Behavior of Cells Seeded in Isolated Fibronectin Matrices

PHILIP HSIEH and LAN BO CHEN
Dana Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT Cell-free fibronectin matrix (FN-matrix) isolated from chick embryo fibroblasts was used to study cell-matrix interaction. After 24 h, most fibroblastic cells, including those without cell surface fibronectin, adopted bipolar fusiform morphology. Cells grew in parallel arrays and aligned with each other apparently along FN-matrix. Since the orientation of fibronectin fibers was determined by chick embryo fibroblasts, our results suggested that intercellular organization of "matrix-using" cell type may be influenced by "matrix-producing" cell type. Whereas the elongation and alignment effects induced by FN-matrix have been detected in fibroblasts (both normal and transformed), myoblast, aortic endothelial cells, neural cell lines (B103 and RT4D1), and cardiac muscle cells, similar effects are not detected in bone marrow hemopoietic cells, circulating lymphocytic T and B cells, and sympathetic neurons. For epithelial cells, FN-matrix has varying effects. Elongation and alignment effects are detected only in transformed epithelial cells with a great reduction in keratin expression. The morphology of normal or transformed epithelial cells with abundant keratin appears unaffected by FN-matrix. FN-matrix reduced the growth of several transformed fibroblastic lines up to 25%, but did not restore the appearance of actin stress fibers and the normal migratory activities of Rous sarcoma virus-transformed rat cells.

Most adherent eukaryotic cells in vivo are bathed in an environment made of extracellular matrices. The importance of this environment in relation to differentiation, migration, morphogenesis, and disease has long been recognized (12). The matrices are composed of collagens, glycosaminoglycans, proteoglycans, glycoproteins, and other minor components. The exact functions of these components and the mode of interaction among them are still unclear. To help clarify these questions, particularly the poorly understood cell-matrix interaction, cell culture system has gradually become useful. Since the early report that fibroblasts in culture produce fibrillar materials (18), it has been recognized that several other cell types also express extracellular matrices in culture. For example, intercellular fibronectin matrices (FN-matrices) are expressed not only in cultured fibroblasts (27) but also in cultured myoblasts (4, 15), some epithelial cells (5), vascular endothelial cells (3, 16), and corneal endothelial cells (10, 26).

To understand the role of cell-matrix interaction, cell-free matrices might be useful since chemical characterization of the matrix as well as its biological effects can be conveniently studied. Thus far, FN-matrices from chick embryo fibroblasts (6) and human foreskin fibroblasts (13), and extracellular matrices containing collagen, fibronectin, and laminin from corneal endothelial cells (10) have been isolated, characterized, and used for studying cell-matrix interaction. In this report, we describe the effect on the elongation and alignment of transformed and normal fibroblastic cells of cell-free fibronectin matrices isolated from chick embryo fibroblasts. The effects of FN-matrix on cell growth, actin stress fibers, and migratory activities of tumorigenic fibroblastic cells were investigated. In addition to fibroblastic cells, the interaction of FN-matrices with cardiac muscle cells, aortic endothelial cells, neural cell lines (B103 and RT4D1), bone marrow hemopoietic cells, circulating lymphocytic T and B cells, sympathetic neuron, normal epithelial cells, carcinogen-transformed epithelial cells, and carcinoma-derived cells was also studied.

MATERIALS AND METHODS

Cell Cultures: All cells were cultured in Dulbecco's modified Eagle's medium (DME) supplemented with 5% calf serum (Gibco Laboratories, Gibco Div., Grand Island, NY). Primary chick embryo fibroblasts (CEF) were prepared according to Rein and Rubin (19). Other cell lines used were: CCL47, a Rous Sarcoma Virus (RSV)-transformed rate cell line, and CCL64, a mink fibroblast line (American Type Culture Collection, Rockville, MD); AnAn, established from a tumor induced by injecting RSV into newborn Lewis rat and passaged in vivo three times (25); t.s. RSV-Rat, Rat 1 transformed with a temperature-sensitive mutant in src gene of RSV-B77, established by J. Wyke (Imperial Cancer Research Fund, England) and provided by R. O. Hynes (Massachusetts Institute of Technology); SV80, SV40-transformed human fibroblast, from D.

THE JOURNAL OF CELL BIOLOGY · VOLUME 96 MAY 1983 1208-1217

Livingston (Harvard Medical School); 3T3, a mouse fibroblast line and Py3T3, polyoma virus-transformed mouse 3T3 fibroblast, from H. Green (Harvard Medical School); 64F3, feline sarcoma virus-transformed mink cells from M. Essex (Harvard School of Public Health); B103 and RT4D1, rat neural tumor lines induced by nitrosourea, from K. Sueoka (Universisy of Colorado); and Rat 1, a rat fibroblast line, from W. Topp (Cold Spring Harbor Laboratory). Rat cardiac muscle cells and mouse bladder epithelial cells were kindly provided by T. Lampidis and I. C. Summerhayes, respectively, of this laboratory. Rat sympathetic neurons were obtained from P. Petterson, T and B lymphocytes from C. Terhorst, mouse bone marrow cultures from U. Reincke, and bovine aortic endothelial cells from B. Zetter, all of Harvard Medical School. Normal epithelial cells, transformed epithelial cells, and carcinoma-derived cells were described previously (21–23).

For measuring cell growth, identical numbers of cells (10^4) were plated on plain or FN-matrix-coated dishes (35 mm). Cells were trypsinized after 1 wk, with culture medium being changed twice. Cell number was counted by a Coulter counter (Coulter Electronics Inc., Hialeah, FL). The cell counts were given as a mean of triplicated dishes.

Isolation of Fibronectin Matrices: Confluent monolayers of primary chick embryo fibroblasts on 12-mm glass coverslips or on T25 culture flasks (Corning Glass Works., Corning, NY) were washed with 0.1 M phosphate buffer (pH 9.6) and then lysed at 37°C for 1 h with the same buffer supplemented with 1% Triton X-100 (lysis buffer). After washing, fresh lysis buffer was added. The treated culture can be left in lysis buffer for up to 24 h or processed immediately by rinsing alternately with 0.3 M KCl and H_2O until clean matrix is obtained. The fiber density of the FN-matrix is dependent on the age and density of chick cells in culture. To obtain consistent results, we recommend the use of exponentially growing primary cells within 4 d in culture at a final density of 5×10^4/cm^2. It is difficult to prepare FN-matrix from over-confluent cultures, especially on glass coverslips. These matrices tend to peel off from the coverslips. Once prepared, the matrices can be stored in phosphate-buffered saline (PBS) at 4°C for up to 6 mo. Before use, the matrices are rinsed with cultured medium extensively.

Light Microscopy: A Leitz Diavert phase contrast microscope with a ×10 objective was used for most of the studies. For time-lapse studies, cells were cultured in a screw-tight T25 flask (Corning Glass Works) and maintained at 37°C by a Zeiss air stream incubator. Photographs were taken on Kodak Technical Pan film 2415 by a Pentax ME camera directly attached to the Diavert microscope. Time-lapse cinematography (16 mm) was made on Opti Quip Model 220 (Opti-Quip, Highland Mills, NY).

