EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Safety of Injectable Autologous Human Fibroblasts

G. Keller, J. Sebastian, U. Lacombe, K. Toft, G. Lask, and E. Revazova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 8, pp. 203-206, August, 2000
Original article submitted June 2, 2000

Autologous dermal fibroblasts after propagation in cell culture were used for face soft tissue augmentation. Twenty patients aged 37-61 years with facial rhytides and atrophic scars were treated with autologous fibroblasts from cell culture. Significant sustained clinical improvement was observed. Cells of early passages (4, 5, 6) were used for injection. The study showed that cultured fibroblasts were functionally active and produced large quantities of type I collagen. *In vitro* studies of scar formation potency of injectable fibroblasts showed that these cells possessed normal collagen gel contraction capacity. *In vivo* experiments showed that cultured fibroblasts exhibited no oncogenic properties and induced no tumors in nude mice.

Key Words: *autologous fibroblasts; type I collagen; soft tissue augmentation; contraction; oncogenicity*

The use of autologous fibroblasts for soft tissue augmentation represents a potentially exciting natural alternative to the use of processed bovine collagen or other foreign materials, which all have some benefits, but also have shortcomings [2,13]. The use of autologous fibroblasts ensures sustained clinical improvement due to decreased degradation of autologous collagen [6] and is not accompanied by allergic or immune reaction to foreign agents. However, it is very important to control functional activity of cultured fibroblasts used for injection (production of collagen and collagen contraction). Normal fibroblasts usually produce components of the extracellular matrix including type I collagen [1,8]. Previous studies showed that fibroblasts lose their ability to synthesize matrix proteins after serial passages *in vitro* [7]. Another drawback of cultured autologous fibroblasts is the possibility of their transformation into pathological fibroblasts characterized by abnormal synthesis of fibronectin [5] and glycosaminoglycans [4]. These cells are responsible for fibrosis and scar formation because they induce more pronounced rearrangements and contraction of extracellular collagen fibrils compared to normal fibroblasts, which modify collagen only at certain points [9-11]. Oncogenic transformation of *in vitro* cultured fibroblasts cannot also be excluded. If injected cells are functionally active and not transformed, the graft will produce patient's own collagen for a long time.

We investigated functional activity, contraction capacity, and potential oncogenic properties of cultured autologous human fibroblasts used for augmentation of facial soft tissues.

MATERIALS AND METHODS

Twenty patients aged 37-61 years with facial rhytides and atrophic facial scars were treated with autologous fibroblasts. Biopsy specimens (4 mm) were taken from the retroauricular area and transported to the laboratory in sterile tubes with 10 ml culture medium (Eagle medium supplemented with 10% fetal bovine serum, 0.1 g/liter sodium pyruvate, and antibiotic and antimicotic drugs). The specimens were transferred to

Department of Head and Neck Surgery, University of California, Los Angeles School of Medicine, Los Angeles, California. ***Address for correspondence:*** G. Keller. UCLA Medical Center. 200 Medical Plaza #550. Los Angeles, Ca. 90095.

