Endocrine, *vol. 10, no. 1, 35–41, February 1999* 0969–711X/99/10:35–41/$11.75

Cell-to-Cell Communication and Expression of Gap Junctional Proteins in Human Diabetic and Nondiabetic Skin Fibroblasts

Effects of Basic Fibroblast Growth Factor

Kay M. Abdullah,[1,2] Girish Luthra,[1] Jerzy J. Bilski,[3] S. Ahmed Abdullah,[2] Lawrence P. Reynolds,[3,4] Dale A. Redmer,[4] and Anna T. Grazul-Bilska,[3,4]

[1]Department of Surgery, University of North Dakota; [2]Plastic Surgery Institute; [3]Cell Biology Center; and [4]Department of Animal and Range Sciences, North Dakota State University, Fargo, ND

Wound healing involves the interactions of many cell types, and is controlled in part by growth factors. Intercellular communication mediated by gap junctions is considered to play an important role in the coordination of cellular metabolism during the growth and development of tissues and organs. Basic fibroblast growth factor (bFGF), known to be important in wound healing, has been found to increase Cx43 expression and intercellular communication in endothelial cells and cardiac fibroblasts. It has been proposed that an increased coupling is necessary for the coordination of these cells in wound healing and angiogenesis, and that one of the actions of bFGF is to modulate intercellular communication. The aim of our study was to evaluate the effects of bFGF on gap junctional intercellular communication (GJIC) in vitro, and the presence of gap junctional proteins connexin (Cx) 26, Cx32, and Cx43 in fibroblasts of diabetic and nondiabetic individuals. Fibroblast cell lines ($n = 10$) were cultured for 3 d in serum-free media with or without bFGF (3 ng/mL). Cells were evaluated for the rate of GJIC by using laser cytometry, and for the presence of Cx26, Cx32, and Cx43 by immunohistochemical and Western analyses. All cell types communicated via contact-dependent mechanisms. The rate of GJIC was greater ($p < 0.01$) for diabetic than for nondiabetic fibroblasts (4.1 ± 0.01 vs 3.3 ± 0.01 %/min). bFGF increased ($p < 0.01$) the rate of GJIC for diabetic (4.9 ± 0.01 vs 4.1 ± 0.01%) and nondiabetic (4.1 ± 0.01 vs 3.3 ± 0.01%) fibroblasts. Immunohistochemistry identified Cx26 in the cytoplasm, Cx32 was not detected, and Cx43 was present on the cellular borders in all cultures. Image analysis of immunofluorescent staining demonstrated that bFGF increased ($p < 0.05$) Cx43 expression in diabetic and nondiabetic fibroblasts. Western immunoblot analysis revealed bands at 43–46 kD that were similar in volume for diabetic and nondiabetic fibroblasts. Thus, gap junctions involving Cx43 and GJIC among fibroblasts appear to be targets for bFGF. Fibroblasts of diabetic individuals appear to have an increased rate of cell–cell coupling, correlating with a decreased rate of proliferation.

Key Words: Connexins; gap junctions; cell-to-cell communication; bFGF; fibroblasts; diabetes; wound healing.

Introduction

Individuals with diabetes continue to encounter problems with poor wound healing, contributing to an increased risk of infection, wound dehiscence, and anastomotic failures *(1)*. Wound healing is initiated whenever tissue is injured, and consists of several responses involving migration, proliferation, differentiation and apoptosis of multiple cell types *(2)*. It is becoming increasingly apparent that a number of growth factors play important roles during wound healing, and exploration of their potential therapeutic usefulness is in progress *(3–5)*. Among the first growth factors to be identified were fibroblast growth factors (FGF). It has been demonstrated that basic FGF (bFGF) has a wide distribution, and a broad specificity for a number of target cells *(6)*. bFGF affects connective tissue and endothelial cells, mediating wound repair by directing the migration and proliferation of fibroblasts and endothelial cells into the wound environment *(7–9)*. It has been demonstrated that the direct application of bFGF on full thickness wounds of streptozotocin-induced diabetic rats resulted in increased biomechanical strength and histologically mature wounds *(10)*.

Recieved September 8, 1998; Revised November 6, 1998; Accepted December 1, 1998.
Author to whom all correspondence and reprint requests should be addressed: Dr. Kay M. Abdullah, UND Department of Surgery, 1919 N. Elm Street, Fargo, ND 58102; E-mail: kaymabd@aol.com

Fibroblasts enter the wound and begin to lay down a network of collagen and fibronectin, forming the extracellular matrix that maintains the physical integrity of the wound. In diabetic models, fibroblasts have been shown to senescence prematurely, with delayed entry into the wound site and reduced collagen production *(11,12)*. The local wound environment and systemic factors interact to modulate fibroblast function. These factors may interact through contact-dependent (gap junctional) and contact-independent pathways *(13)*.

