibrils. Finally, the fibers take on a more filamentous quality with an increase in size of the fibers and a parallel loss of the small conglomerates. Recent studies in cultures of RFL-6 cells (Kozel et al., 2004) revealed a similar time course, although during a different time frame.

Here we present a computational study on the dynamics of supracellular (10–100 μm) elastic fiber formation in cell culture. We found that cells and elastin-containing ECM are mechanically coupled, as revealed by the local positive correlation between their respective displacements. This coupling, however, is transient: bTE-Timer data indicate that fiber formation often involves the relative motion of adjacent cells during which ECM is released from the secreting cell and relocated by a nearby cell. The larger ECM structures are not destroyed by the random motility of adjacent cells, as the aggregate size increases, cells even 150 μm apart tend to move in the same direction. This long-range correlated sheet motion of cells is indicated by the positive velocity–velocity correlation. Therefore, cell motility seems to be required for the formation of elastic fibers, although some molecular steps, like the specific association between the secreted tropoelastin and the ECM scaffold, can take place also in the absence of cell activity (Kozel et al., 2004).

Figure 8 summarizes our model for elastic fiber formation. Cells move, and thereby extend and align a network of microfibrils, as well as interact with adjacent elastin-containing aggregates. Interestingly, substantial bending or compression of the fibers does not occur, either as a consequence of their mechanical rigidity, or a mechanical stress-dependence of the ECM–cell interaction. As a result of coordinated cell motility, two distinct elastic fiber aggregates can approach each other and attach to the same cell. Subsequently, by local ECM reorganization at the cell surface the two ECM structures can unite to form a larger composite structure. By repeating the ECM movements and aggregation steps over time, progressively larger units emerge. The confluent, possibly multi-layered cultures increase the

Fig. 8. Cartoon of large-scale elastic fiber assembly. Cells can join adjacent ECM fibers (a), or rearrange an existing network by cell motion (b). However, the rearrangements tend to increase the filament length, and folding back (c) is never observed. Based on these elementary steps, the ECM network formation is summarized in Part (d). After the random deposition of short fibrils, cell motion (represented by blue and green arrows) creates elongated fibers. As cell movements become more correlated, they can convey larger pieces of ECM aggregates. When two of these aggregates meet by chance, they become associated or connected and a larger aggregate is formed. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
likelihood that a given cell interacts simultaneously with two ECM aggregates, or an ECM aggregate is attached to multiple cells. Moreover, in subconfluent cell cultures, if the secreted ECM accumulates between the cells and the substrate, then attachment to the substrate may prevent further reorganization of the ECM fibrils (Ohashi et al., 2002). Parallel to this large-scale, hierarchical assembly, newly secreted elastin-containing globules may also be added to the aggregates (Kozel et al., in press). Beyond these two assembly models, we found no evidence for additional potential growth mechanisms. Most importantly, we always found the direct extension (growth) of established fibers coupled with a concurrent decrease in fiber diameter. Thus, deposition of secreted elastin monomers directly on established fibrils does not seem to substantially increase elastic fiber length.

The resolution of this study does not allow the direct observation of the presence or absence of chemical crosslinks between adjacent fibers. Rather, we deduce the existence of these interactions based on the lack of substantial distance changes between adjacent fibers, indicating some sort of stable mechanical connection. Similar linking of adjacent fibronec fibbers was also observed as the resolution of fiber type differentiation in Xenopus laevis cap assays (L. Davidson, personal communication), and in osteoblast cell cultures (S. Dallas, personal communication). We speculate further, that the increasing size of ECM aggregates imposes coordinated cell sheet movement. The large expansion of the domain in which velocities are correlated occurs in 24 h, during which cell density and other cell culture parameters remained similar. The correlation of cell velocities may increase as a result of mechanotransduction-related signaling (Chen et al., 2004), driven by ECM-mediated mechanical coupling between distant (non-adjacent) cells: One may speculate that the divergent motion of two cells, both attached to the same ECM aggregate, would generate mechanical stress within the cell–ECM composite material. This mechanical stress could be sensed by appropriate receptors, and the resulting biochemical signaling would alter the cells’ direction of movement.

This body of work addresses the dynamics of cell and elastic fiber interactions in vitro. Computational data on cell–ECM motion indicates that over time, cells and the ECM form increasing large, coherently moving units. The presented results imply that cell motility plays a fundamental and ongoing role in the formation of fibrillar ECM networks.

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LITERATURE CITED


