*Proc. Natl. Acad. Sci. USA*
Vol. 93, pp. 4219–4223, April 1996
Cell Biology

# Myofibroblasts differentiate from fibroblasts when plated at low density

**(transforming growth factor β/cornea/integrins/fibroblasts/smooth muscle α-actin)**

S. K. MASUR†‡§, H. S. DEWAL†, T. T. DINH†, I. ERENBURG†, AND S. PETRIDOU†

Departments of †Ophthalmology and ‡Cell Biology/Anatomy, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029-6574

*Communicated by Salome G. Waelsch, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, December 26, 1995 (received for review September 29, 1995)*

**ABSTRACT** **Myofibroblasts, defined by their expression of smooth muscle α-actin, appear at corneal and dermal incisions and promote wound contraction. We report here that cultured fibroblasts differentiate into myofibroblasts by a cell density-dependent mechanism. Fibroblasts seeded at low density (5 cells per mm$^2$) produced a cell culture population consisting of 70–80% myofibroblasts, 5–7 days after seeding. In contrast, fibroblasts seeded at high density (500 cells per mm$^2$) produced cultures with only 5–10% myofibroblasts. When the myofibroblast-enriched cultures were subsequently passaged at high density, the smooth muscle α-actin phenotype was lost within 3 days. Furthermore, initially 60% of the low-density-cultured cells incorporated BrdUrd compared to 30% of cells passaged at high density. Media from myofibroblast-enriched cultures had more latent and active transforming growth factor β (TGF-β) than did media from fibroblast-enriched cultures. Although there was a trend towards increased numbers of myofibroblasts after addition of exogenous TGF-β, the results did not reach statistical significance. We conclude that myofibroblast differentiation can be induced in fibroblasts by plating at low density. We propose a cell density-dependent model of myofibroblast differentiation during wounding and healing in which at least two factors interact: loss of cell contact and the presence of TGF-β.**

Myofibroblasts are essential to wound contraction and healing (1). They differentiate from fibroblasts and are characterized by the presence of stress fibers containing the α-actin isoform that is expressed in smooth muscle (SM) cells (2–5). In the cornea, fibroblasts adjacent to an incision quickly become myofibroblasts (6). Myofibroblasts disappear with the completion of healing; the continuous presence of myofibroblasts is characteristic of abnormal healing as in fibrotic lesions (7).

In the normal cornea, quiescent fibroblasts are connected to one another via gap junctions (8, 9). Immediately after wounding, fibroblasts become activated, exhibit protein synthesis, and lose cell–cell contact (10, 11). Cell–cell connections are also disrupted when quiescent corneal fibroblasts are placed in culture and become activated. Cultured corneal fibroblasts have been used to model aspects of the wounded cornea. For example, we found that *de novo* expression *in situ* of the fibronectin receptor in corneal fibroblasts after wounding is mimicked by *de novo* expression of the fibronectin receptor in fibroblasts cultured from corneal fibroblasts (12).

We have used cultured corneal fibroblasts as a model system with which to examine mechanisms of differentiation during wound healing (13, 14). In the current study, we passaged corneal fibroblasts at low density (absent contact) or high density (immediate contact). We report that two factors interact for the conversion of fibroblasts to myofibroblasts: loss of cell–cell contact and action of transforming growth factor β (TGF-β). Thus, although TGF-β is necessary to this process, it is not sufficient.

## MATERIALS AND METHODS

**Preparation of Cultured Corneal Fibroblasts.** After removing the epithelium and endothelium from extirpated rabbit corneas, we prepared and passaged corneal fibroblasts as described previously and used them in passages 2–8 (12). For studies of cells passaged at low density or high density, cells were counted after trypsinization and passaged at $10^3$ cells per ml (5 cells per mm$^2$) or $10^5$ cells per ml (500 cells per mm$^2$), respectively, in a single well of a 24-well plate. For the comparable cell density in a 100-mm dish, we passaged $\approx 4 \times 10^4$ cells (low density) and $4 \times 10^6$ cells (high density), respectively. Cells were cultured in 1:1 (vol/vol) Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 medium containing 10% (vol/vol) fetal bovine serum (FBS) and antibiotics (100 units of penicillin, 100 μg of streptomycin, 0.25 μg of amphotericin B, and 50 μg of gentamicin per ml).