Immunofluorescence Microscopy: Fibronectin in FN-matrices prepared on 12-mm glass coverslips can be visualized by staining with fluoresceinated gelatin or with rabbit antibody to human plasma fibronectin followed by rhodamine-B-conjugated goat antibody against rabbit immunoglobulin G (Cappel Laboratories, Cochranville, PA). The specificity of the fibronectin antibody and the FITC-gelatin-staining procedure has been described previously (14). Actin antibody, generously provided by K. Burridge (University of N. Carolina), was used to localize actin in cultured cells (29). Keratin antibody from T. T. Sun (New York University) and J. J. Rheinwald (Harvard Medical School), raised against human callus total keratin, was used as described previously (21, 22). Keratin content of different cell lines was compared by monitoring the fluorescence of cells stained with keratin antibody with a Nanospec-10S microspectrophotometer (Nanometrics, Inc., Sunnyvale, CA) attached on a Zeiss Photomicroscope III equipped with Planapo objective lens (×63). The average fluorescence was estimated from 100 randomly chosen areas (625 μm^2) of each coverslip. Fluorescence unit >200 was represented by (++++) and <50 by (+) in Table I. Photographs were taken as described previously (17).

Scanning Electron Microscopy: Procedures were described previously (6).

RESULTS

Morphological Effect of the FN-Matrix

Soluble fibronectin has been shown to restore normal morphology to most but not all transformed cell lines (1, 30, 31). AnAn, a rat tumor line induced by Rous sarcoma virus and without detectable cell surface fibronectin, is unresponsive to soluble fibronectin with regard to morphological reversion. However, after 2 d in cell-free FN-matrix, AnAn cells are bipolarized to adopt fusiform morphology and aligned in parallel array (Fig. 1). Fig. 2 is a scanning electron micrograph of AnAn cells growing on glass coverslips with or without FN-matrix. As seen, FN-matrix changes the shape of AnAn cells drastically. The three elongated cells were bipolarized in the direction apparently along the orientation of fibronectin fibers. Besides AnAn cells, FN-matrix also affects the morphology and alignment of a variety of cell lines and cell types (Fig. 3). The bipolarization effect of the matrix is detectable on transformed cells even at a high density (Fig. 3*f*). The effects of FN-matrix survive the pretreatments of matrix with bacterial collagenase (37°C for 48 h), air drying (22°C for 5 min), or heat (80°C for 5 min). It is, however, very sensitive to trypsin treatment (0.5 μg/ml for 5 min).

Spreading and Attachment

We used time-lapse cinematography (16-mm film) to compare the spreading of CCL47 cells on plastic substratum to those on FN-matrices. Although CCL47 is RSV-transformed rat cell, within 24 h after seeding on plastic the spreading and morphology of this cell line are similar to those of normal cells. In the absence of FN-matrices, CCL47 cells attach to the dish within 15 min. By 30 min, 80% of the cells spread radially and have extensive marginal ruffles (lamellipodia). No bipolarity is detected in these spreading cells. Bipolarized cells are rarely detected until 12 h later. On FN-matrix, all of the cells attach to the substratum in <15 min. However, spreading does not occur immediately. By 2 h, only 40% of the cells spread out, but marginal ruffles are not readily detectable. Cells appear to be bipolarized soon after spreading. By 5 h, 80% of the attached cells have spread out, and most of them are also bipolarized.

A series of phase-contrast photographs on the spreading of CCL47 cells on plastic substratum and on FN-matrix is shown in Fig. 4. The results on the spreading are identical to those obtained with 16-mm time-lapse cine. 10 min after plating CCL47 cells on FN-matrix, the first photograph was taken (Fig. 4*a*). Identical positions in these figures are marked as reference points by arrows, open or closed squares, circles or numbers. In Fig. 4*a*, cell no. *1* starts to spread, but no marginal ruffle is seen; two pairs of cells marked no. *2* and *3* are still in round shape. On the plastic dish at 10 min, many cells are spreading radially and marginal ruffles are readily detectable (cells no. *4* and no. *5* in Fig. 4*b*). By 40 min, many cells start to spread on FN-matrices, but the membrane extension seems to be inhibited. Marginal ruffles are rarely detected. Cell no. *1* is bipolarized as it spreads out. On the plastic dish, cells no. *4*, *5*, and *6* remain radially spread at 40 min. 95% of the spreading cells do not have a defined bipolarity. By 2 h, more bipolarized cells are seen on FN-matrix, and cell pair no. *2* starts to spread out (Fig. 4*e*). On the plastic substratum (Fig. 4*f*), membrane extension of spreading cells almost reaches the maximum. Cells no. *5* and *6* are fully spread out at this time point, yet still without bipolarity.

By 5 h, 95% of the cells on FN-matrix are spread out, and almost all of them are bipolarized. Cells within each cell pair, no. *2* and *3*, are bipolarized in a parallel direction; cell no. *1* is fully bipolarized (Fig. 4*g*). On the plastic dish some cells start to gain their bipolarity (Fig. 4*h*). One of the two cells in pair no. *5* is partially bipolarized, but not the other. Cell no. *6* is bipolarized and starts to migrate towards the white dot marked by a small open circle. Cell no. *4* still remains radially spread.

By 12 h, cells on FN-matrix are extending their processes. Cell no. *1* is migrating away from the open square and in the same direction as the initial bipolarity; all cells in each pair, no. *2* and *3*, are extending their processes (Fig. 4*i*). On the plastic, cell no. *6* is reaching out for the white dot, and one of the cells in cell pair no. *5* has divided. Still, there are cells (i.e.,

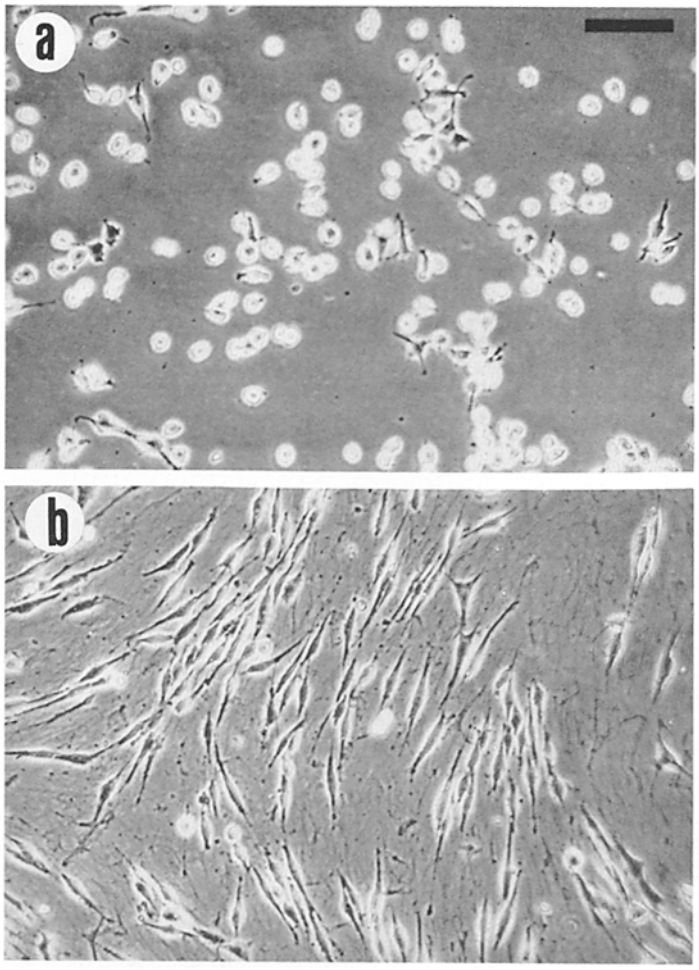

FIGURE 1 Phase-contrast micrographs of AnAn (RSV-rat tumor) cells on (*a*) plastic dish and (*b*) cell-free fibronectin matrices. Bar, 120 μm.

