Skin Biopsy Analysis Reveals Predisposition to Stretch Mark Formation

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Background: Stretch marks are a disfiguring skin condition of yet unknown etiology.

Objective: We wanted to compare the functional disposition of dermal fibroblasts, derived from unaffected skin of patients with stretch marks and fibroblasts from normal age-matched subjects, and develop a test to predict predisposition to stretch mark development.

Methods: Skin biopsies from normal subjects (NS), stretch-marked skin (SM), and normal-looking skin from patients with stretch marks (NL) were analyzed by histochemistry and assays of total protein, DNA, and elastin. Cellular migration, proliferation, and matrix production were also measured in primary cultures of biopsy-derived fibroblasts.

Results: We found that NL skin contained less DNA, protein, and elastin than NS skin (–16%, –36%, –44%, respectively) and that such deficiencies were more profound in SM skin (–55%, –64%, –80%, respectively). Both NL- and SM-derived cells had slower than normal outgrowth of their fibroblasts, which also demonstrated low migration and proliferation rates, and produced less elastin, fibrillin 1, collagen 1, and fibronectin than NS-derived cells in primary cultures. All these aberrant features, indicating a dormant phenotype of NL- and SM-derived fibroblasts, were reversed and normalized in the fourth passage of all tested fibroblasts.

Conclusions: A series of in vitro tests led to the discovery of a dormant phenotype in dermal fibroblasts from patients with stretch marks. The described tests may serve as a diagnostic tool for predicting predisposition to stretch marks. The reported reversibility of impaired fibroblast phenotypes opens a new perspective for preventive treatments for people predisposed to stretch mark formation. (Aesthetic Surg J 2005;25:593-600.)
lysyl oxidase-dependent cross-linking of their lysine residues.\textsuperscript{24-26} Mature (insoluble) elastin, synthesized almost exclusively during late gestation and early childhood,\textsuperscript{27} is metabolically inert and may last over the entire human lifespan in undisturbed tissues.\textsuperscript{28,29}

We hypothesize that stretch marks are induced by excessive mechanical stretching of skin to the point of rupturing dermal elastic fibers, and that local fibroblasts are unable to adequately repair or replace those ECM components that are solely responsible for the resilience of skin. Since most patients with stretch marks do not display any obvious signs of known genetic diseases permanently affecting connective tissue, we anticipate that these lesions develop as a consequence of certain acquired metabolic disturbances that significantly diminish the reparatory abilities of dermal fibroblasts and, in particular, impair their ability to produce new ECM containing elastic fibers.

Our current studies were aimed at delineation of the possible functional differences between dermal fibroblasts derived from biopsies of unaffected skin regions of patients with stretch marks and fibroblasts derived from skin of normal age-matched individuals. We hoped that the disclosure of eventual functional differences between fibroblasts derived from these 2 groups would constitute a reliable test that would allow prediction of an individual’s predisposition for development of \textit{striae distensae} in skin challenged by stretching during pregnancy. Establishing that the predicted functional impairment of dermal fibroblasts can be reversible would open a new avenue for development of preventive treatment(s) for individuals diagnosed with predisposition to stretch mark development and eventual treatment of fully developed lesions.

\textbf{Materials and Methods}

Small punch biopsies were taken from “healthy” skin regions of 10 female patients with stretch marks, aged 24 to 47 years, and from skin of 3 age-matched individuals without stretch marks. Additional biopsies were taken directly from the stretch-marked skin of 3 patients. Results of the routine blood tests (complete blood count [CBC] and Chem-20 panels) showed values within normal ranges in all tested subjects. None of the patients reported a history of dermatologic problems or underlying genetic or metabolic systemic physiologic conditions. None of the tested subjects were actively taking any prescription medications. Institutional review board approval and patient informed consent were obtained for this study. Guidelines set by the Department of Health and Human Services for the protection of human subjects were strictly followed.