In one series of experiments, we used plasminogen-free FBS obtained by two passages of the serum through a lysine-Sepharose column (15). Plasminogen-free serum was tested for residual plasminogen (plasmin) in a chromogenic assay for plasmin after acid treatment (to inactivate plasmin inhibitors) supplemented with urokinase plasminogen activator (16).

**Immunocytochemistry.** We identified myofibroblasts by immunodetection of SM α-actin (17). The cells were fixed on coverslips with 3% *p*-formaldehyde in PBS containing 2% sucrose and permeabilized in 20 mM Hepes/300 mM sucrose/0.5% Triton/50 mM NaCl/3 mM $MgCl_2$, pH 7.4, for 3 min at 0°C (18); aldehyde-induced fluorescence was quenched with 50 mM $NH_4Cl$. After blocking nonspecific binding with 3% (vol/vol) normal serum, we incubated the cells with mouse monoclonal antibodies against SM α-actin and with rabbit polyclonal antibodies against vinculin (Sigma). Primary antibodies were visualized with rhodamine-labeled anti-mouse IgG and fluorescein-labeled anti-rabbit IgG. F-actin-containing stress fibers were visualized with rhodamine- or fluorescein-labeled phalloidin (Sigma). Coverslips were viewed with a Zeiss Axiomat microscope equipped for epifluorescence and differential interference contrast and were photographed on Kodak TMAX 3200 (exposed at 6300 ASA). Each experimental condition was repeated at least three times. To quantitate the number of myofibroblasts and fibroblasts in each experimental condition, we counted the SM α-actin-stained cells and the total number of cells per coverslip in at least three randomly chosen microscopic fields at ×50 (total cells, >100). Differences were analyzed by the two-tailed *t* test; results were considered significant if $P < 0.05$.

**Immunoblot Analysis.** Cells plated at low and high density in 100-mm diameter dishes were grown for 7 days and lysed in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TGF-β, transforming growth factor β; SM, smooth muscle; FNR, fibronectin receptor.

§To whom reprint requests should be addressed.

1% SDS sample buffer. Equal amounts of protein, determined with the Pierce micro-BCA (bicinchoninic acid) method, were separated by reducing electrophoresis on 10% polyacrylamide gels.

Proteins were transblotted to nitrocellulose membrane (Bio-Rad), and SM α-actin was detected by an isoform-specific mouse monoclonal antibody (Sigma), followed by anti-mouse IgG–alkaline phosphatase detection (Pierce). Nonmuscle calponin was detected with rabbit anti-acidic calponin [D. Applegate (19)], followed by anti-rabbit IgG–alkaline phosphatase treatment. Coomassie blue staining of companion gels was used to confirm that similar amounts of protein were loaded onto each lane.

**Cell Proliferation.** Either 1 or 18 hr before fixation, cells cultured on coverslips were incubated with BrdUrd. Incorporated BrdUrd was detected immunocytochemically with fluorescein isothiocyanate (FITC)-labeled anti-BrdUrd after DNA denaturation (4 M HCl for 30 min at room temperature) and viewed and quantitated as described above.

**Bioassay for TGF-β.** We used a specific nonradioactive bioassay for active TGF-β1, -β2, and -β3 based on the induction by TGF of a construct carrying the gene for plasminogen activator inhibitor 1 and the luciferase promoter (PAI-1/L) in Mink lung epithelial cells (MLECs) (20). MLECs stably transfected with PAI-1/L were plated in 96-well tissue culture dishes at $1.6 \times 10^4$ cells per well and allowed to attach (for 3 hr at 37°C under 95% air/5% $CO_2$). The medium was replaced with DMEM/F-12 containing 0.1% bovine serum albumin (BSA) and TGF-β (Becton Dickinson) at 5, 10, 25, 100, 250, or 500 pg/ml or replaced by test samples for 14 hr at 37°C. Test samples were media from high- or low-density cell cultures, cultured for up to 7 days in DMEM/F-12 containing 1% FBS. (Myofibroblast content of the cultures was verified by immunofluorescent detection of SM α-actin in fixed cells at day 7.) To measure total (latent plus active) TGF-β, an aliquot of each sample was heat-activated (80°C for 5 min) prior to testing. We assayed luciferase activity by using a kit (Analytical Luminescence Laboratory, San Diego) and standard curve generated with known concentrations of TGF-β.

**Exogenous TGF-β.** Cells were diluted for high- or low-density cell passage and suspended in DMEM/F-12 containing 1% FBS and 2 ng of TGF-β (Becton Dickinson) per ml. Furthermore, in two sets of experiments, the medium was replaced every other day with the same fresh medium.

