BioTechniques Rapid Dispatches

Benchmarks

Adhesive-tape soft lithography for patterning mammalian cells: application to wound-healing assays

Anil B. Shrirao[1], Ali Hussain[2], Cheul H. Cho[2], and Raquel Perez-Castillejos[2]
[1]Department of Electrical and Computer Engineering, and [2]Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ, USA

This paper introduces a benchtop method for patterning mammalian cells—i.e., for culturing cells at specific locations—on planar substrates. Compared with standard cell culture techniques, which do not allow the control of what areas of a monolayer are populated by one type of cell or another, techniques of cell patterning open new routes to cell biology. Researchers interested in cell patterning, however, are oftentimes hindered by limited access to photolithographic capabilities. This paper shows how cells can be patterned easily with sub-millimeter precision using a non-photolithographic technique that is based on the use of office adhesive tape and poly(dimethylsiloxane) (PDMS). This method is fast (~4 h to go from a layout to have the cells patterned in the shape of such layout) and only requires materials and tools readily available in a conventional biomedical laboratory. A wound-healing assay is presented here that illustrates the potential of the technique (which we call tape-based soft lithography) for patterning mammalian cells and studying biologically significant questions such as collective cellular migration.

Keywords: tape; soft lithography; clean room-free; cell patterning; collective cell migration; microvascular; endothelial cell; MVEC

We describe the use of adhesive tape as a mold to fabricate poly(dimethylsiloxane) (PDMS) compartments with sub-millimeter dimensions that we ultimately employed for patterning primary rat heart microvessel endothelial cells (MVECs) on a culture-treated Petri dish in order to perform a wound healing assay. Compared with conventional techniques of cell culture, cell patterning (see References 1 and 2 for reviews of cell patterning techniques) makes it possible to select the areas of a culture dish that are populated by one type of cell or another or by no cells. The ability to control the spatial organization of mammalian cells has opened new routes for tissue engineering (3,4), for the study of intracellular mechanisms in single cells (5,6), intercellular communication in patterned co-cultures (7,8), and the development of cell-based sensors (9,10).

For patterning cells, researchers can opt for microfabrication techniques (namely micropatterning) capable to produce patterns with dimensions similar to those of biological cells (1–10); that is, down to a few micrometers. Micropatterning, however, requires the use of the photolithographic equipment typically available in a clean room, which limits its use by researchers who lack an expertise in microfabrication. Alternatively, a number of non-photolithographic approaches (for a review see Reference 11) have been proposed to pattern cells that do not require access to photolithography. Most of these clean room-free approaches involve *(i)* preparing a mold (normally called master) by non-photolithographic means, and *(ii)* casting PDMS to form a replica of the master—this later step is known as soft lithography (12). Reported non-photolithographic methods to fabricate masters include transferring the layout of the master onto a transparency with a photocopier (13), with a printer of wax (14) or toner (15), onto a thermoplastic polymer (Shrinky Dinks; 16) or a printed circuit board (17). The use of these methods for patterning cells, however, is limited in some cases because (13–15) the masters are too shallow—less than ~15 μm—and the resulting replicas do not allow cells to flow along such shallow cavities; in other cases (16,17), the materials required for producing the master are not commonly found in conventional biomedical laboratories.

We recently demonstrated that office adhesive tape, patterned by hand with a blade to the required shape, produced masters for soft lithography (18) quickly (~30 min), inexpensively (~$1 per master), readily (only materials and tools typically found in biomedical laboratories are required), and reproducibly (more than 50 PDMS replicas have been obtained from one single adhesive-tape master). In this paper, we show that soft-lithographic

Method summary:

This paper introduces a benchtop method for patterning mammalian cells that does not require access to photolithographic capabilities. This paper shows how cells can be patterned easily with sub-millimeter precision using a non-photolithographic technique that is based on the use of office adhesive tape and PDMS. This method is fast, biocompatible, reliable, safe, inexpensive, and suitable for biomedical researchers, as it only requires materials and tools commonly found in a biomedical laboratory. We believe this tape-based soft lithography can empower biologically oriented researchers to produce their own microfluidic devices, freeing them from the need to use a clean room.