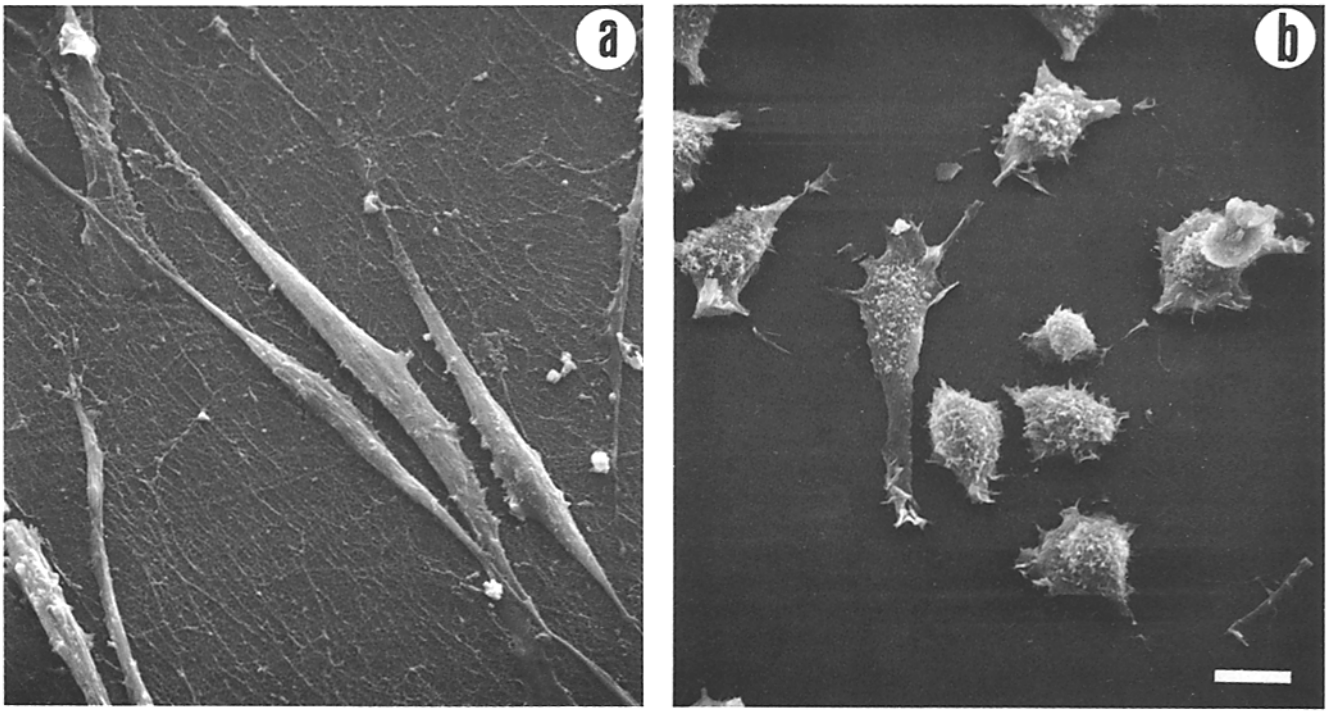

FIGURE 2 Scanning electron micrographs of AnAn (RSV-rat tumor) cells on (*a*) cell-free fibronectin matrices and (*b*) glass coverslip. Bar, 35 μm.

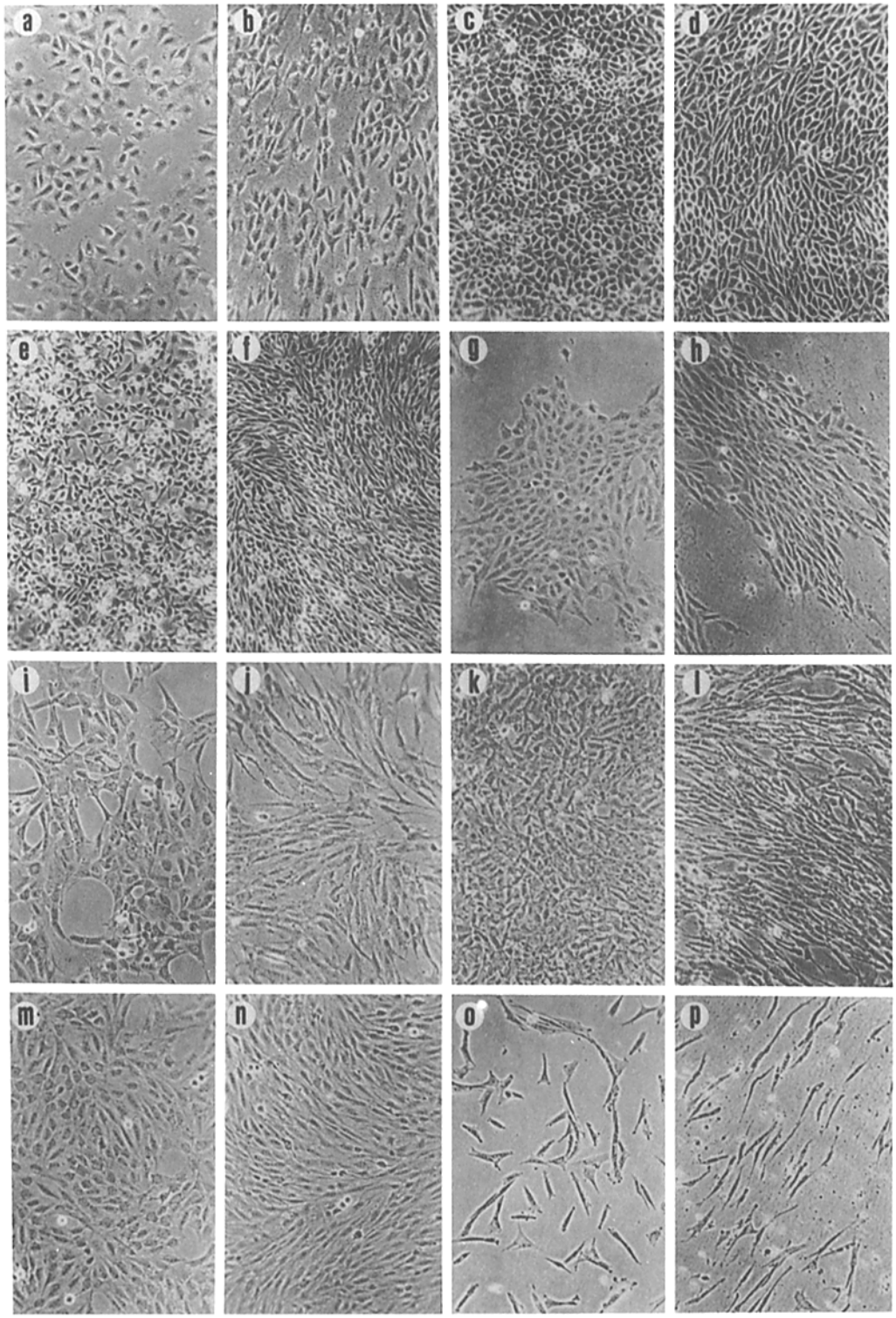

FIGURE 3 Phase-contrast micrographs of CCL47 at a lower density (*a* and *b*); CCL47 at a higher density (*c* and *d*); t.s. RSV-Rat at permissive temperature (36°C) (*e* and *f*) and nonpermissive temperature (40.5°C) (*g* and *h*); 3T3 (*i* and *j*); Py 3T3 (*k* and *l*); Rat-1 (*m* and *n*); chick embryo fibroblasts (*o* and *p*). *a, c, e, g, i, k, m,* and *o* are on plastic dish. *b, d, f, h, j, l, n,* and *p* are on cell-free fibronectin matrices.

cell no. *4*) remaining radially spread without obvious bipolarity (Fig. 4*j*).