\textbf{Histopathology}

To justify the clinical relevance of our in vitro studies, we examined the distribution of ECM components in 4-μm-thick histologic sections of each obtained skin biopsy. All histologic sections were stained with Movat’s pentachrome,\textsuperscript{30,31} which shows elastin as black, glycosaminoglycans as green, collagen as yellow, smooth muscle as red, and nuclei as dark blue. Previous studies have confirmed that the distribution of black-stained material with Movat’s method entirely overlaps with immunodetectable elastin.\textsuperscript{32-34}

\textbf{Biochemical analyses of skin biopsies}

The content of total DNA was analyzed using the DNeasy Tissue System (Qiagen Inc, Valencia, CA), and total protein levels were assessed with the Bio-Rad protein assay Dye Reagent (Bio-Rad Laboratories, Hercules, CA) according to the manufacturers’ instructions. The obtained values were then normalized per 1 mg wet weight of the tested samples. The content of insoluble elastin in the biopsies was assessed after 1-mm\textsuperscript{2} tissue samples were weighed and boiled in 0.5 mL of 0.1 N NaOH for 45 minutes to solubilize all matrix components except elastin. The resulting pellets containing the insoluble elastin were then solubilized in 200 μL of 0.25 M hot oxalic acid for 1 hour, and the protein content was assessed with the Bio-Rad protein assay Dye Reagent kit. The results were normalized per DNA content in each biopsy.

\textbf{Cell cultures}

Multiple fragments of each obtained biopsy (explants) were first placed in culture dishes and maintained in alpha-minimum essential medium supplemented with 20 mM Heps, 1% antibiotics/antimycotics, 10% fetal bovine serum (FBS), and 1% L-Glutamate for the initial outgrowth of skin fibroblasts. Fibroblasts growing out of all primary explants were detached by trypsinization, maintained in monolayer cultures, and analyzed through their 4 consecutive passages. Cell proliferation was assessed by incorporation of [\textsuperscript{3}H]-thymidine, and production of the major components of ECM—elastin, fibrillin 1, fibronectin, and collagen 1—were assessed by immunohistochemistry. The production of new elastin was also assessed after metabolic labeling of cultured fibroblasts with radioactive valine.
Cell migration assays  

Migration from the initial explants. The migratory abilities of dermal fibroblasts present in the skin biopsies were assessed initially by observation of their initial outgrowth from the multiple small explants (1 mm²) placed in the tissue culture dishes. The explants were observed by inverted microscopy with Nomarski optics, and images were collected daily for 21 consecutive days. Images of each sample were captured, and the number of migrating cells detected in 25-mm² squares surrounding each explant were counted using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Five explants derived from the single biopsy were analyzed.

Migration into scratch-gaps of primary cultures. Fibroblasts obtained by trypsinization of the initial outgrowth from primary explants (passage 1) were plated on 30-mm culture dishes and incubated in medium supplemented with 10% FBS for 6 days until fully confluent, at which time a 5-mm scratch-gap was introduced using a rubber policeman as previously described. Cultures were then incubated in media supplemented with 5% FBS for 7 days, during which time their migration into the gaps was monitored using an inverted microscope. Five microscopic (2-mm²) fields in each scratch-gap injury were evaluated under inverted microscope. Images of each sample were captured, and the number of cells migrating into each field was counted using the Image-Pro Plus program.

Cell proliferation assays  
The first and fourth passages of fibroblasts derived from each analyzed skin biopsy were suspended in alpha-MEM (minimum essential medium) containing 5% FBS and initially plated in 6 well dishes at a density of 50,000/cells per well. The medium was changed 24 hours later, and parallel cultures were maintained for the next 48 hours. The cell density was roughly estimated in each culture under the inverted microscope with Nomarski optics, and then cells were trypsinized and counted in a hemocytometer. Parallel sextuplicate cultures, incubated as previously described, were also exposed to [³H]-thymidine (2 μCi/well) for the last 24 hours. These cultures were then washed in phosphate-buffered saline and treated with cold trichloroacetic acid twice for 10 minutes at 4°C. Then, 0.5 mL of 0.3 N NaOH was added to all dishes for 30 minutes, and subsequently, 200-μL aliquots of each culture were mixed with scintillation fluid and counted.

Immunocytochemistry  
Seven-day-old confluent cultures of fibroblasts derived from the first and fourth passage of all analyzed skin biopsies, fixed in cold 100% methanol, were incubated for 1 hour with 20 μg/mL of polyclonal antibody to tropoelastin (Elastin Products, Owensville, MO); 20 μg/mL of polyclonal antibody to fibrillin 1 (Chemicon, Temecula, CA); 10 μg/mL of polyclonal antibody to collagen type I (Chemicon); and 2 μg/mL of monoclonal antibody to fibronectin (Sigma, St. Louis, MO). All cultures were then incubated with appropriate fluorescein-conjugated secondary antibodies (GAR-FITC or GAM-FITC) for an additional hour. Nuclei were counterstained with propidium iodide. Morphometric analysis of all cultures immunostained with individual antibodies recognizing ECM components was performed using an Olympus AH-3 microscope (Olympus, Markham, Ontario, Canada) attached to a CCD camera (Optronix, Oxford, England) and a computerized video analysis system (Image-Pro Plus software 3.0 for Macintosh).