## RESULTS

**Cell Density-Dependent Myofibroblast Differentiation.** When corneal fibroblasts were plated at low density (5 cells per $mm^2$), they required 7–10 days to reach confluence, at which time the majority of cells were found to be myofibroblasts. Fig. 1*A* depicts a representative culture, fixed at 7 days after low-density cell passaging: 80% of the cells were identified as myofibroblasts, based on SM α-actin expression. In contrast, Fig. 1*B* shows that the fibroblast phenotype was maintained when fibroblasts were passaged at confluence by splitting at 1:2 or 1:3 dilution, yielding 500 cells per $mm^2$ (21).

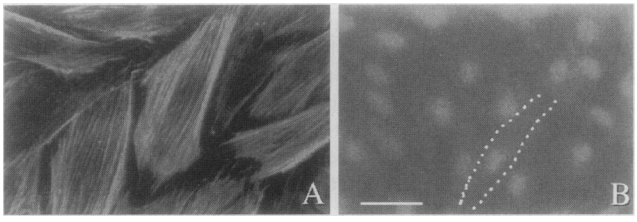


FIG. 1. Myofibroblasts predominate in cultures derived from cells passaged at low density. Immunocytochemical localization of SM α-actin in cultures 7 days after passage as low-density (*A*) or as high-density (*B*) cultures. Fibroblasts passaged at low density (*A*) have become myofibroblasts as indicated by SM α-actin staining of stress fibers in all of the cells in the field. These are lacking in the high-density cultures (*B*). In *B* the dotted lines indicate the borders of an individual fibroblast. (Bar = 50 μm.)

Cells at the high density seedings (500 cells per $mm^2$) reached confluence in 2–3 days. In two representative corneal fibroblast cultures fixed on days 3, 5, or 7 after high-density passaging, 3%, 0%, and 6% of the fibroblasts, respectively, were identified as myofibroblasts whereas 28%, 61%, and 80% were myofibroblasts after low-density passaging. Immunoblots of lysates of confluent cells demonstrated SM α-actin in the low-density cultures, but not in the high-density cultures (Fig. 2).

When cells were plated at an intermediate density, 50 cells per $mm^2$, the percent myofibroblasts in the culture was also intermediate—e.g., on day 5 70% of the cells stained for SM α-actin in a low-density culture, 35% in an intermediate-density culture, and 3% in a high-density culture.

We did not directly assess the role of growth factors present in FBS in the density-dependent myofibroblast differentiation. However, comparable percentages of SM α-actin-positive cells were seen in low-density cultures grown in DMEM/F-12 containing either 1% or 10% FBS when the cells were first allowed to attach in DMEM/F12 with 10% FBS for several hours prior to decreasing the concentration of FBS. It is unlikely that this is solely an effect on cell adhesion, since plating the cells on fibronectin-coated coverslips cannot substitute for the presence of 10% serum (data not shown).

Furthermore, the myofibroblast phenotype was maintained in cultures that were allowed to grow for 7 days past confluency (data not shown). Of interest, subsequent low-density plating of myofibroblast cultures perpetuated the myofibroblast phenotype: SM α-actin-positive cultures that arose from cells grown at low density in medium containing 10% FBS that were then replated at low density yielded a confluent culture in which SM α-actin expression remained high.

However, the myofibroblast phenotype is not a state of terminal differentiation. Cells of an SM α-actin-positive culture, subsequently trypsinized and plated at high density—e.g., 500 cells per $mm^2$—exhibited a dramatic decrease in SM α-actin expression in the resultant confluent cultures (Fig. 3). Thus, the fibroblast and myofibroblast phenotype continued to be dependent upon the cell density at plating.

Myofibroblasts also differentiated from noncorneal fibroblasts when they were plated as low-density cultures. In cultures grown for 5–7 days after low-density plating, myofi-

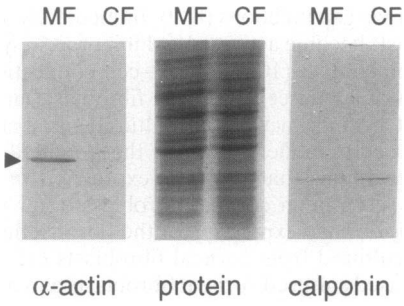


FIG. 2. Anti-SM α-actin detects a protein in lysates of myofibroblasts (lanes MF) but not fibroblasts (lanes CF) (arrowhead). In each lane, 20 μg of protein was electrophoresed and either stained with Coomassie blue or transferred to nitrocellulose and detected with anti-SM α-actin or anti-acidic calponin (nonmuscle calponin). No significant difference is seen in the pattern of protein from both lysates in the Coomassie blue-stained gel or in immunoblot detection of acidic calponin, a nonmuscle form of an actin-binding protein which is present in similar quantities in both lysates. In contrast, SM α-actin is detected in lysates of myofibroblasts (MF) but not fibroblasts (CF).