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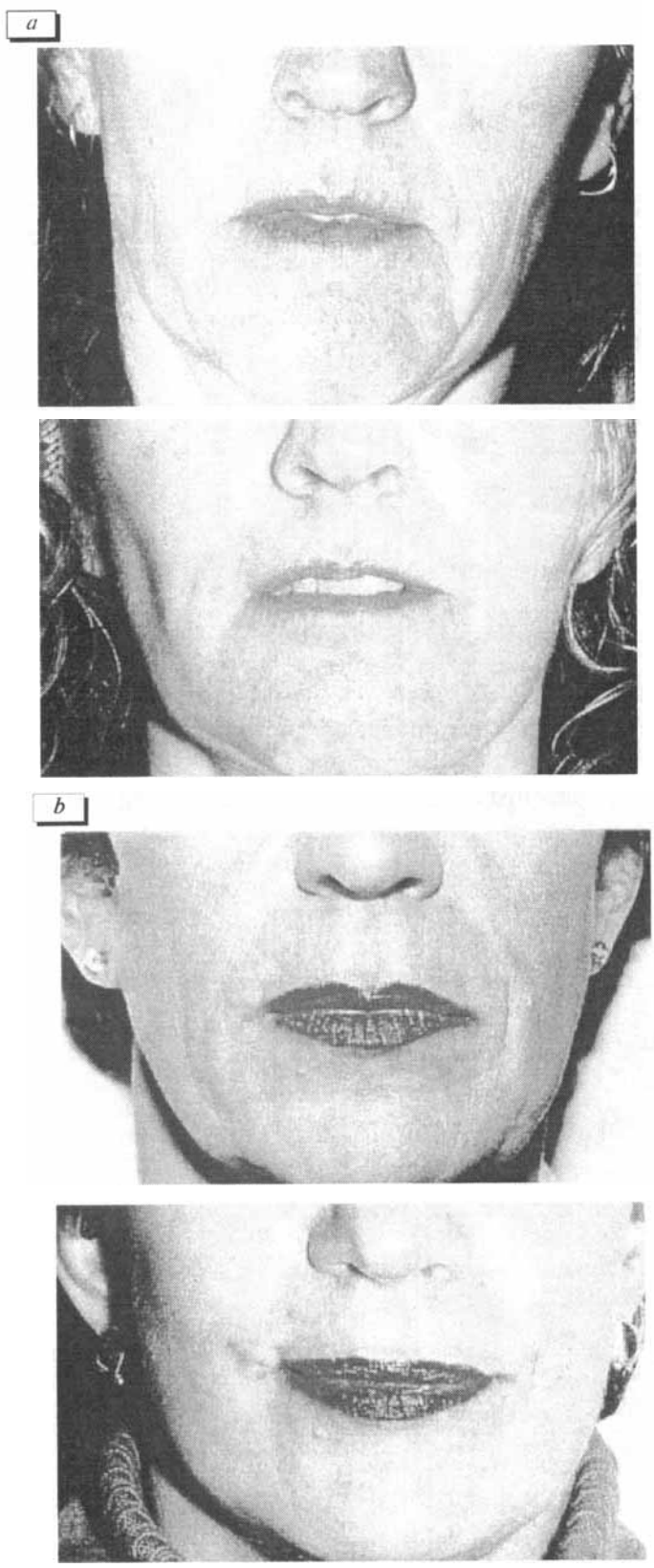

Fig. 1. Use of autologous fibroblasts for correction of nasolabial folds. *a*) 60-year-old woman before (top photo) and 7 months after 3 injections of autologous fibroblasts (bottom photo); *b*) 61-year-old woman before (top photo) and 12 months after 3 injections of autologous fibroblasts (bottom photo).