By 24 h, cells on FN-matrix remain bipolarized with long processes. Cell no. *1* has moved farther away from the open square. Both cells in pair no. *2* have divided and the resulting four daughter cells are also bipolarized with processes (Fig. 4 *k*). On the plastic dish, cell no. *6* has migrated farther down, and only by this time are some of the cells partially polarized (Fig. 4 *l*).

Taking these observations together, it appears, therefore, that FN-matrix may delay cell spreading, inhibit radial membrane extension, and promote bipolarization.

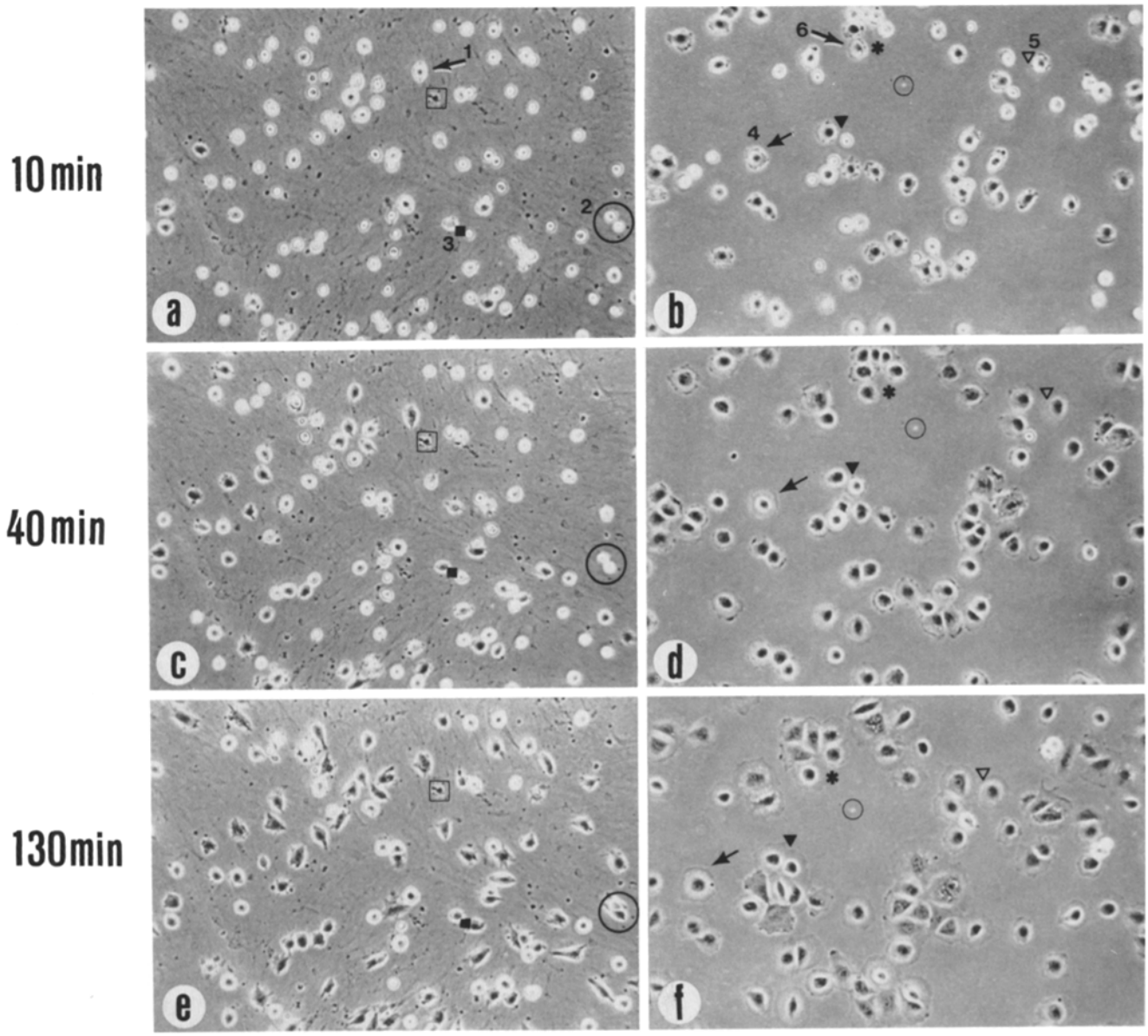

FIGURE 4 Spreading of CCL47 (RSV-Rat) cells in cell-free fibronectin matrices (*a*, *c*, *e*, *g*, *i*, and *k*) and in a plastic dish (*b*, *d*, *f*, *h*, *j*, and *l*) after 10 min (*a* and *b*,); 40 min (*c* and *d*); 130 min (*e* and *f*); 5 h (*g* and *h*); 12 h (*i* and *j*); and 24 h (*k* and *l*).

Migration

In view of the above results, it is of interest to compare, by time-lapse cinematography, the migrations of cells seeded on substrata with or without FN-matrices. FN-matrix did not promote migration of cells such as AnAn and CCL47, which normally have little displacement on plastic. It appears that bipolarization of these cells by FN-matrix did not improve them with respect to undertaking migration. However, cells that normally migrate on plastic dishes, such as primary fibroblasts of various origins, tend to move along the fibers of FN-matrix. The guiding effect of FN-matrix is most readily detectable in CEF. On plastic substratum, CEF migrate randomly. On FN-matrix, the migration pathway of CEF tends to follow the fibronectin fiber.

Cytoskeleton

It has been reported that soluble cellular fibronectin induces the formation of actin-containing stress fibers in transformed cells (1, 29). We therefore examined whether stress fibers can also reappear in RSV-transformed rat cells grown in FN-matrices. The staining of actin in AnAn cells growing on plastic substratum is diffuse, whereas filament bundles were detected in untransformed Rat-1 cells with actin antibody (Fig. 5 *a* and *b*). The staining of actin in bipolarized AnAn cells growing on FN-matrix is still diffuse, and no stress fiber is detected (Fig. 5 *c*).

Growth

Fibronectin matrix has little effect on the growth of untransformed fibroblastic cell lines (i.e., Rat 1, 3T3, and CCL64). These cells have a similar growth rate whether grown on FN-matrix or not. The growth of secondary chick embryo fibroblasts and rat embryo fibroblasts is slightly enhanced when they are seeded on FN-matrix. For transformed cells, unexpectedly, the FN-matrix has negative effects on their growth. Up to 25% less of transformed cells grown on FN-matrix is observed when compared with those grown on plastic (Fig. 6).