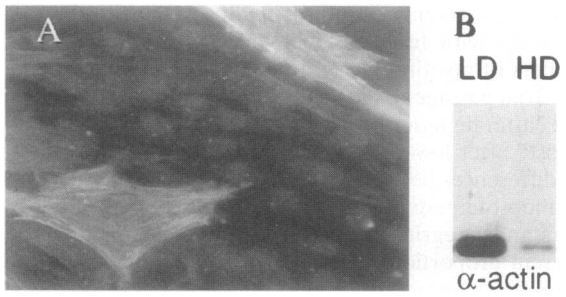


FIG. 3. The myofibroblast phenotype is not a terminal differentiation. Immunocytochemical and immunoblot detection of SM α-actin antibody. Myofibroblasts were passaged at high density and cultured for 5 days. (*A*) The majority of cells lack organized stress fibers and lack SM α-actin staining. The few SM α-actin-staining cells appear to be growing on top of the confluent fibroblasts. (*B*) Immunoblot (30 μg of protein per lane) of myofibroblasts lysed 7 days after low-density passage (lane LD) or passaged at high density and cultured for an additional 5 days as in *A* (lane HD). Immunodetectable SM α-actin was greatly decreased after high-density passage.

broblasts were the majority cell type in primary cultures of rabbit dermal fibroblasts, mouse corneal fibroblasts, mouse dermal fibroblasts, human breast fibroblasts, and rat 6 cloned fibroblasts (data not shown). Similarly SM α-actin-positive cells were the majority cell type 7–10 days after freshly isolated SM α-actin-negative breast fibroblasts were plated as single cells (22).

**Characteristics of Myofibroblasts.** Myofibroblasts were significantly larger than fibroblasts (Fig. 1, 50 μm vs. 25 μm wide,

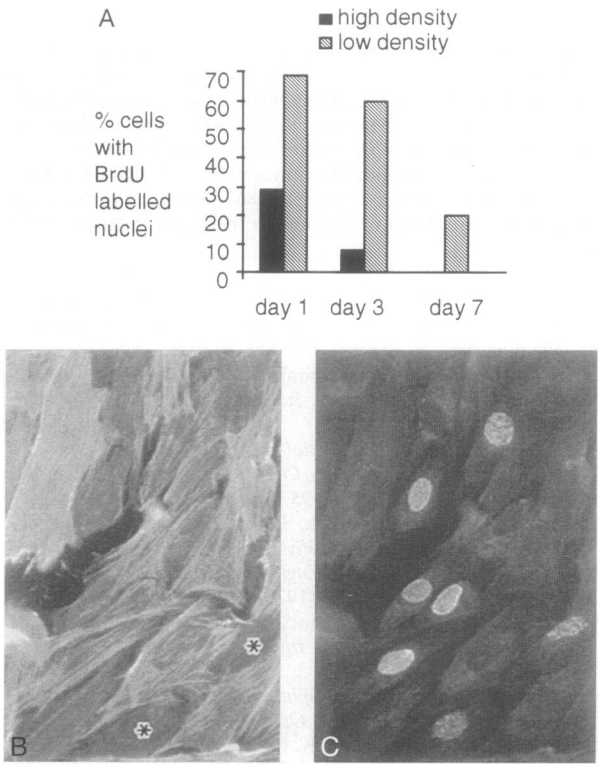


FIG. 4. Low-density cultures have actively proliferating cells. Incorporation of BrdUrd added to the cultures for 18 hr before fixation identifies actively proliferating cells in high- and low-density cultures (*A*). BrdUrd was detected by using anti-BrdUrd conjugated to fluorescein isothiocyanate (*B* and *C*). Cells from low-density culture were fixed 7 days after plating and double labeled for SM α-actin and BrdUrd incorporation. Whereas the majority of cells in the culture were myofibroblasts (*B*), two nonmyofibroblasts had BrdUrd-labeled nuclei (compare asterisks in *B* with *C*).