Gap junctions are membrane channels between adjacent cells that permit the exchange of ions and other small molecules. They consist of two hemi-channels called connexons. Each connexon consists of six subunit proteins called connexins (Cx). There are several different types of connexins, encoded by a multigene family *(14,15)*. Gap junctions are believed to be critical in regulating growth and development of organs and tissues, and are found in almost all mammalian tissues, except circulating red blood cells and adult skeletal muscle *(16)*. Two connexins, Cx43 and Cx26, have been demonstrated in dermal fibroblasts, and the pattern of cell–cell communication has been characterized, demonstrating an extensive coupling of dermal fibroblasts and epidermal keratinocytes. This study also revealed junctional cell–cell communication taking place in normal and fully differentiated human skin *(17)*. Studies of the effects of wounding and bFGF on gap junction function have implicated the gap junctions as being involved in cell–cell coordination of wound healing *(18–20)*. It has been demonstrated that connexin expression and intercellular communication are altered in response to epidermal wounding, with an upregulation of Cx26 in cells proximal to the wound, but downregulation of Cx26 at the wound edge *(18)*. bFGF has been found to increase the expression of Cx43 and intercellular communication in cardiac fibroblasts *(19)*, as well as microvascular endothelial cells in human skin *(20)*, leading to speculation that it may have the same effect following injury.

The gap junction is a channel with multiple functions and likely plays a role in the coordination of wound healing *(21)*. The role of gap junctional communication in the coordination of fibroblast function in diabetic versus nondiabetic individuals has not been previously evaluated. The aim of this study was to investigate the effects of bFGF on gap junctional intercellular communication (GJIC) and the expression of gap junctional proteins in fibroblasts from diabetic and nondiabetic individuals. We hypothesized that fibroblasts from diabetic individuals may display altered intercellular communication and/or connexin expression contributing to delayed fibroblast function in wound repair.

Results

All of the diabetic and nondiabetic fibroblasts communicated via a contact-dependent (gap junctional) pathway (Fig. 1). The rate of GJIC was greater ($p < 0.01$) for diabetic than for nondiabetic fibroblasts (4.1 ± 0.01 vs 3.3 ± 0.01 %/min during the first 4 min after photobleaching; Fig. 1). In addition, bFGF increased ($p < 0.01$) the rate of GJIC for diabetic (4.9 ± 0.01 vs 4.1 ± 0.01%) and nondiabetic (4.1 ± 0.01 vs 3.3 ± 0.01%) fibroblasts (Fig. 1).

Across all cultures, there was no effect on the rate of GJIC for cells which were not photobleached (positive control), or for cells which were photobleached but not in contact with other cells (negative control). The rate of dye transfer for these cells was negligible (0.02 ± 0.001%, $n = 329$ for positive control and 0.03 ± 0.001%, $n = 38$ for negative control over the first 4 min of the assay), showing that nonspecific photobleaching or leakage of fluorescent probe from the cells was negligible.

Connexin 43 was localized primarily on the cellular borders of all cells types, but cytoplasmic staining was also observed (Fig. 2A,B). Image analysis demonstrated that bFGF increased ($P < 0.01$) Cx43 expression in diabetic and non-diabetic fibroblasts (Fig. 3). Connexin 26 was localized in the cytoplasm of all cell types (Fig. 2C), and the pattern of expression was similar in control and bFGF-treated cultures. Preabsorbtion of the antibodies with their corresponding connexin peptide resulted in a complete lack of staining for Cx26 or Cx43 (similar to Fig. 2D). Cx32 was not detected in cultured fibroblasts (data not shown).

Western immunoblot analysis for Cx43 revealed bands in the range of 43–46 kD in diabetic and nondiabetic fibroblasts. Statistically significant effects of bFGF were not observed, probably owing to the high variation among samples (Figs. 4 and 5). Rat heart, used as a positive control in the Cx43 assay, revealed one band at 43 kD (data not shown). Similar to immunohistochemistry, Cx32 was not detected in cultured fibroblasts by Western analysis (data not shown).

Discussion

Many of the acute metabolic abnormalities in diabetes are a result of insulin deficiency, but the mechanisms underlying the connective tissue changes of the diabetic state, including poor wound healing, are poorly understood. Fibroblasts are important cells in the wound healing process, functioning to lay down an extensive network of collagen and fibronectin, leading to a mature, well-healed wound *(22)*. Being key players in wound healing, detailed evaluation of their metabolism and functions, including cellular interactions and their regulation, should lead to a better understanding of the healing process in general, and the impairment characteristic of diabetic wounds.