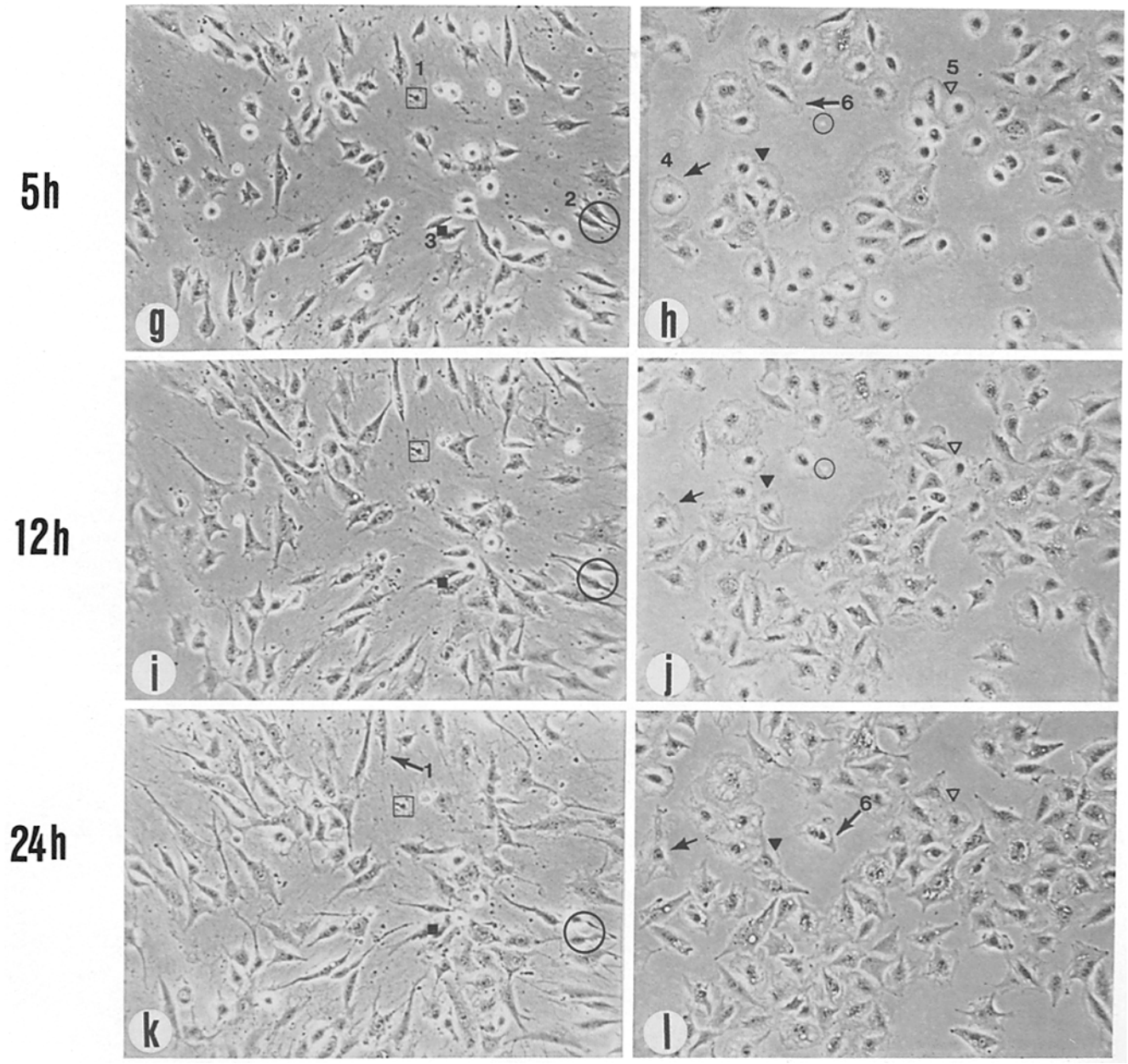

FIGURE 4 *Continued.*

These transformed cell lines include CCL47, AnAn, t.s. RSV-Rat, Py3T3, and 64F3 (FeSV-mink).

Epithelial Cells

The above experiments were carried out on fibroblastic cells. It is of interest to determine whether epithelial cells will respond to FN-matrix similar to fibroblastic cells. Fig. 7 shows that normal mouse bladder epithelial cells are not bipolarized by FN-matrix, and no alignment effect is detected. Since these epithelial cells are known to contain abundant keratin, and since we previously observed that many transformed epithelial cell lines with a significant reduction in keratin expression adopt fibroblastic morphology in culture (21, 22), we examined the polarization and alignment effects of FN-matrix on various carcinogen-transformed epithelial cell lines and carcinoma-derived cell lines. Table I summarizes the results and Fig. 8 illustrates an example of the effect of FN-matrix on benzo(a)pyrene-transformed rabbit bladder epithelial line, RBC-1 (22). It appears that for epithelial cells there is a correlation between a decrease in keratin content and an increase in propensity to be polarized and aligned along FN-matrix.

Other Cell Types

We also examined the morphological effect of FN-matrix on bone marrow cells, cardiac muscle cells, aortic endothelial cells, neural cell lines, lymphocytic cells, and sympathetic neurons. The FN-matrix promotes the adhesion and polarization of some bone marrow cells to the substratum. These adherent cells are probably histiocytes and reticulum cells. Attachment and polarization of bone marrow hemopoietic cells on FN-matrix are not detected. Rat cardiac muscle cells also become