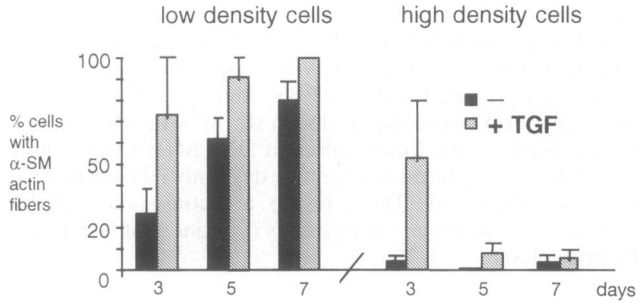


FIG. 5. Addition of exogenous TGF-β increased the number of cells staining for SM α-actin over non-TGF-β-treated cultures, but the increase was not significant ($P = 0.30$). Cells were plated in DMEM/F-12 containing 10% FBS, and then the medium was changed within 2 hr to DMEM/F-12 containing 1% FBS without (black bars) or with (hatched bars) the addition of TGF-β at 2 ng/ml. In the high-density cultures, the SM α-actin staining tended to be diffuse rather than restricted to microfilaments.

respectively) and contained more protein per cell (1.5–2.0 mg per $3 \times 10^6$ cells vs. 0.8–1.0 mg per $3 \times 10^6$ cells, respectively).

Although stress fibers of myofibroblasts stained for SM α-actin, both fibroblasts and myofibroblasts were immunocytochemically negative for desmin, basic (muscle) calponin, and SM-20, a vascular smooth muscle protein (data not shown) (23).

To determine the proliferative state of low-density and high-density cultures, we evaluated BrdUrd incorporation into nuclei. At 1, 3, and 7 days after plating, a larger proportion of cells in the low-density cultures incorporated BrdUrd (Fig. 4). In an apparent paradox, it was rare for a myofibroblast with strong fibrillar SM α-actin staining to have a nucleus labeled with BrdUrd. In fact, when they achieved confluence, neither fibroblasts nor myofibroblasts incorporated BrdUrd.

**TGF-β Effect.** Previous studies have shown that TGF-β can induce myofibroblast differentiation in dermal, breast, and corneal fibroblasts (24–27). We also found that addition of exogenous TGF-β (2 ng/ml) at the time of plating increased the number of myofibroblasts. For cells seeded at high density, the effect of TGF-β was greatest at day 3; however, at the end of 7 days there were few or no myofibroblasts, even when fresh TGF-β was added every other day. In contrast, exogenous TGF-β added to cells plated at low density enhanced the appearance of SM α-actin throughout the culture period (day 3 > day 5 > day 7) (Fig. 5). However, the increases were not significant ($P = 0.30$).

Because fibroblasts grown on plastic secrete TGF-β (28), we reasoned that autocrine TGF-β secretion might induce corneal myofibroblast differentiation. At day 3 after plating, we determined active TGF-β secretion, using a specific and sensitive bioassay (20). In two separate experiments, the low-density conditioned medium had active TGF-β of 100 pg/ml, whereas the high-density culture medium produced an activity of 5 or 25 pg/ml. (Total TGF-β, measured after heat activation of the medium, was also higher in conditioned medium from cells plated at low density compared with high-density plating: 250 pg/ml, compared with 25 or 50 pg/ml, respectively.) Thus, although active TGF-β is present in the medium of cells plated at high density, it is not effective at inducing myofibroblast formation under these conditions. This is consistent with the lack of myofibroblast differentiation in high density cultures after the addition of exogenous TGF-β described above.

As indicated above, TGF-β is secreted in a latent form that is activated *in vivo* by proteolysis (29). Many proteases, including plasmin and its precursor, plasminogen, are found in FBS. Plasminogen activator, found in culture medium, activates the serum plasminogen, which can then activate TGF-β (30). To reduce TGF-β activation in one set of experiments, we plated

and grew cells at low density in medium prepared with plasminogen-depleted FBS (1% FBS*). In addition we added aprotinin, a protease inhibitor. After 7 days, the cultures grown in medium prepared with 1% FBS* and aprotinin contained 30% myofibroblasts compared with the 85% in DMEM/F-12 containing 1% FBS. Furthermore, if DMEM/F-12 containing 1% FBS* was replaced every other day, only 20% of the cells were myofibroblasts. These results are consistent with SM $\alpha$-actin induction by an endogenous cytokine that is activated by proteolysis.