The present data demonstrated that diabetic and nondiabetic fibroblasts communicated through gap junctional, contact-dependent mechanisms, and GJIC was greater in diabetic than nondiabetic cells. Gap junctions enable cells to share a metabolic load, and therefore assist with tissue homeostasis. The number of plaques of gap junctions

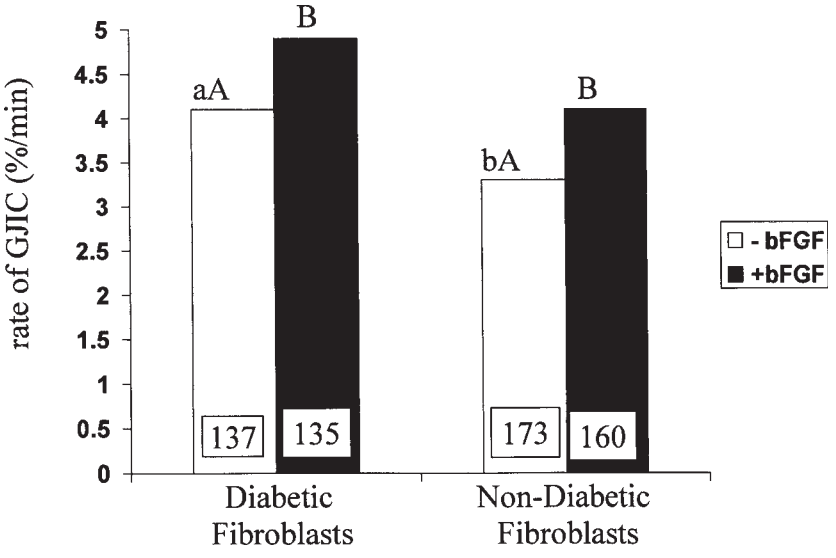

Fig. 1. Effects of bFGF on GJIC of diabetic and nondiabetic fibroblasts. SEM = 0.1-0.2 across cell types and treatments. **(A,B)** Values differ ($p < 0.01$) within diabetic or nondiabetic fibroblasts (with bFGF vs without bFGF); a,b values differ ($p < 0.01$) between types of fibroblasts cultured without bFGF. Numbers within bars indicate number of cells evaluated.

between adjacent cells seems to be proportional to the metabolic cooperation between these cells *(23)*. This could account for the increased GJIC seen in diabetic fibroblasts. In addition, it has been demonstrated that there is an inverse correlation of cell replication and GJIC in some cell lines *(23)*. In our experiment, diabetic fibroblasts had a doubling time of 97.3 ± 25.4 h ($n = 5$ cultures), but nondiabetic fibroblasts had a doubling time of 77.6 ± 22.8 h ($n = 5$ cultures; Abdullah et al., unpublished observation), suggesting that the greater rate of GJIC of diabetic fibroblasts may be correlated with their slower rate of proliferation.

Connexins have been shown to be specific gap junction proteins, therefore, the localization of connexins is widely used for identification of gap junctions in a variety of tissues *(23–25)*. In the present study, Cx26 and Cx43 were detected in diabetic and nondiabetic fibroblasts, and their expression was similar in both. Cx43 was localized primarily on the cellular borders of fibroblasts, Cx26 appeared to be present in the cytoplasm, and Cx32 was not detected. In numerous other tissues and cell types, Cx43 has also been localized on cellular borders *(24–29)*. Studies have indicated that Cx26 is present either on cell borders or in the cytoplasm, depending on the cell type *(25)*. Cx26, Cx32, Cx40, and Cx43 have been identified in mouse skin *(30)*. Cx43 and Cx26 have been identified in the dermis and epidermis of human skin *(17)*. In addition, it has been suggested that Cx43 could be the predominant gap junctional protein in keratinocyte-to-keratinocyte communication *(17)*. In agreement with these observations, our data suggest that Cx43 rather than Cx26 is more important for GJIC of fibroblasts, since Cx43 but not Cx26 expression was regulated by bFGF.

Growth factors have been identified as playing an important role in initiating and modulating wound healing. Several studies have indicated that bFGF functions to attract fibroblasts into the wound, stimulate their proliferation, influence extracellular matrix deposition, and augment tissue repair in impaired healing models *(4,7)*.

The present study demonstrated that bFGF increased GJIC among diabetic and non-diabetic fibroblasts and upregulated the expression of Cx43. These results demonstrate that bFGF functions to upregulate gap junction function and formation in both human diabetic and non-diabetic fibroblasts.

Several studies have indicated the role of the gap junctions in wound healing. Pepper and Meda *(20)* demonstrated an increased coupling and Cx43 expression after mechanically wounding a confluent layer of endothelial cells, and this effect was reduced by antibodies to bFGF. These results suggest that bFGF effects on cellular coupling may at least partially mediate the coordination of endothelial cells during angiogenesis. Goliger and Paul *(18)* demonstrated an altered connexin expression in response to epidermal wounding, and Doble and Kordami *(19)* showed that bFGF may modulate fibroblast communication and Cx43 expression during cardiac repair after infarct. These data demonstrate that gap junction function is affected by bFGF. This suggests that gap junctions are regulated by growth factors that may be involved in the cellular coordination and activity in wound repair.