Metabolic labeling and quantification of newly deposited insoluble elastin  
The first and fourth passages of fibroblasts derived from each analyzed skin biopsy were grown to confluency in 30-mm cell culture dishes in quadruplicates. Three days later, 20 μCi of [³H]-valine was added to each dish along with fresh media. Cultures were then incubated for another 72 hours, and soluble and insoluble elastin were assessed separately in each culture. At the end of each experiment, media were removed and cell layers containing deposited extracellular matrix were then scraped in 0.1 N NaOH, sedimented by centrifugation, and boiled in 0.5 mL of 0.1 N NaOH for 45 minutes to solubilize all matrix components except elastin. The resulting pellets containing the insoluble elastin were then solubilized by boiling in 200 μL of 5.7 N HCl for 1 hour, and the aliquots were mixed with scintillation fluid and counted. Final results reflecting amounts of metabolically labeled, insoluble elastin were expressed as CPM/μg DNA. DNA was determined with the DNeasy Tissue System from Qiagen.

Statistical Analysis  
Differences between groups were analyzed with 1-way analysis of variance (ANOVA). If analysis of variance demonstrated significant differences between groups, individual differences were analyzed with a 2-tailed unpaired t test.

Results  
Histologic analysis of skin biopsies taken from “normal-looking” (NL) skin regions of 10 women with stretch
marks revealed existence of peculiar ECM. In all 10 analyzed cases, this matrix was more compact than that of normal individuals but contained fewer elastic fibers. The fibers were also thinner and often fragmented (Figure 1, A and B). Moreover, the distribution of elastic fibers was uneven. In 7 out of 10 analyzed cases, the deficiency in elastic fiber content in the subepidermal zones was striking and resembled biopsies obtained from the actual stretch mark (SM) sites (Figure 1). The latter were further characterized by a very thin epidermis and visible loss of dermal papillae, as well as by a striking deficiency of collagen bundles and increased levels of proteoglycans (Figure 1, C).

Biochemical analysis of biopsies derived from NL skin contained an average 16% less DNA and 36% less protein per 1 mg of their wet weight than biopsies obtained from “normal” healthy skin. Biopsies derived from the actual stretch marks demonstrate an even more pronounced deficiency of total DNA and total protein as compared to “normal” skin tissues.

Monitoring of the initial outgrowth of fibroblasts from the primary explants of the skin biopsies demon-
stratified that in contrast to NS biopsies, in which fibroblasts started to migrate within the first week in culture, NL biopsies and SM biopsies demonstrated a significantly delayed (6 and 11 days, respectively) and less efficient fibroblast outgrowth (Figure 4).

Results of the scratch-gap migration assay (Figure 5) and proliferation assays (Figure 6) performed in first passage cultures also confirmed that NL fibroblasts and SM fibroblasts maintained their proportionally slower migration and proliferation rates as compared to NS fibroblasts.

Results of quantitative morphometric analysis of immunostained 7-day-old cultures of NL fibroblasts (first passage) and SM fibroblasts clearly demonstrated that cells from both experimental groups produce proportionally less ECM components than NS fibroblasts (Figure 7, A). Immunostaining with anti-elastin antibody (Figure 8) and results of quantitative analysis of [3H]-valine–labeled insoluble elastin (Figure 9, A) indicated that first passages of NL fibroblasts deposited 70% to 80% less elastin than NS fibroblasts, and that SM fibroblasts deposited only traces of detectable elastin.

It is of particular importance that results of parallel assays performed in fourth-passage cultures of the respective dermal fibroblasts (NL and SM) derived from all tested patients with stretch marks demonstrated a practical normalization of their aberrant proliferation rate (detected in the first passage) (Figure 6, B) and a spectacular recovery in their matrix production to levels observed in cultures of normal skin fibroblasts (Figures 7, B; 8; and 9, B).