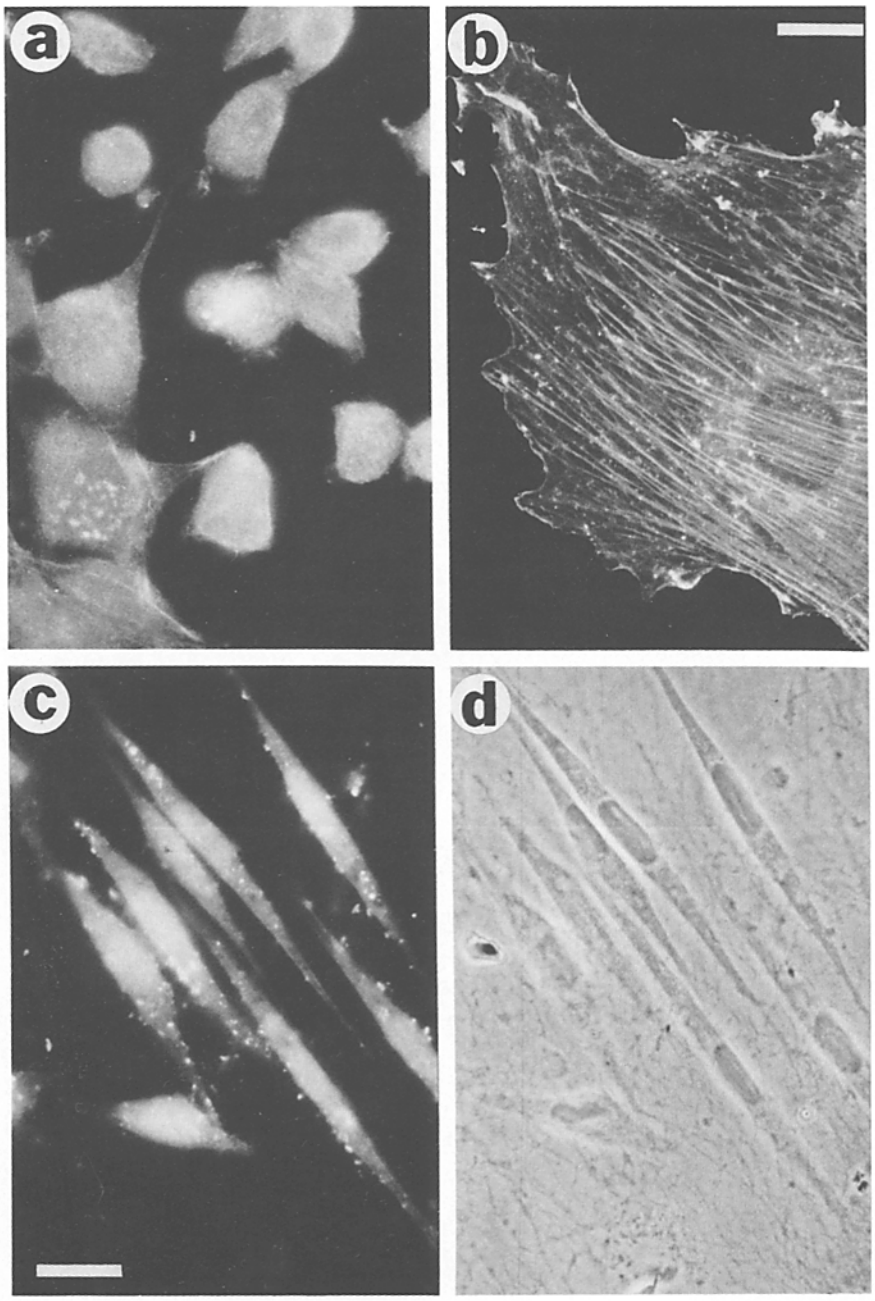

FIGURE 5 Immunofluorescence staining with actin antibody of (*a*) AnAn cells on a glass coverslip; (*b*) Rat 1 cells on a glass coverslip; (*c*) AnAn cells on a coverslip containing cell-free fibronectin matrices; and (*d*) phase-contrast micrograph of (*c*). Bars: *a*, *c*, and *d*, 40 μm; *b*, 20 μm.

more elongated and aligned on FN-matrix. Bovine aortic endothelial cells and neural cell lines (B103 and RT4D2) are polarized and aligned by the matrix as well. Sympathetic neurons with or without nerve growth factor are not affected by FN-matrix, and the neurite outgrowth does not follow the fibronectin fibers in general. The morphology of mouse lymphocytic T and B cells is not affected by the FN-matrix.

DISCUSSION

We reported previously the isolation of cell-free fibronectin matrix from cultured chick embryo fibroblasts (6). In this report, we investigated further the biological effects of cell-free fibronectin matrices. It appears that fibronectin matrix is able to bipolarize all fibroblastic cells into fusiform morphology and to align them in a parallel array, similar to those seen in the histological sections of connective tissues. All tested transformed fibroblasts irrespective of the expression of cell surface fibronectin also respond to fibronectin matrix by reversing their morphology to a more normal spindle shape and aligning in a parallel array.

Whether fibronectin molecule alone is responsible for the effect of FN-matrix described here or proteoglycans are also required needs further investigation. Our rabbit anti-fibronectin antibody preparation does not block the biological effects of FN-matrix (data not shown). However, Rovasio et al. (20) have shown that the antibody against 160-kdalton cell-binding fragment of fibronectin does block the biological effect of FN-matrix on neural crest cell migration. Our antibody may not recognize the cell-binding site of fibronectin. Although actin and myosin are present as contaminants in cell-free FN-matrix (6), it is unlikely that they play a role in the effects of FN-matrix described here. These contaminants are present in glob-

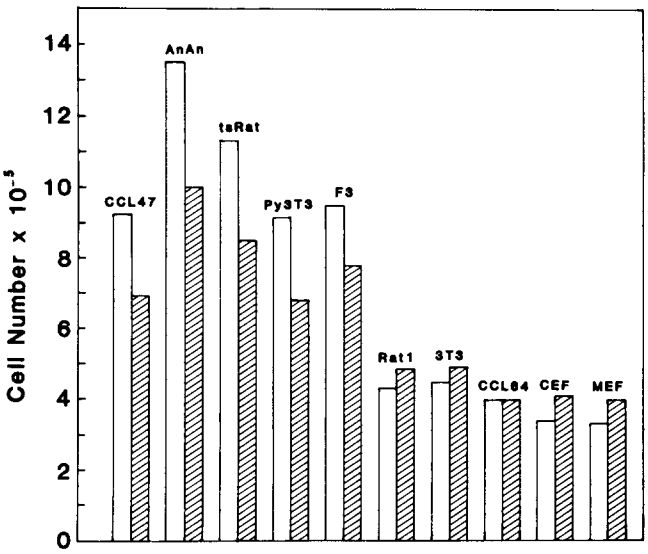

FIGURE 6 Effect of cell-free fibronectin matrices on the cell growth of various cell lines and primary fibroblasts. White bars, control; cross-hatched bars, matrix.

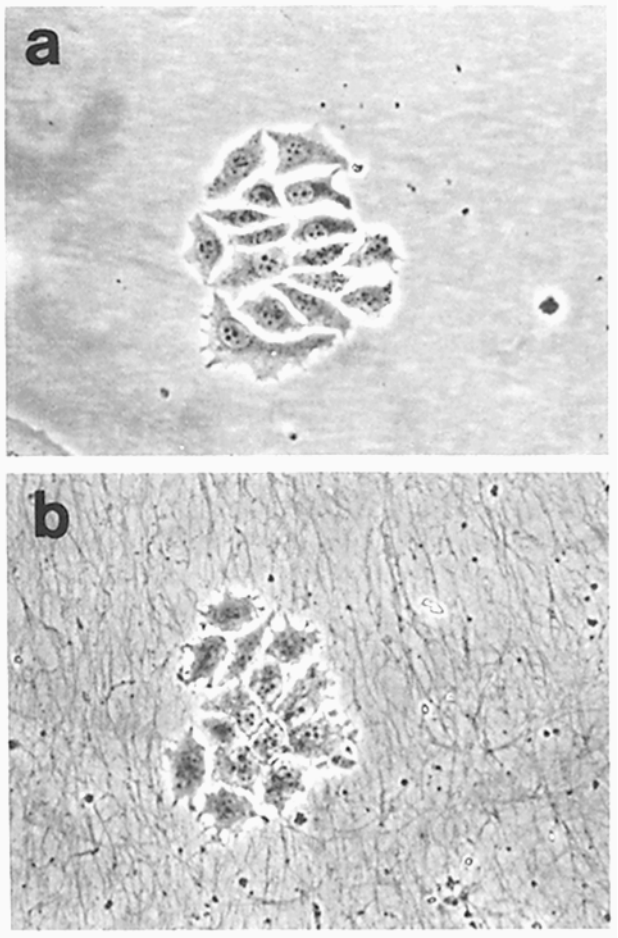

FIGURE 7 Human breast carcinoma-derived cell line, MCF-7, on (*a*) a plastic dish and (*b*) cell-free fibronectin matrices.

ular structures randomly distributed in FN-matrix. Areas free from actin and myosin are readily detectable by immunofluorescence with actin and myosin antibodies. Fig. 5*c* is an example of area of FN-matrix devoid of actin but still supporting the bipolarization of RSV-rat cells. Recently, we have