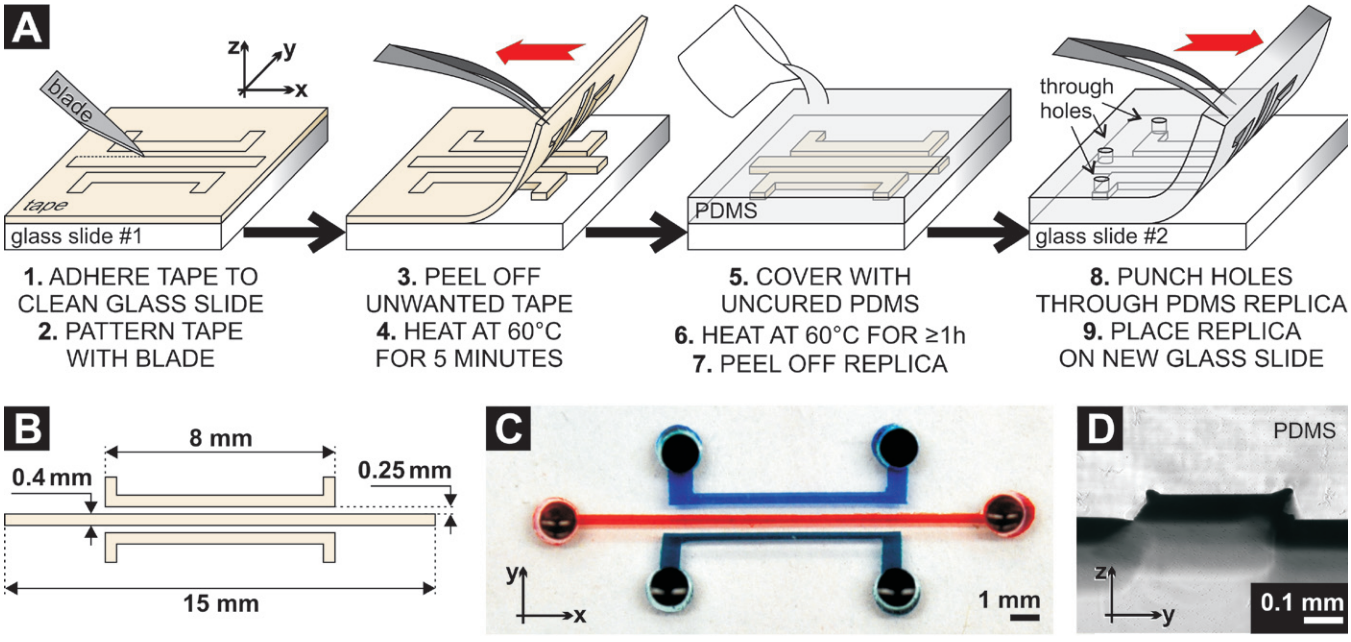

Figure 1. Microfluidic compartments made by casting PDMS on patterned adhesive tape. (A) Sequence of steps to prepare a master with adhesive tape (steps 1–4) and subsequently, to fabricate a system of microfluidic compartments by replicating the tape-based master with PDMS (steps 5–9). (B) Layout of the system of three adjacent compartments used here to pattern mammalian cells. Photos of the three-compartment system after fabrication: (C) top view, with inks filling the compartments for ease of visualization, and (D) cross-sectional view of the central compartment.

replicas of adhesive-tape masters can be used for patterning mammalian cells on standard polystyrene cell culture-treated Petri dishes. Specifically, we describe the fabrication of tape-based masters and their replication. We used the PDMS replicas to pattern MVECs on Petri dishes for studying their collective migration. The pattern of cells consisted of three adjacent, albeit separated, areas populated with MVECs—these areas are schematized in yellow in Figure 1B.

In order to restrict the regions of the Petri dish where cells would be allowed to attach, we created a system of PDMS compartments with the same planar dimensions as the desired cell-covered areas. The PDMS compartments were prepared by casting PDMS in a mold (master) made of adhesive tape. Briefly, we attached a layer of adhesive tape (Scotch 3650, 3M, St. Paul, MN, USA) on a glass slide (Figure 1A, step 1; Fisher Scientific, Morris Plains, NJ, USA) and then, using a blade (Fisher Scientific), patterned the adhesive tape in the shape chosen for the areas that will be ultimately covered by cells (Figure 1A, step 2). The tape surrounding the chosen layout was removed with tweezers (Figure 1A, step 3) and the resulting construct of patterned tape attached to the glass slide was rinsed with isopropanol (to remove any residues of adhesive) and then placed in an oven at 65°C for ~5 min to strengthen the adhesion of the tape to the slide (Figure 1A, step 4).