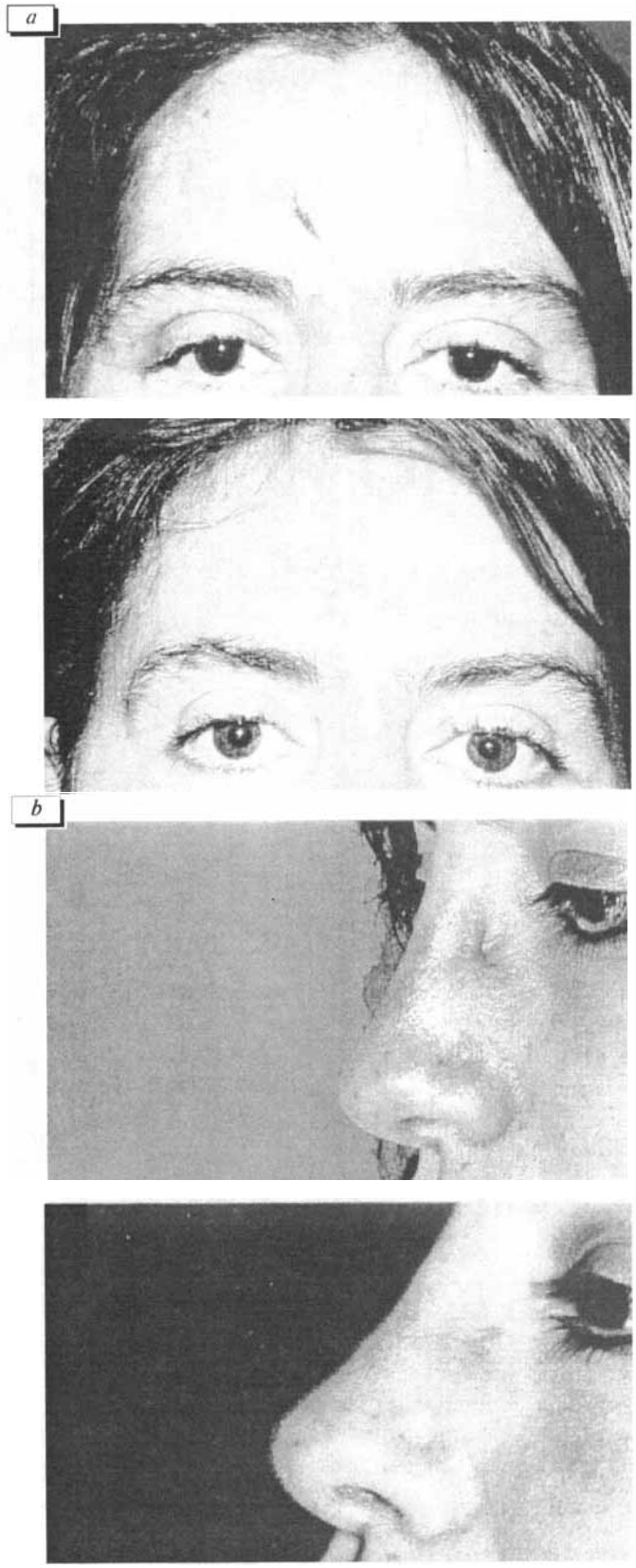

Fig. 2. Use of autologous fibroblasts in patients with scars. *a*) 40-year-old woman with scar on the nose and forehead before (top photo) and 12 months after 3 injections of autologous fibroblasts (bottom photo); *b*) 39-year-old woman with scar on the nose before (top photo) and 7 months after 3 injections of autologous fibroblasts (bottom photo).

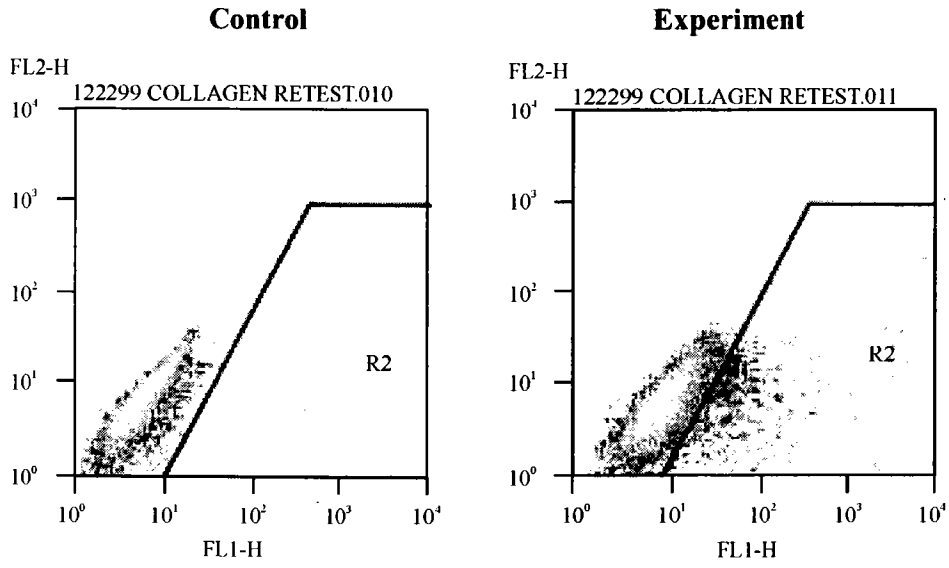

Fig. 3. Expression of type I collagen in fibroblasts after 6 passages in culture.

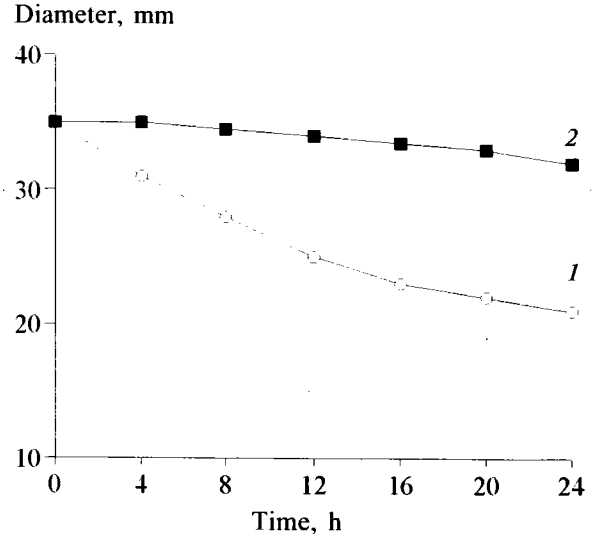

Fig. 4. Contraction of collagen gel after 24-h incubation with fibroblasts isolated from hypertrophic scar (*1*) and normal skin (*2*). Differences are significant ($p<0.05$) in each time point.