In summary, the present data demonstrated that diabetic and nondiabetic fibroblasts communicate via contact-dependent mechanisms, that the rate of communication was greater in diabetic than nondiabetic fibroblasts, and that

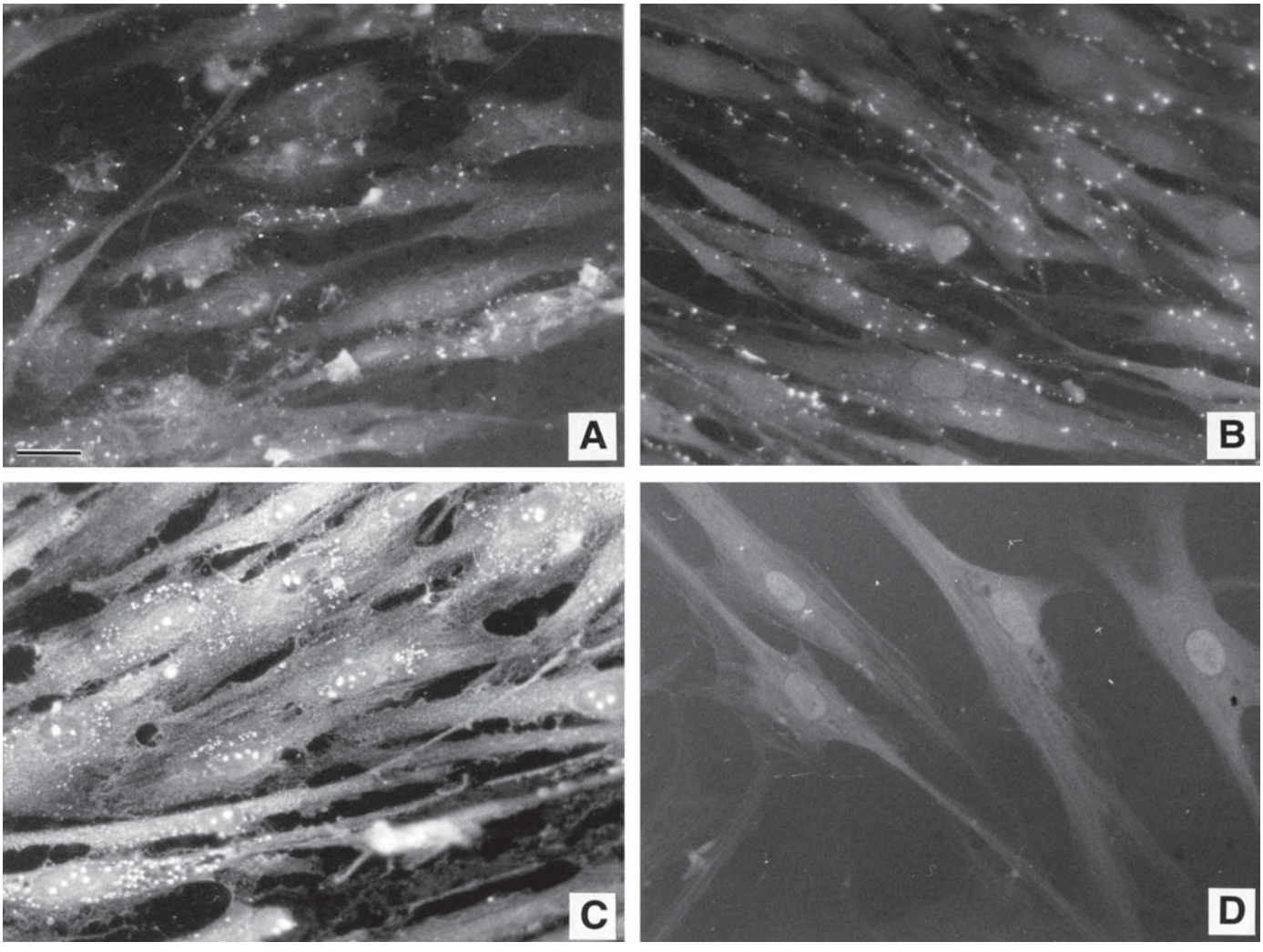

Fig. 2. Immunofluorescent localization of Cx43 in nondiabetic fibroblasts cultured **(A)** without and **(B)** with bFGF, and **(C)** Cx26 in nondiabetic fibroblasts cultured without bFGF. Micrographs show representative staining. **(D)** represents control staining. Bar = 20 µm.