TABLE I

Effects of Fibronectin Matrices on the Elongation and Alignment of Epithelial Cells with Different Keratin Contents

Epithelial cell lines	Species	Organs	Keratin content	Elongation and alignment
MCF-7	Human	Breast Carcinoma	++++	−
RT112	Human	Bladder Carcinoma	++++	−
64-24	Rat	Breast Carcinoma	++++	−
THE	Hamster	Stomach-SV40	++++	−
PtK1	Rat-kangaroo	Kidney Normal	++++	−
MB331	Mouse	Bladder-DMBA	++++	−
Primary	Mouse	Bladder Normal	++++	−
EJ	Human	Bladder Carcinoma	+	+
J82	Human	Bladder Carcinoma	+	+
RBC-1	Rabbit	Bladder-Benzo(a)pyrene	+	+
MB48B	Mouse	Bladder-DMBA	+	+
MB49	Mouse	Bladder-DMBA	+	+

prepared FN-matrix from cytochalasin-B-treated cells. Very large areas of FN-matrix totally free from actin and myosin are available for studies. Cells seeded in these areas behave as do those in areas contaminated with actin and myosin. Thus, actin and myosin appear not to be involved in the biological effects of cell-free FN-matrix.

We have attempted to analyze the mechanism involved in the bipolarization and alignment of cells in FN-matrix. On the basis of the results presented in this report, the simplest explanation seems to be that FN-matrix exerts a contact guidance effect on those responding cells. The contact guidance phenomenon was first characterized by Weiss (28) and subsequently confirmed in several other studies (2, 7, 8). Time-lapse cinematography and phase-contrast micrographs taken at longer time intervals (Fig. 4) of cells seeded in FN-matrix indicate that fibronectin fibers may provide the physical structure for contact guidance. If a cell type tested is normally migratory on plastic substratum, once a contact guidance effect is exerted by fibronectin fiber the cells tend to migrate along the fibronectin fibers. However, if a cell type tested is normally nonmigratory, such as CCL47, although cells are bipolarized and aligned by fibronectin fibers the migratory activity is not enhanced. It appears as if contact guidance effect has been exerted by FN-matrix on CCL47 cells, but the migratory activity of these cells has not been improved even after the cells have become spindle-shaped.

The influence of cell shape on cell proliferation has been elegantly studied by Folkman's laboratory. The inhibitory effect of FN-matrix on the growth of some transformed cell lines observed here may be related to the findings reported by Folkman and Moscona (9). It is conceivable that, by adopting a more normal shape, transformed fibroblasts may also proliferate more like normal fibroblasts to a lower final density.

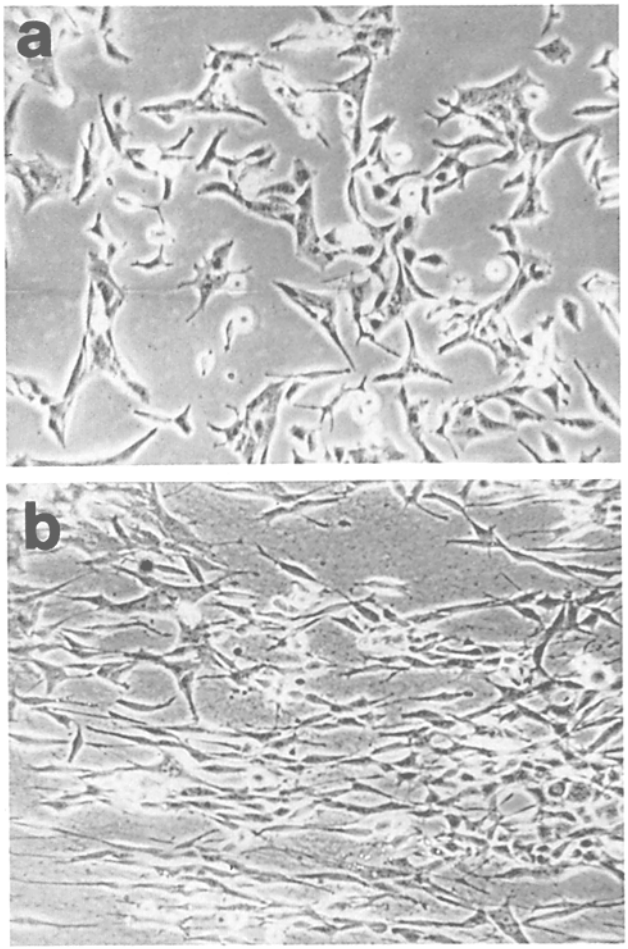

FIGURE 8 Benzo(a)pyrene-transformed rabbit bladder epithelial cell line, RBC-1, on (*a*) a plastic dish and (*b*) cell-free fibronectin matrices.

The lack of influence on the morphology of T and B lymphocytes and hemopoietic cells from bone marrow by FN-matrices may be expected since the shape of these cells is spherical, and the differentiation programs probably preclude them from spreading into spindle shapes. That nerve cells such as sympathetic neurons, which are able to send out long neurites, are also unresponsive to FN-matrices is more disappointing. Most intriguing, however, is the unresponsiveness of keratin-abundant epithelial cells to FN-matrix. It appears that the expression of abundant keratin in such cell types is related to the unresponsiveness to FN-matrix for bipolarization and alignment. Epithelial cells with a great reduction in keratin content will respond to FN-matrix and grow in parallel array. Greenburg and Hay (11) have reported recently that in three-dimensional, native collagen gels, certain adult and embryonic epithelial cells tested adopt an elongated shape, detach from epithelia explant, and migrate as individual cells. It would be of interest to determine the keratin content of these epithelial cells as well as their responsiveness to FN-matrix for elongation and alignment.

The more interesting finding of this work, perhaps, is the adaption of added cells to the FN-matrix, resulting in a spatial organization of cells and fibronectin fibers as if the matrix were produced by the added cells. Acellular areas abundant in extracellular matrices are frequently detected in histological sections of various tissues. During metastasis when a single or a clump of tumor cells reaches a distant site, the ability of tumor cells to adapt to the new environment by adopting some of the extracellular matrices as a "surrogate matrix" may influence the probability of its survival. To assume a cell shape similar to that of the neighboring normal cells and to mimic the cellular organization similar to that of the surrounding tissue may be advantageous to the tumor cells at the initial encounter. Only after further cell proliferation leading to a critical mass may the need for "surrogate matrix" then subside.

During tissue regeneration, the influence of pre-existing extracellular matrices on the repopulating cells is another instance where the "surrogate matrix" may be useful. Although in certain cases the components of extracellular matrices may be removed or destroyed before regeneration, in other cases the remaining matrices (often as basement membranes) may serve as a guidance for the regenerating cells to restore the same pattern of cellular organization.

The phenomenon of using "surrogate matrix" by migrating cells may also exist during embryogenesis. Matrices can serve not only as the "temporary surrogate matrix" during contact guidance, such as in the course of the development of neural crest cells demonstrated recently by Thiery and his co-workers (24), but also as a "permanent surrogate matrix" for cells undergoing terminal differentiation. Moreover, since the orientation and organization of "surrogate matrix" were determined by previous cells residing in this environment, the transfer of certain information (cell shape, cellular organization etc.) from the matrix-forming cell type to the matrix-using cell type may be carried out spontaneously. If the matrix is of stable nature, information transfer may even be operatable through a long time interval. The essence of time clock during embryogenesis may reside less on the so-called "intracellular program," and more on the cue encoded in the extracellular matrix, as repeatedly demonstrated by classical embryology.