## DISCUSSION

The major finding of the present study is that fibroblast populations can be directed into either the myofibroblast or fibroblast phenotype by the density at which they are cultured. Myofibroblasts have, as their signature, the expression of SM $\alpha$-actin. Myofibroblasts are not terminally differentiated but can differentiate into fibroblasts upon rapid establishment of cell–cell contact after passage under dense conditions.

TGF-$\beta$ has been implicated as a regulatory agent in switching between fibroblasts and myofibroblasts. In the dermis, myofibroblasts differentiate from fibroblasts after infusion of active TGF-$\beta$ *in situ* (24). In the normal cornea, there is immunocytochemical evidence for several TGF-$\beta$ isoforms (31, 32), and TGF-$\beta$ mRNA appears at corneal wound margins (33). We found that sufficient TGF-$\beta$ is secreted and activated by cultured corneal fibroblasts to induce myofibroblast differentiation.

Our data suggest that the absence of cell–cell contact is the proximate cause of myofibroblast differentiation. Thus, TGF-$\beta$ added to or produced by high-density cells did not cause the fibroblast to myofibroblast differentiation. One hypothesis that would explain our results is that cell–cell contact signals a decrease in the number of cell surface TGF-$\beta$ receptors (34). In any case, it seems likely that TGF-$\beta$ is necessary, but not sufficient, for myofibroblast formation, and that cell–cell contact is an overriding influence. Different regulatory mechanisms are likely for vascular smooth muscle cells in which cell–cell contact promotes the expression of SM $\alpha$-actin expression (35).

In addition to growth factors, the specific matrix composition may have inductive effects. For example, myofibroblasts differentiate from hepatic lipocytes seeded on the EIIIA isoform of fibronectin, an isoform found after hepatic wounding (36). Similarly, since in high-density cultures when we found myofibroblasts they were growing on top of a layer of fibroblasts, we hypothesize that the fibroblast's topography and/or secreted matrix may be capable of inducing myofibroblast differentiation (37).

There is considerable evidence that the myofibroblast is a contractile cell (1, 4). In parallel with the SM $\alpha$-actin expression, the higher expression of integrins is consistent with the greater adhesion of myofibroblasts to matrix protein and with the provision of more insertion sites for the actin-based contractile system of myofibroblasts (26). Also the ability of myofibroblasts to produce tissue contraction could be greatly enhanced by the insertion of actin in cell–cell adhesion molecules (e.g., cadherins). We have found along with SM $\alpha$-actin expression, actin-associated cell junctional proteins (cadherins) are synthesized and localized to myofibroblast cell–cell borders. In contrast, in fibroblasts, the gap junctional protein connexin43 is found in the fibroblast cell–cell junctions (38). Taken together, the *de novo* synthesis in myofibroblast cultures of SM $\alpha$-actin (a contractile form of actin) and the increased expression of cadherins, of integrins, and of matrix are consistent with a role of myofibroblasts in promoting the contraction of wounds (6, 39, 40).

Our finding of density-dependent myofibroblast differentiation has ramifications for the interpretation of experiments using subconfluent "fibroblast" cultures. Specifically, starting a passage with few cells will produce a culture with high numbers of myofibroblasts. In addition, the same "line" may vary from passage to passage. The myofibroblast phenotype is "lost" after high-density passage, and the fibroblast phenotype is "lost" after low-density passage. Since there are physiological differences between myofibroblasts and fibroblasts, (e.g., metalloproteinase secretion patterns, response to phorbol esters and integrin expression), assay of cultures containing different proportions of these two populations could provide conflicting results (26).

These findings have physiological significance for corneal wound healing. Plating cells at very low density mimics the situation in the cornea after wounding, a condition in which cell density is decreased by cell damage, infection, or inflammation (41–44). Microscopically, after corneal wounding, stromal fibroblasts are rounded, suggesting retraction of their cytoplasmic extensions and loss of cell–cell contact (11). We hypothesize that by plating the cells at low density, we have reproduced the disconnection of one corneal fibroblast from another. This allows myofibroblast differentiation in response to TGF-$\beta$. After wounding, both corneal epithelium and stroma are candidates for local TGF-$\beta$ secretion and activation (33, 45, 46). We suggest that, in normal healing, reestablishment of cell–cell contact is similar to culture at high density. In this regard, the rapid turnover of SM $\alpha$-actin mRNA may facilitate rapid switching of phenotypes (47). Further study will be required to evaluate the contribution of several factors, including cell–matrix interaction, the role of integrins in signaling, the role of junctional molecules in cell–cell adhesion and communication, and the impact of cytokines and growth factors in addition to TGF-$\beta$ (28, 48, 49).