bFGF increased Cx43 expression and GJIC in both cell types. Elevated GJIC in diabetic fibroblasts may be due to altered metabolism in conditions of hyperglycemia, and may be one of the mechanisms involved in homeostasis. One of the mechanisms of enhanced healing seen with bFGF may be its effect on gap junctions and intercellular communication. Whether bFGF acts solely via the insertion of newly synthesized Cx43 into the plasma membranes of fibroblasts, or also affects the conductance and permeability of existing gap junctions requires additional studies. Our study suggests that bFGF and its effect on intercellular communication may mediate the coordination of fibroblast function. Our findings also indicate that fibroblasts of diabetic individuals function similarly to non-diabetic fibroblasts in terms of gap junction expression and bFGF mediated intercellular communication. Unraveling the impact of bFGF on gap junction expression and intercellular communication, and the influence of these in normal and impaired wound healing, may lead to a better understanding of the process of wound repair in general, and also of the mechanism of delayed wound healing seen in diabetes.

Materials and Methods

Cell Culture

Skin fibroblasts from mature onset diabetic ($n = 5$) and nondiabetic ($n = 5$; passages 4–7) men (ages 33–46) were purchased from Coriell Institute for Medical Research, Camden, NJ. The day after arrival, the cells were removed from plastic transport dishes by using trypsin (Gibco, Grand Island, NY, 0.1% in phosphate buffered saline containing 0.02% EDTA), counted, and split into two T-75 Falcon dishes. The cells were cultured in minimum essential media Eagle (MEME, Sigma, St. Louis, MO) supplemented with 20% heat inactivated fetal bovine serum (FBS; Gibco), 2 m*M* glutamine (Sigma), nystatin (Sigma), 100 U/mL penicillin-G and 100 µg/mL streptomycin (Gibco) in a humidified atmosphere of 95% air, 5% CO_2 at 37°C for 8–15 d. Media were changed every 2–3 d.

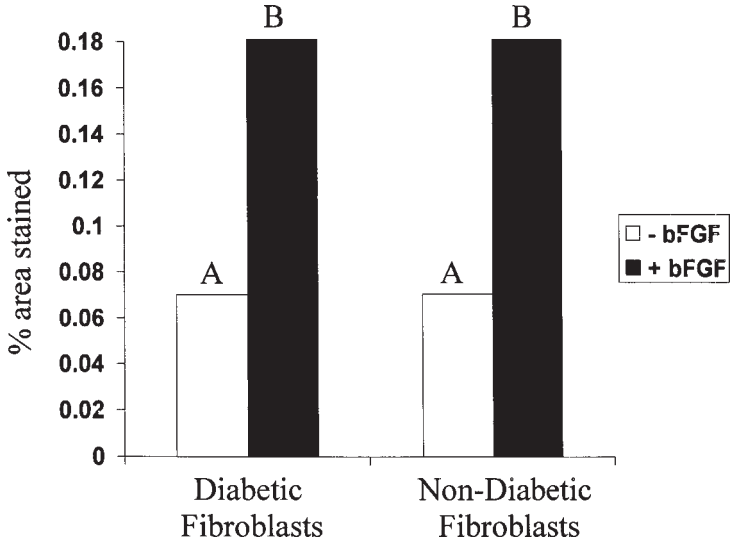

Fig. 3. Effects of bFGF on Cx43 expression evaluated by image analysis of immunofluorescently stained cultured fibroblasts. SEM = 0.003 and 0.01 for fibroblasts cultured without and with bFGF, respectively. **(A,B)** Values differ ($p < 0.01$) within type of fibroblast.

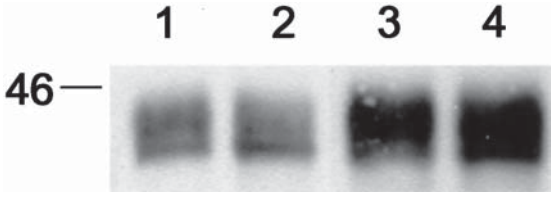

Fig. 4. Western analysis of Cx43 in diabetic and nondiabetic fibroblasts. Lanes 1-4 demonstrate representative samples of (1) diabetic fibroblasts cultured without bFGF; (2) diabetic fibroblasts cultured with bFGF; (3) nondiabetic fibroblasts cultured without bFGF; and (4) nondiabetic fibroblasts cultured with bFGF. Standard is indicated by its mol-wt value ($\times 10^3$).