Discussion

Results of biochemical assays of skin biopsies from actual stretch marks demonstrated a striking decrease in DNA, total protein, and elastin content as compared...
with NS samples. The outgrowth of fibroblasts from explants of these biopsies was initially nonexistent, and required at least 2 to 3 weeks of culture in medium with 10% serum to yield the first wave of outgrowing fibroblasts. These fibroblasts, maintained in secondary cultures, produced small amounts of collagen I and fibrillin 1 but did not deposit elastin. Parallel biochemical tests of NL biopsies and analysis of their cultured fibroblasts also demonstrated a significant decline in their proliferative, migratory, and synthetic abilities. Given these results, we concluded that skin of patients with stretch marks contains a functionally impaired fibroblast phenotype that could be detected even in regions of skin that have never been challenged by physical forces (stretching). Persistent mechanical forces may induce local damage of the skin structure, but permanent lesions occur only in patients whose dermal fibroblasts are incapable of quick repair of these stretch-dependent injuries. Aside from major genetic diseases causing faulty production of elastic fibers, usually diagnosed in early childhood, the influence of relatively mild acquired metabolic deficiencies that may also harm the proper

Figure 7. A, Results of quantitative morphometric analysis of immunostained 7-day-old cultures of fibroblasts from the first passage demonstrate that fibroblasts derived from normal-looking skin of patients with stretch marks and fibroblasts from stretch-marked skin clearly produce proportionally less ECM components than fibroblasts derived from individuals with no stretch marks. B, Results of morphometric analysis of fourth passage cultures demonstrate that fibroblasts from all tested groups deposited very similar amounts of all immunodetectable ECM components.

Figure 8. Representative micrographs demonstrating immunodetectable elastic fibers in 7-day-old cultures of biopsy-derived fibroblasts maintained in the first and fourth passages. The first-passage fibroblasts derived from normal-looking skin of patients with stretch marks produced fewer elastic fibers than normal fibroblasts. Fibroblasts derived from stretch-marked skin did not deposit immunodetectable elastic fibers. The fourth passage fibroblasts derived from all experimental groups produced similar levels of elastic fibers.

Figure 9. A, Results of the quantitative analysis of [3H]-valine-labeled insoluble elastin detected in 7-day-old cultures of biopsy-derived fibroblasts demonstrate that primary cultures (first passage) of fibroblasts derived from normal-looking skin of patients with stretch marks produce much less insoluble elastin than normal fibroblasts, and that those cells derived from the stretch marked skin deposited only traces of insoluble elastin. B, In contrast, results of a similar assay performed in the respective passage four of biopsy-derived fibroblasts demonstrate similar levels of metabolically labeled insoluble elastin in all experimental groups.
production and/or maintenance of dermal elastic fibers should be seriously considered in the pathophysiology of stretch marks. The fact that the impaired fibroblast phenotype detected in passage one was reversible after prolonged exposure to media containing normal FBS seems to confirm our hypothesis that the detected phenotype is not caused by a permanent genetic defect, but rather develops in response to an imbalance of certain humoral factor(s) in serum of affected individuals. Despite the fact that routine blood tests (Chem-20 and CBC with differential) of our skin biopsy donors did not reveal any gross hematologic abnormalities, a more comprehensive analysis involving assessments of trace elements and rare metabolites may be needed for the establishment of metabolic reasons for dermal fibroblast dormancy.

**Conclusion**

Results of our in vitro tests indicate that fibroblasts derived from “healthy-looking” regions of skin of patients with stretch marks are metabolically affected and functionally dormant. Therefore, we suggest that stretch marks develop only in certain individuals whose genetic profile or actual metabolic status is compromised, which leads to functional dormancy of dermal fibroblasts, diminishing their potential for proper response to persistent mechanical stretching and fast repair of damaged ECM. Thus, the described tests involving early passages of biopsy-derived dermal fibroblasts may constitute a useful diagnostic tool allowing for prediction of whether a particular patient would develop stretch marks during pregnancy or substantial weight gain. Moreover, our data indicate that metabolically dormant fibroblasts, even those derived from actual stretch marks, can eventually recover their ability to produce new elastic fibers and proliferate when maintained in the normal serum.

This observation confirms our initial hypothesis that in most patients, a predisposition to stretch marks, detected with the proposed panel of in vitro tests, reflects acquired metabolic disturbances that significantly, but not irreversibly, diminish the functional abilities of dermal fibroblasts for local tissue repair. Thus, identification of the metabolically impaired but “curable” fibroblasts opens a new avenue for development of a preventive treatment aimed at “awakening lazy fibroblasts” in individuals diagnosed with predisposition to stretch mark development and eventual treatment of fully developed lesions. ■

**References**


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