We covered the tape-and-glass master with PDMS prepolymer (Sylgard 184, Dow Corning Corp., Midland, MI, USA) to start the process of soft lithography (Figure 1A, step 5). After curing the PDMS (covering the master) for at least 1 h in an oven at 65°C, the replica was separated from the master and holes were pierced at both ends of each of the three compartments using a puncher (Figure 1A, steps 6–8; Ted Pella Inc., Redding, CA, USA). Finally, the PDMS replica, with the grooves facing down, was laid on a Petri dish (Figure 1A, step 9; Fisher Scientific) and pressure was applied gently to the replica with the fingertips in order to ensure its conformal adhesion to the dish. As a result, the grooves in the PDMS replica were closed by the Petri dish and the compartments became accessible only via the through holes opened in step 8. The compartments could then be filled with liquids, which did not leak among adjacent compartments (Figure 1C). The height of the compartments fabricated with this technique is determined by the thickness of the adhesive tape for the master— the tape we used in this study (Scotch Moving and Storage Tape, Cat. No. 3650) was ~60 µm thick (Figure 1D). For fabricating taller compartments one can prepare a taller master using *(i)* a thicker tape or *(ii)* several layers of thinner tape. (See Supplementary Materials for a detailed protocol of the fabrication of the tape-based master and its replication by soft lithography.)

We conformally adhered the PDMS replica (Figure 1B) to a Petri dish and filled the three resulting compartments with a suspension of primary rat heart MVECs (VEC Technologies, Rensselaer, NY, USA); this procedure was similar to that in Figure 1C although here we used a cell suspension instead of the colored inks of Figure 1C. MVECs were delivered into the PDMS compartments (Figure 2B) at a concentration of 25×10^6 cells/mL, which resulted in a surface cell density of 1,500 cells/mm^2 after gravity seeding; the surface cell density is the product of the volumetric cell concentration times the height of the compartment: $(25 \times 10^6$ cells/mL$) \times 60$ µm = 1,500 cells/mm^2. This concentration of the suspension was chosen so that, after gravity seeding for only 2 h in a 5% CO_2 humidified incubator, MVECs reached confluence in the areas of the dish under the PDMS compartments (Figure 2A). Two hours after delivering the cell suspension into the compartments, the PDMS replicas were gently peeled off from the Petri dishes, which were then kept in the incubator for up to 96 h (4 days).

Collective cell migration is essential in multiple physiopathological mechanisms, including organogenesis, cancer metastasis, and regeneration and wound healing (19). The conventional approach to study collective cell migration is known as the scratch wound healing assay in which a cellular monolayer is "wounded"