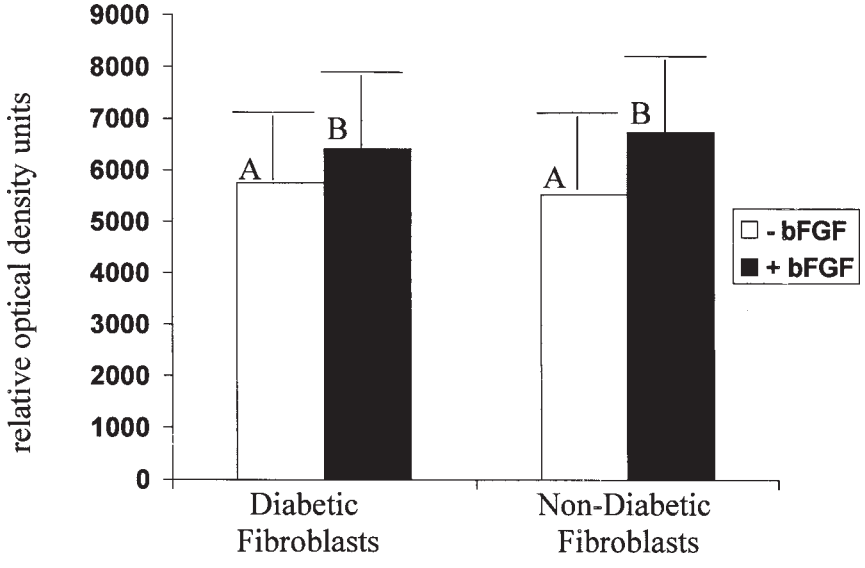

Fig. 5. Densitometric evaluation of Western immunoblot analysis of Cx43 in diabetic and nondiabetic fibroblasts. Data are expressed as relative optical density units.

After the cells reached confluency, they were removed from the dishes by using trypsin, counted and plated on 30-mm Petri dishes at a concentration of $3–4 \times 10^4$ cells/cloning cylinder (Id. 5 mm; Belco Glass, Vineland, NJ). Cloning cylinders were used to facilitate cell–cell contact by concentrating the cells in a small area. After 24 h of incubation, serum-containing media was replaced with serum-free media (MEME, 2 m*M* glutamine, nystatin and antibiotics) and cultured for an additional 3 d with or without human bFGF (3 ng/mL; Boehringer Mannheim, Indianapolis, IN). The dose of bFGF was chosen on the basis of preliminary and previous experiments (*31,32,* Boehringer Mannheim). Cells from each culture were then used for evaluation of GJIC by using laser cytometry, and for immunohistochemical localization of gap junctional proteins Cx26, Cx32, and Cx43, as described below. In addition, cells from each culture were cultured on 60-mm Petri dishes at a concentration of 1×10^6 cells/dish, treated as described above, then used for gap junctional protein detection using Western immunoblot analysis, as described below. For all cultures, for GJIC, immunohistochemistry and Western immunoblot every treatment was performed in duplicate.

Analysis of Gap Junctional Intercellular Communication (GJIC)

GJIC between cells in culture was monitored with an ACAS 570 laser cytometer (Meridian Instruments, Okemos, MI) using a fluorescence recovery after photobleaching (FRAP) technique as described previously *(33–35)*. Briefly, after incubation with treatments, medium was removed from each dish, and medium containing a fluorescent probe (CFDA-AM, 20 μ*M*; Molecular Probes, Eugene, OR) was added. After a 15-min incubation (22°C), dishes were rinsed three times with serum-free medium to remove excess CFDA-AM. Dishes were then placed onto the interactive laser cytometer, and three fields (180×180 μm/field) on each dish were identified for scanning. For each field, 6–12 cells were selected and analyzed for initial fluorescence intensities. Immediately after measurement of initial fluorescence, the fluorescent probe was photobleached in 4–8 selected cells in each field. To determine the rate of FRAP, the fluorescence intensity of all selected cells was quantified every 4 min for 8 min after photobleaching. As reported previously, recovery of fluorescence could only be due to transfer of the fluorescent probe from the adjacent cells that were not photobleached, and only the linear portion of fluorescence recovery curve (first 4 min after photobleaching) was used for statistical analysis. Treatment did not affect the initial fluorescence intensities of the fibroblasts, indicating that uptake of the fluorescent probe was similar among treatment groups.

Immunohistochemistry

The presence of Cx26, Cx32, and Cx43 in cultured fibroblasts was evaluated by using an immunofluorescent method as described previously *(36)*. Fibroblasts were fixed in ethanol:glacial acetic acid (5.7:1) for 20 min, and then were treated for 20 min. with blocking buffer consisting of PBS (0.01 *M* phosphate, 0.14 *M* NaCl, pH 7.3) containing 0.3 % (v/v) Triton X-100 (Malinckrodt, Paris, KY), and 1% (v/v) normal goat serum (Vector Labs., Burlingame, CA). Cells were then incubated with a polyclonal antibody against Cx26, Cx32, or Cx43 (Zymed, San Francisco, CA) overnight at 4°C. Detection of primary antibodies was accomplished by using FITC-conjugated secondary antibody (goat antirabbit IgG; Boehringer Mannheim, Indianapolis, IN). Control staining consisted of replacing the primary antibody with the same dilution of rabbit serum *(36)*. To evaluate specificity of staining for Cx26 and Cx43, antibodies (Zymed) were preabsorbed by using Cx26 or Cx43 peptides (Zymed). Antibodies were mixed with the appropriate peptide at the ratio of antibody:peptide recommended by the manufacturer, and incubated for 3 h at room temperature. The cultured cells were treated as described above, but the primary antibody was replaced by the mixture of preincubated antibody:peptide.