We are most grateful to our colleagues, listed in the text, who generously provided various cell lines, cell types, and antisera. Scanning electron microscopy (Fig. 2) was kindly performed by Dr. Tom Wright, Harvard Medical School. Keratin content was determined by Dr. I. C. Summerhayes of this laboratory.

This work has been supported by a grant from National Cancer Institute, CA22659 to L. B. Chen. P. Hsieh was supported by a Muscular Dystrophy Association postdoctoral fellowship. L. B. Chen is the recipient of an American Cancer Society Faculty Research Award.

Received for publication 8 November 1982, and in revised form 17 January 1983.

REFERENCES

1. Ali, I. U., V. Mautner, R. Lanza, and R. O. Hynes. 1977. Restoration of normal morphology, adhesion and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. *Cell.* 11:115–126.
2. Bard, J. B. L., and E. D. Hay. 1975. The behavior of fibroblasts from the developing avian cornea: a study of cell movement *in vivo*. *J. Cell. Biol.* 67:400–418.
3. Birdwell, C. R., D. Gospodarowicz, and G. L. Nicolson. 1978. Identification, localization and role of fibronectin in cultured bovine endothelial cells. *Proc. Natl. Acad. Sci. USA.* 75:3273–3277.
4. Chen, L. B. 1977. Alteration in cell surface LETS protein during myogenesis. *Cell.* 10:393–400.
5. Chen, L. B., N. Maitland, P. H. Gallimore, and J. K. McDougall. 1977. Detection of the large external transformation sensitive protein on some epithelial cells. *Exp. Cell Res.* 106:39–46.
6. Chen, L. B., A. Murray, R. A. Segal, A. Bushnell, and M. L. Walsh. 1978. Studies on intercellular LETS glycoprotein matrices. *Cell.* 14:377–391.
7. Ebendal, T. 1977. Extracellular matrix fibrils and cell contacts in the chick embryo. *Cell Tissue Res.* 175:439–458.
8. Elsdale, T., and J. B. L. Bard. 1972. Collagen substrata for studies on cell behavior. *J. Cell. Biol.* 54:626–637.
9. Folkman, J., and A. Moscona. 1978. Role of cell shape in growth control. *Nature (Lond.).* 273:345–349.

10. Gospodarowicz, D., D. Delgado, and I. Vlodavsky. 1980. Permissive effect of the extracellular matrix on cell proliferation *in vitro*. *Proc. Natl. Acad. Sci. USA*. 77:4094–4098.
11. Greenburg, G., and E. D. Hay. 1982. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J. Cell Biol.* 95:333–339.
12. Hay, E. D. 1981. Cell Biology of Extracellular Matrix. Plenum Press, New York. 1–333.
13. Hedman, K., M. Kurkinen, K. Alitalo, A. Vaheri, S. Johansson, and M. Hook. 1979. Isolation of the pericellular matrix of human fibroblasts cultures. *J. Cell Biol.* 81:83–91.
14. Hsieh, P., R. Segal, and L. B. Chen. 1980. Studies of fibronectin matrices in living cells with fluoresceinated gelatin. *J. Cell Biol.* 87:14–22.
15. Hynes, R. O., G. S. Martin, M. Shearer, D. R. Critchley, and C. J. Epstein. 1976. Viral transformation of rat myoblast: effects on fusion and surface properties. *Dev. Biol.* 48:35–46.
16. Jaffee, E. A., and D. F. Mosher. 1978. Synthesis of fibronectin by cultured human endothelial cells. *J. Exp. Med.* 147:1779–1791.
17. Johnson, L. V., M. L. Walsh, and L. B. Chen. 1980. Localization of mitochondria in living cells with rhodamine 123. *Proc. Natl. Acad. Sci. USA*. 77:990–994.
18. Porter, K. R., and P. Vanamee. 1949. Observations on the formation of connective tissue fibers. *Proc. Soc. Exp. Biol. Med.* 71:513–534.
19. Rein, A., and H. Rubin. 1868. Effect of local cell concentrations upon the growth of chick embryo cells in tissue culture. *Exp. Cell Res.* 49:666–678.
20. Rovasio, R. A., A. Delouvee, K. M. Yamada, R. Timpl, and J. P. Thiery. 1983. Neural crest cell migration: requirements for exogenous fibronectin and high cell density. *J. Cell Biol.* 96:462–473.
21. Summerhayes, I. C., and L. B. Chen. 1982. Localization of a M_r 52,000 keratin in basal epithelial cells of the mouse bladder and expression throughout neoplastic progression. *Cancer Res.* 42:4098–4109.
22. Summerhayes, I. C., Y. S. E. Cheng, T. T. Sun, and L. B. Chen. 1981. Expression of keratin and vimentin intermediate filaments in rabbit bladder epithelial cells at different stages of benzo(a)pyrene-induced neoplastic progression. *J. Cell Biol.* 90:63–69.
23. Summerhayes, I. C., T. J. Lampidis, S. D. Bernal, J. J. Nadakavakaren, K. K. Nadakavakaren, E. L. Shepherd, and L. B. Chen. 1982. Unusual retention of rhodamine 123 by mitochondria in muscle and carcinoma cells. *Proc. Natl. Acad. Sci. USA*. 79:5292–5296.
24. Thiery, J. P., J. L. Duband, and A. Delouvee. 1982. Pathways and mechanism of avian trunk neural crest cell migration and localization. *Dev. Biol.* 93:324–343.
25. Tsen, S. D., R. Lee, B. Damiani, P. Hsieh, and L. B. Chen. 1980. F-actin inhibits protein kinase activity associated with the src-gene product. *Cold Spring Harbor Symp. Quant. Biol.* 44:967–974.
26. Vlodavsky, I., G. M. Lui, and D. Gospodarowicz. 1980. Morphological appearance, growth behavior and migratory activity of human tumor cells maintained on extracellular matrix versus plastic. *Cell.* 19:607–616.
27. Wartiovaara, J., E. Linder, E. Ruoslahti, and A. Vaheri. 1974. Distribution of fibroblast surface antigen: association with fibrillar structures of normal cells and loss upon viral transformation. *J. Exp. Med.* 140:1522–1533.
28. Weiss, P. 1961. Guiding principles in cell locomotion and cell aggregation. *Exp. Cell Res. Suppl.* 8:260–281.
29. Willingham, M. C., K. M. Yamada, J. Pouyssegur, and I. Pastan. 1977. Microfilament bundles and cell shape are related to adhesiveness to substratum and are dissociable from growth control in cultured fibroblasts. *Cell.* 10:375–380.
30. Yamada, K. M., K. Olden, and I. Pastan. 1978. Transformation sensitive cell surface protein: isolation, characterization, and role in cellular morphology and adhesion. *Ann. N. Y. Acad. Sci.* 312:256–277.
31. Yamada, K. M., S. S. Yamada, and I. Pastan. 1976. Cell surface protein partially restores morphology, adhesiveness and contact inhibition of movement to transformed fibroblasts. *Proc. Natl. Acad. Sci. USA*. 73:1217–1221.