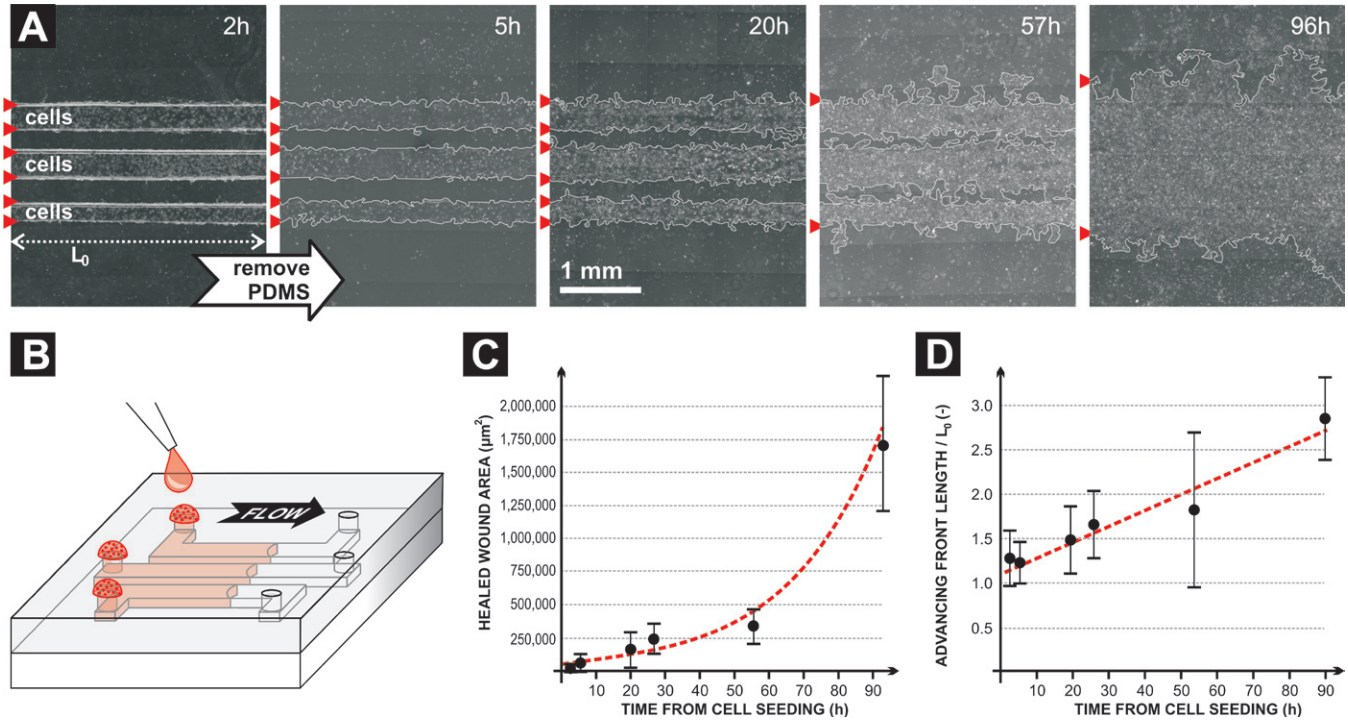

Figure 2. Wound healing assay prepared by adhesive tape-based soft lithography. (A) Sequence of phase-contrast images of the wound healing assay at different times. Red arrowheads indicate the advancing fronts that were analyzed in each time point: 6 advancing fronts at 2, 5, 20, 27, and 57 h; 2 advancing fronts at 96 h, when the smallest gaps had already disappeared. (B) Schematic describing the process to deliver a cell suspension to the PDMS compartments. (C) Progression of healed wound areas with time. (D) Ratio of advancing front length to initial front length (L_0) as a function of time. In (C) and (D), error bars indicate the SD for the measurements performed on multiple advancing fronts and associated healed wound areas: 12 advancing fronts (6 advancing fronts in 2 independent assays) were analyzed at 2, 5, 20, 27, and 57 h; 4 advancing fronts were analyzed at 96 h (2 advancing fronts in 2 independent assays). Time $t = 0$ is taken at the time of cell seeding.

by scratching it with a sharp instrument that removes the cells from the area of the scratch (20). Cells are then observed as they proceed to "heal" the wound by recoating the gap left by the scratch. The significance of the scratch wound healing assay, however, is limited in that the scratch *(i)* damages the substrate and *(ii)* ruptures cells that then release their intracellular content, which may interfere with the process of wound healing. Alternatively, in the barrier wound healing assay, a block of material (e.g., hydrogel or PDMS) is temporarily adsorbed to the substrate of cell culture before seeding the cells (21). The block acts as a mask to cell attachment by preventing cells from accessing the area to which the block is adsorbed. Upon removal of the adsorbed block, the cell monolayer is presented with a gap that resembles a wound in need of healing. The use of microfabrication and soft lithography has made it possible to prepare cell masking barriers (21) with high precision (in the range of the few hundreds of microns required for the wound healing assay) and repeatability (as tens of replicas can be obtained from a single master). Here, we demonstrate that the use of a master made of hand-patterned adhesive tape makes it possible to achieve the precision and repeatability required in barrier wound healing assays while avoiding the need for photolithography and access to a clean room.

Upon removal of the PDMS replica from the Petri dish (2 h after seeding), the MVECs appeared patterned in three elongated, adjacent islands (Figure 2A). We took images of the "wounded" area at several times during the healing process and analyzed them using the public domain software ImageJ (22). Shortly after removing the PDMS barriers, the MVECs started migrating from the patterned islands into the gaps. The only cue required by the MVECs to start migrating was their exposure to the gaps; this observation agrees with that made by van Horssen and colleagues (23). We determined the "healed wound" areas—that is, the areas of the initially cell-free gaps that eventually became covered with cells—using ImageJ. The increase of healed wound areas with time was found to follow an exponential law (Figure 2C, $R2 = 0.9102$), which indicates that cells did not stop their healing activity after closing the smaller gaps but they accelerated their invasion of the remaining free areas. Cellular migration was not uniform along the borders of the cellular islands but roughening of the borders was observed, with multiple fingering protrusions (also called fingers or digitations; 21) emerging from the cellular advancing front and extending toward the cell-free areas. We quantified the evolution of the fingering destabilization of the borders by comparing the length of each advancing front (L; indicated by red arrowheads in Figure 2A), including the perimeter of its fingers, at a given time with the initial length of the front, L0, as defined in Figure 2A. (For example, if 5 fingers—approximated as rectangles of width = L0/10 and height = L0/5—appear along an advancing front, the actual length of the advancing front will be L = L0+10(L0/5) = 3L0, which results in a ratio L:L0 = 3.) We observed that the ratio of the advancing front length to L0 increases linearly with time ($R2 = 0.9454$). The linear correlation corresponds to a sustained increase of fingering with time (Figure 2D). Notably, the formation of fingers at the edges does not disrupt the cohesion of the cellular monolayers. Others have noted that the pulling forces created by the cells leading the movement