Image Analysis

For cultured fibroblasts, the percentage of the total cellular area that exhibited immunofluorescent staining for Cx43 was evaluated quantitatively with an image analysis system (VIDAS ver. 2.5; Roche Image Analysis System, Los Altos, CA) as described previously *(36)*. For each culture, seven randomly chosen fields (0.025 mm^2 per field) were evaluated in duplicate plates of nontreated or bFGF-treated culture dishes ($n = 28$ measurements/culture). Background fluorescence was minimal and was adjusted to the same level for each section by the image analysis system. The data are reported as the mean percentage ± SEM of the total area within each field that exhibited positive staining.

Western Immunoblot Analysis

Western analysis was performed as described previously in detail *(36)*. After culture, cells were removed from petri dishes by using a rubber policeman, then homogenized in buffer (1% cholic acid [w/v], 0.1% SDS [w/v] in PBS), and sonicated with an ultrasonic processor (Sonics & Materials, Danbury, CT). Samples of protein from cultured fibroblasts (100 μg), and a mouse liver lysate (Zymed, used as a positive control in Cx32 detection) or rat heart (30 μg, used as a positive control in Cx43 detection) were added to loading buffer, boiled for 2 min, then applied to a 12% polyacrylamide gel with a 3% stacking gel *(37)*. After electrophoresis, separated proteins were electroblotted onto an Immobillon-P membrane (Milipore, Bedford, MA), then immunoblotted with monoclonal or polyclonal antibodies against Cx32 or Cx43 (Zymed) respectively. Membranes were then incubated with an antimouse antibody or peroxidase-labeled antirabbit (Amersham International plc, Little Chalfont, England) followed by detection by using ECL

reagents (Amersham). Densitometry was used to measure the intensity of bands, and was performed by using a densitometer (Model PDSI; Molecular Dynamics, Sunnyvale, CA) as described previously *(34)*. After scanning the autoradiograph, a grid was drawn so that each sample was contained within rectangles of equal size, and the densitometric area contained within each rectangle was given a volume measurement.

Statistical Analysis

Data for the rates of GJIC, image analysis of Cx43 in cultured fibroblasts and densitometry of Western immunoblots were analyzed by using the general linear models (GLM) analysis of variance procedure, with the effects of culture type (diabetic vs nondiabetic) and treatment (no treatment vs bFGF-treatment) and the culture type x treatment interaction included in the model *(38)*. When an F-test was significant ($p < 0.05$) differences between specific means were evaluated by Bonferroni's multiple comparison procedure (*39*).

Acknowledgments

We wish to acknowledge Diane Schoeszler for typing the manuscript, and Mr. James D. Kirsh, and Kim C. Kraft for expert technical assistance. Presented at the Annual Meeting of Association for Academic Surgery, Dallas, Texas, November 6-8, 1997. This work is supported by Grants No. 1813-0497-2090 and No. 6371-8183-2661 from University of North Dakota, Grand Forks, ND to K. M. A. and A. T. G.-B.