Antibodies to acidic (nonmuscle) calponin were supplied by Dr. Dianne Applegate, New York Blood Center (New York). We thank S. Antohi, J. Weissberg, and A. Dokun for excellent laboratory help and Drs. C. H. Damsky, R. Majeska, L. Ossowski, and V. Schuster for stimulating discussions. This research was supported by the National Eye Institute of the National Institutes of Health (RO1-EY09414 to S.K.M. and a Core Center Grant EY01867) and an unrestricted grant from Research to Prevent Blindness to the Department of Ophthalmology.

1. Skalli, O. & Gabbiani, G. (1988) in *The Molecular and Cellular Biology of Wound Repair*, eds. Clark, R. A. F. & Henson, P. M. (Plenum, New York), pp. 373–401.
2. Darby, I., Skalli, O. & Gabbiani, G. (1990) *Lab. Invest.* **63,** 21–29.
3. Sappino, A. P., Schürch, W. & Gabbiani, G. (1990) *Lab. Invest.* **63,** 144–161.
4. Grinnell, F. (1994) *J. Cell Biol.* **124,** 401–404.
5. Rønnov-Jessen, L., Petersen, O. W., Koteliansky, V. E. & Bissell, M. J. (1995) *J. Clin. Invest.* **95,** 859–873.
6. Jester, J. V., Petroll, W. M., Barry, P. A. & Cavanagh, H. D. (1995) *Invest. Ophthalmol. Visual Sci.* **36,** 809–819.
7. Friedman, S. L. (1993) *N. Engl. J. Med.* **328,** 1828–1835.
8. Hasty, D. L. & Hay, E. D. (1977) *J. Cell Biol.* **72,** 667–686.
9. Jester, J. V., Barry, P. A., Lind, G. J., Petroll, W. M., Garana, R. & Cavanagh, H. D. (1994) *Invest. Ophthalmol. Visual Sci.* **35,** 730–743.
10. Weimar, V. (1957) *Am. J. Ophthalmol.* **44** (Oct., pt. 2), 173–182.
11. Nakayasu, K. (1988) *Jpn. J. Ophthalmol.* **32,** 113–125.
12. Masur, S. K., Cheung, J. K. H. & Antohi, S. (1993) *Invest. Ophthalmol. Visual Sci.* **34,** 2690–2698.
13. Lauweryns, B., Van den Oord, J. J., Volpes, R., Foets, B. & Missotten, L. (1991) *Invest. Ophthalmol. Visual Sci.* **32,** 2079–2085.
14. Masur, S. K., Idris, A., Michelson, K., Antohi, S., Zhu, L.-X. & Weissberg, J. D. (1995) *Invest. Ophthalmol. Visual Sci.* **36,** 1837–1846.
15. Deutsch, D. G. & Mertz, E. T. (1970) *Science* **170,** 1095–1096.
16. Busso, N., Masur, S. K., Lazega, D., Waxman, S. & Ossowski, L. (1994) *J. Cell Biol.* **126,** 259–270.