of the fingers are transmitted to the rest of the cells in the monolayer through strong cell-cell adhesions (24). The good agreement we found between the results of our wound healing assay and those of others (21, 23) strongly supports our thesis that tape-based masters mimic the potential of other barrier wound healing assays (including photolithographic ones) in the study of cellular collective migration.

We have shown that a 60 μm-thick adhesive-tape master was capable of producing PDMS replicas that we ultimately used for patterning MVECs. Compared with other methods for patterning cells, the tape-based soft lithographic method we describe here presents a distinct collection of advantages. It is *(i)* fast—requiring ~4 h to pattern the cells, which includes ~30 min to prepare the master, ~1 h to cure the PDMS replica, ~30 min to prepare the compartments and seed the cells in them, and ~2 h to allow that the cells attach to the substrate; *(ii)* biocompatible—retaining the viability and functional activity of cells after patterning, as shown by the motility of MVECs right after removing the PDMS barriers in the wound healing assays; *(iii)* reliable—producing more than 50 replicas from a single tape master; *(iv)* simple—entailing no previous knowledge of microfabrication by the user; *(v)* safe—not involving the use of harmful chemicals when used as directed; *(vi)* inexpensive—costing less than $1 for the master and a PDMS replica; and *(vii)* suitable for biomedical researchers—necessitating only materials and tools commonly found in a biomedical laboratory. Construction of a tape-based master may be limited by the manual dexterity of the user with a handheld scalpel. This limitation, however, can be circumvented by utilizing a laser or a craft cutter to pattern the tape. The potential of this technique for biological studies was informed here by the development of a wound healing assay to study the collective migration of MVECs on standard polystyrene cell culture-treated Petri dishes. Our observations of the collective migration of MVECs matched well those published previously by other researchers. In conclusion, we believe that tape-based soft lithography can empower biologically oriented researchers to produce their own PDMS replicas and microfluidic devices, freeing them from the need to fabricate their masters by photolithography in a clean room.

Acknowledgments

This work was performed at the Perez-Castillejos' Tissue Models Laboratory and Cho's Stem Cells and Tissue Engineering Laboratory, at the New Jersey Institute of Technology (NJIT). The authors want to thank Dr. Eric T. Mack for critically reviewing the manuscript. This work was supported by NJIT startup funds.

Competing interests

The authors declare no competing interests.