60-mm culture dishes and incubated in a CO_2 incubator (5% CO_2). After 2 weeks the cells were trypsinized and seeded to 25-cm^2 flasks. After 5, 6, and 7 weeks in culture, the cells (passages 4, 5, and 6) were injected to the patient in a dose of 4×10^7 cells.

For evaluation of collagen production, 5×10^5 fibroblasts (passage 6) were washed with PBS containing 0.1% sodium azide (Sigma) at 2% fetal bovine serum and stained using mouse monoclonal antibodies against human type I collagen (clone MAB 1340, Chemicon) and FITC-labeled goat polyclonal antibodies against mouse IgG/IgM (36484D-MO38768, BP Phar). The samples incubated only with FITC-labeled goat antimouse antibodies and unstained cells were used as the control. The cells were resuspended with antibodies in 50 μl medium for 30 min on ice [4]. No less than 10^5 cells were analyzed on a Becton Dickenson flow cytometer.

For evaluation of fibroblast contraction potency, 0.2% atelocollagen gel was prepared. To this end, 0.3% pepsin-processed type I atelocollagen (pH 7.3) was mixed with 6-fold Minimum Essential Medium and 10% embryo calf serum (4:1:1). Fibroblasts (passage 6, 10^5 cells/ml) dispersed with 0.05% trypsin and 0.02% EDTA (Gibco) in PBS were mixed 1:1 with 0.2% collagen gel (CG) and the mixture was transferred to 35-mm dishes (3 ml per dish). The dishes were incubated at 37°C and 5% CO_2. The diameter of CG was measured every 4 h for 24 h. Cultured fibroblasts from hypertrophic scar (after passage 6) were used as the control.

For evaluation of oncogenicity of injectable fibroblasts, the cells (passage 7) were injected subcutaneously in a dose of 4×10^7 to two 1.5-month BALB/c nude mice.

RESULTS

Stable clinical improvement was observed in patients receiving injections of AF for correction of nasolabial folds (Fig. 1) and in patients with scars (Fig. 2) 7 and 12 months after the 3rd AF injection.

Flow cytometry showed that cultured fibroblasts actively produced type I collagen (fluorescence intensity 8.49 *vs.* 0.09 arb. units in the control, Fig. 3).

In vitro studies confirmed normal contraction capacity of cultured fibroblasts (for passage 6 fibroblasts the diameter of CG after 24-h incubation was 31.8±0.9 *vs.* 20.9±0.7 mm in the control. Moreover, the dynamics of gel contraction by fibroblasts from normal

skin was characterized by prolonged lag-period from the start of incubation: 6 *vs.* 2 h ($p<0.05$) for fibroblasts from hypertrophic scar (Fig. 4).

Cultured fibroblasts (4×10^7 cells per mouse) induced no tumors in nude mice within 2 months after transplantation.

Thus, we demonstrated that AF used for augmentation of facial soft tissues are characterized by normal collagen production and collagen contraction properties and exhibit no oncogenic potency.

Fibroblasts obtained from 60-61-year-old patients retained their proliferation and collagen-producing capacities in culture. These cells produced type I collagen. We assume that these natural collagen fibers are more resistant to proteases than cross-linked bovine collagen with cleaved telopeptidases [6]. All injectable fibroblasts were characterized by normal collagen-contracting capacity *in vitro* and did not promoted scar formation and fibrosis in patients. Collagen production and contraction did not change during culturing. Moreover, even after 7 passages fibroblasts exhibited no oncogenic potency in nude mice.

However, some drawbacks related to the use of autologous human collagen system were revealed. AF produced a weaker clinical effect compared to other methods, in particular, injection of bovine collagen. Some patients expecting immediate improvement were dissatisfied. However, viable autologous fibroblasts will permanently produce collagen and this newly synthesized collagen will accumulate in target areas. Moreover, the use of patient's own fibroblasts eliminates the risk of immune reaction.

Fibroblasts have a finite lifespan and limited capacity to repair damages, therefore fibroblasts from young patients are more effective. Fibroblast culture can be cryo-stored for future use so that older patient can receive fibroblasts taken from him at a younger age, which will repair defects and rejuvenate skin more effectively.

Thus, AF retaining their functional activity are an ideal filler for face soft tissue correction.

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