17. D'Alessio, M., Ramirez, F., Suzuki, H. R., Solursh, M. & Gambino, R. (1990) *J. Biol. Chem.* **265,** 7050–7054.
18. Zambruno, G., Marchisio, P. C., Melchiori, A., Bondanza, S., Cancedda, R. & De Luca, M. (1993) *J. Cell Sci.* **105,** 179–190.
19. Applegate, D., Fang, W., Green, R. S. & Taubman, M. B. (1994) *J. Biol. Chem.* **269,** 10683–10690.
20. Abe, M., Harpel, J. G., Metz, C. N., Nunes, I., Loskutoff, D. J. & Rifkin, D. B. (1994) *Anal. Biochem.* **216,** 276–284.
21. Freshney, R. I. (1987) *Culture of Animal Cells: A Manual of Basic Technique* (Liss, New York).
22. Rønnov-Jessen, L., van Deurs, B., Celis, J. E. & Petersen, O. W. (1990) *Lab. Invest.* **63,** 532–543.
23. Wax, S. D., Rosenfield, C. L. & Taubman, M. B. (1994) *J. Biol. Chem.* **269,** 13041–13047.
24. Desmoulière, A., Geinoz, A., Gabbiani, F. & Gabbiani, G. (1993) *J. Cell Biol.* **122,** 103–111.
25. Rønnov-Jessen, L. & Petersen, O. W. (1993) *Lab. Invest.* **68,** 696–707.
26. Dewal, H. S., Dinh, T. T., Erenburg, I. & Masur, S. K. (1994) *Mol. Biol. Cell* **5,** 59a (abstr.).
27. Barry, P. A., Cavanagh, H. D., Petroll, W. M. & Jester, J. V. (1994) *Mol. Biol. Cell* **5,** 464a (abstr.).
28. Streuli, C. H., Schmidhauser, C., Kobrin, M., Bissell, M. J. & Derynck, R. (1993) *J. Cell Biol.* **120,** 253–260.
29. Roberts, A. B. & Sporn, M. B. (1990) in *Peptide Growth Factors and Their Receptors*, eds. Sporn, M. B. & Roberts, A. B. (Springer, Berlin), pp. 419–472.
30. Lopez-Alemany, R., Mirshahi, S., Faure, J. P., Pouliquen, Y., Burtin, P. & Mirshahi, M. (1995) *Fibrinolysis* **9,** 223–229.
31. Pasquale, L. R., Dorman-Pease, M. E., Lutty, G. A., Quigley, H. A. & Jampel, H. D. (1994) *Invest. Ophthalmol. Visual Sci.* **34,** 23–30.
32. Wilson, S. E., Schultz, G. S., Chegini, N., Weng, J. & He, Y.-G. (1994) *Exp. Eye Res.* **59,** 63–72.
33. Trinkaus-Randall, V., Brown, C., Nugent, M. & Latkany, R. (1994) *Mol. Biol. Cell (Suppl.)* **5,** 308a (abstr.).
34. Rizzino, A., Kazakoff, P., Ruff, E., Kuszynski, C. & Nebelsick, J. (1988) *Cancer Res.* **48,** 4266–4271.
35. Owens, G. K., Loeb, A., Gordon, D. & Thompson, M. M. (1986) *J. Cell Biol.* **102,** 343–352.
36. Jarnagin, W. R., Rockey, D. C., Koteliansky, V. E., Wang, S.-S. & Bissell, D. M. (1994) *J. Cell Biol.* **127,** 2037–2048.
37. Chou, L., Firth, J. D., Uitto, V.-J. & Brunette, D. M. (1995) *J. Cell Sci.* **108,** 1563–1573.
38. Petridou, S. & Masur, S. K. (1996) *Invest. Ophthalmol. Visual Sci.*, in press.
39. Montesano, R. & Orci, L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4894–4897.
40. Jester, J. V., Petroll, W. M., Barry, P. A. & Cavanagh, H. D. (1995) *J. Anat.* **186,** 301–311.
41. Jester, J. V., Petroll, W. M., Feng, W., Essepian, J. & Cavanagh, H. D. (1992) *Invest. Ophthalmol. Visual Sci.* **33,** 3255–3270.
42. Awata, T., Nishida, T., Nakagawa, S. & Manabe, R. (1989) *Jpn. J. Opthalmol.* **33,** 132–143.
43. Berman, M. B. (1993) *Cornea* **12,** 420–432.
44. Frangieh, G. T., Hayashi, K., Teekhasaenee, C., Wolf, G., Colvin, R. B., Gipson, I. K. & Kenyon, K. R. (1989) *Arch. Ophthalmol.* **107,** 567–671.
45. Berman, M., Leary, R. & Gage, J. (1980) *Invest. Ophthalmol. Visual Sci.* **20,** 1204–1221.
46. Khaw, P. T., Schultz, G. S., MacKay, S. L. D., Chegini, N., Rotatori, D. S., Adams, J. L. & Shimizu, R. W. (1992) *Invest. Ophthalmol. Visual Sci.* **33,** 3302–3306.
47. Kumar, C. C., Bushel, P., Mohan-Peterson, S. & Ramirez, F. (1992) *Cancer Res.* **52,** 6877–6884.
48. Damsky, C. H. & Werb, Z. (1992) *Curr. Opin. Cell Biol.* **4,** 772–781.
49. Greenwel, P., Iraburu, M. J., Reyes-Romero, M., Meraz-Cruz, N., Casado, E., Solis-Herruzo, J. A. & Rojkind, M. (1995) *Lab. Invest.* **72,** 83–91.