References

1. **Fink, J., M. Théry, A. Azioune, R. Dupont, F. Chatelain, M. Bornens, and M. Piel.** 2007. Comparative study and improvement of current cell micro-patterning techniques. Lab Chip *7*:672-680.
2. **Goubko, C.A. and X. Cao.** 2009. Patterning multiple cell types in co-cultures: A review. Mater. Sci. Eng. C *29*:1855-1868.
3. **McBeath, R., D.M. Pirone, C.M. Nelson, K. Bhadriraju, and C.S. Chen.** 2004. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment several studies have noted that changes in cell shape themselves can alter the differentiation of precommitted mesenchymal lineages. Dev. Cell *6*:483-495.
4. **Yuan, B., Y. Jin, Y. Sun, D. Wang, J. Sun, Z. Wang, W. Zhang, and X. Jiang.** 2012. A strategy for depositing different types of cells in three dimensions to mimic tubular structures in tissues. Adv. Mater. *24*:890-896.
5. **Théry, M., A. Jiménez-Dalmaroni, V. Racine, M. Bornens, and F. Jülicher.** 2007. Experimental and theoretical study of mitotic spindle orientation. Nature *447*:493-496.
6. **Khatau, S.B., C.M. Hale, P.J. Stewart-Hutchinson, M.S. Patel, C.L. Stewart, P.C. Searson, D. Hodzic, and D. Wirtz.** 2009. A perinuclear actin cap regulates nuclear shape. Proc. Natl. Acad. Sci. USA *106*:19017-19022.
7. **Cho, C.H., J. Park, A.W. Tilles, F. Berthiaume, M. Toner, and M.L. Yarmush.** 2010. Layered patterning of hepatocytes in co-culture systems using microfabricated stencils. BioTechniques *48*:47-52.
8. **Kim, J., M. Hegde, and A. Jayaraman.** 2010. Co-culture of epithelial cells and bacteria for investigating host-pathogen interactions. Lab Chip *10*:43-50.
9. **Håkanson, M., M. Textor, and M. Charnley.** 2011. Engineered 3D environments to elucidate the effect of environmental parameters on drug response in cancer. Integr. Biol. (Camb). *3*:31-38.
10. **Khetani, S.R. and S.N. Bhatia.** 2008. Microscale culture of human liver cells for drug development. Nat. Biotechnol. *26*:120-126.
11. **Marlon, S.T., B. Millare, J.M. Clift, D. Bao, C. Hong, and V.I. Vullev.** 2010. Print-and-Peel Fabrication for Microfluidics: What's in it for Biomedical Applications? Ann. Biomed. Eng. *38*:21-32.
12. **Xia, Y. and G.M. Whitesides.** 1998. Soft lithography. Annu. Rev. Mater. Sci. *28*:153-184.
13. **Tan, A., K. Rodgers, J.P. Murrihy, C. O'Mathuna, and J.D. Glennon.** 2001. Rapid fabrication of microfluidic devices in poly(dimethylsiloxane) by photocopying. Lab Chip *1*:7-9.
14. **Kaigala, G.V., S. Ho, R. Penterman, and C.J. Backhouse.** 2007. Rapid prototyping of microfluidic devices with a wax printer. Lab Chip *7*:384-387.
15. **Bao, N., Q. Zhang, J.-J. Xu, and H.-Y. Chen.** 2005. Fabrication of poly(dimethylsiloxane) microfluidic system based on masters directly printed with an office laser printer. J. Chromatogr. A *1089*:270-275.
16. **Grimes, A., D.N. Breslauer, M. Long, J. Pegan, L.P. Lee, and M. Khine.** 2008. Shrinky-Dink microfluidics: rapid generation of deep and rounded patterns. Lab Chip *8*:170-172.
17. **Abdelgawad, M., M.W.L. Watson, E.W.K. Young, J.M. Mudrik, M.D. Ungrin, and A.R. Wheeler.** 2008. Soft lithography: masters on demand. Lab Chip *8*:1379-1385.
18. **Shrirao, A.B., R. Perez-Castillejos.** 2010. Simple fabrication of microfluidic devices by replicating Scotch-tape masters. Lab Chip: Chips & Tips.
19. **Friedl, P. and D. Gilmour.** 2009. Collective cell migration in morphogenesis, regeneration and cancer. Nat. Rev. Mol. Biol. *10*:445-457.
20. **Gov, N.S.** 2007. Collective cell migration patterns: follow the leader. Proc. Natl. Acad. Sci. USA *104*:15970-15971.
21. **Poujade, M., E. Grasland-Mongrain, A. Hertzog, J. Jouanneau, P. Chavrier, B. Ladoux, A. Buguin, and P. Silberzan.** 2007. Collective migration of an epithelial monolayer in response to a model wound. Proc. Natl. Acad. Sci. USA *104*:15988-15993.
22. **Rasband, W.S.** 1997-2012. ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/.
23. **van Horssen, R., N. Galjart, J.A.P. Rens, A.M.M. Eggermont, and T.L.M. ten Hagen.** 2006. Differential effects of matrix and growth factors on endothelial and fibroblast motility: Application of a modified cell migration assay. J. Cell. Biochem. *99*:1536-1552.
24. **Trepat, X., M.R. Wasserman, T.E. Angelini, E. Millet, D.A. Weitz, J.P. Butler, and J.J. Fredberg.** 2009. Physical forces during collective cell migration. Nat. Phys. *5*:426-430.

Received 29 June 2012; accepted 17 August 2012.

Address correspondence to Raquel Perez-Castillejos, Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ, USA. Email: RaquelPC@njit.edu

Supplementary material for this article is available at www.BioTechniques.com/article/113928.