References

1. Goodson III, W. H., and Hunt, T. K. (1979). *Surg. Gynecol. Obstet*. **149,** 600.
2. Schaffer, C. J., and Nanney, L. B. (1996). *Int. Rev. Cytol.* **169,** 151.
3. Greenhalgh, D. G., Sprugel, K. H., Murray, M. J., and Ross, R. (1990). *Am. J. Pathol*. **136,** 1235.
4. Greenhalgh, D. G. (1996). *J. Trauma, Inj., Infect. Crit. Care.* **41,** 159.
5. Knighton, D.R., Ciresi, K., Fiegel, V. D., Schumerth, S., Butler, E., Cerra, F. (1990). *Surg. Gynecol. Obstet.* **170,** 56.
6. Burgess, W. H., and Maciag, T. (1989). *Annu. Rev. Biochem.* **58,** 575.
7. Servold, S. A. (1991) *Clin. Podiatr. Med. Surg.* **8,** 937.
8. Nissen, N. N., Polverini, P. J., Gamelli, R. L., and DiPietro, L. A. (1996). *Surgery.* **119,** 457.
9. Davidson, J., Buckley, A., Woodward, S., et al. (1988). In *Growth Factors and Other Aspects of Wound Healing, Biological and Clinical Implications.* A. Barbul, E. Pines, M. Caldwell, et al. (eds.), Adam R. Liss, New York, pp. 63.
10. Phillips, L. G., Abdullah, K., Geldner, P. D., Dobbins, S., Ko, F., Linares, H. A., Broemeling, L. D., and Robson, M. C. (1993). *Ann. Plast. Surg.* **31,** 331.
11. Rowe, D. W., Starman, B. J., Fujimoto, W. Y., and Williams, R. H. (1977). *Diabetes.* **26,** 284.
12. Goodson III, W. H., and Hunt, T. K. (1977). *J. Surg. Res.* **22,** 221.
13. Salomon, D., Saurat, J., and Meda, P. (1988). *J. Clin. Invest.* **82,** 248.
14. Willecke, K., Haubrich, S. (1996). *J. Bioenerg. Biomembr.* **28,** 319.
15. Yamasaki, H. and Naus, C. G. (1996). *Carcinogenesis.* **17,** 1199.
16. Kumar, N. M., and Gilula, N. B. (1996). *Cell.* **84,** 381.
17. Salomon, D., Masgrau, E., Vischer, S., Ullrich, S., Dupont, E., Sappino, P., Saurat, J. H., and Meda, P. (1994). *J. Invest. Dermatol.* **103,** 240.
18. Goliger, J. A., and Paul, D. L. (1995). *Mol. Biol. Cell*. **6,** 1491.
19. Doble, B. W., and Kardami E. (1995). *Molec. Cell. Biochem.* **143,** 81.
20. Pepper, M.S., and Meda, P. (1992). *J. Cell. Physiol.* **153,** 196.
21. Larson, D. M. (1990). In *Cell Intercommunication.* Walmor C. DeMello (ed.), CRC Press, Boca Raton, Florida, pp. 93.
22. Martin, P., Hopkinson-Woolley, J., McCluskey, J. (1992). *Prog. Growth Factor Res.* **4,** 25.
23. Holder, J. W., Elmore, E., and Barrett, J. C. (1993). *Cancer Res.* **53,** 1475.
24. Grazul-Bilska, A. T., Reynolds, L. P., Redmer, D. A. (1997). *Biol. Reprod.* **57,** 947.
25. Grazul-Bilska, A.T., Redmer, D. A., Reynolds, L. P. (1998). *Seminar Reprod. Endocrinol.* **15,** 383.
26. Lee, S.W., Tomasetto, C., Paul, D., Keyomarsi, K., Sager, R. (1992). *J. Cell Biol.* **118,** 1213.
27. Matesic, D. F., Germak, J. A., Dupont E., and Madhukar, B. V. (1993). *Neuroendocrinology.* **58,** 485.
28. Meda, P., Pepper, M.S., Traub, O., Willecke, K., Gros, D., Beyer, E., Nicholson, B., Paul, D., and Orci, L. (1993). *Endocrinology.* **133,** 2371.
29. Kojima, T., Yamamoto, M., Tobioka H., Mizuguchi, T., Mitaka, T., Mochizuki, Y. (1996). *Exp. Cell Res.* **223,** 314.
30. Butterweck, A., Elfgang, C., Willecke, K., and Traub, O. (1994). *Eur. J. Cell Biol.* **65,** 152.
31. Grazul-Bilska, A. T., Redmer, D. A., Jablonka-Shariff, A., Biondini, M. E., Reynolds, L. P. (1995). *Can. J. Physiol. Pharmacol.* **73,** 491.
32. Grazul-Bilska, A. T., Redmer, D. A., Zheng, J., Killilea, S. D., and Reynolds, L. P. (1995). *Growth Factors* **12,** 131.
33. Burghardt, R.C., Barhoumi, R., Doolittle, D. J., and Philips, T. D. (1994). In *Principles and Methods of Toxicology.* 3rd ed., A.W. Hayes (ed.), Raven Press, New York, pp. 1231.
34. Grazul-Bilska, A.T., Reynolds, L.P., Kirsch, J.D., Redmer, D.A. (1996). *Biol. Reprod.* **54,** 538.
35. Redmer, D. A., Grazul-Bilska, A. T., Reynolds, L. P. (1991). *Endocrinology*. **129,** 2757.
36. Grazul-Bilska, A. T., Redmer, D. A., Johnson, M. L., Jablonka-Shariff, A., Bilski, J. J., and Reynolds, L. P. (1996). *Biol. Reprod.* **54,** 1279.
37. Laemmli, E. K. (1970). *Nature.* **227,** 680.
38. SAS. (1985). User's Guide, Statistics, 5th Edition. Statistical Analysis System Institute, Cary, NC.
39. Kirk, R. E. (1982). *Experimental Design, Procedures in Behavioral Sciences*, 2nd Ed., Brooks/Cole, Belmont, CA.