EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

The Dynamics of Fibronectin Synthesis in Cultured Human Fibroblasts

E. V. Glushchenko, T. L. Zayats, and G. G. Serov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 575-577, May, 1996
Original article submitted March 22, 1995

Two qualitatively different stages in fibronectin production by confluent cultures of human fibroblasts are demonstrated by immunomorphological and immunobiochemical methods. In the first stage fibronectin is accumulated exclusively in the cytoplasm, while excretory synthesis predominates in the second stage. The maximum production of fibronectin is observed on day 3 in culture. It is concluded that 3-day-old cultures of human fibroblasts are preferable for wound treatment.

Key Words: *fibronectin; fibroblasts*

The treatment of extensive thermal burns with the use of cultured human fibroblasts (FB) has found wide application in clinical practice [1,4-6]. The method is based on the stimulatory effect of FB on the adhesion and proliferation of epidermocytes. Fibronectin (FN), a high-molecular-weight glycoprotein produced by grafted FB, is a key factor in these processes. It stimulates both adhesion and proliferation of epidermocytes [2], and therefore, the level of FN synthesis should be regarded as an important determinant of the optimal times for FB transfer onto a wound. Our objective was to study the dynamics of FN synthesis in a confluent culture of human FB and to determine the times of maximum FN production.

MATERIALS AND METHODS

The dynamics of FN synthesis by human FB was studied in 4th passage cultures. A primary culture was initiated from fragments of the derma [3]. Cells were cultured in Eagle's medium with 10% fetal calf serum and 2% glutamine at 37°C in a CO_2 incubator (Flow). Quantitative and qualitative analyses of the FN content were performed on days 1, 3, and 6 of culturing using immunomorphological and immunobiochemical methods.

Fibronectin was identified by the immunoperoxidase method. Murine mononclonal antibodies against human FN (Antifibronectin, cell attachment fragment, Boehringer Mannheim, No. 1087720) were used. Cell cultures were fixed with acetone for 10 min at -12°C, rinsed with neutral phosphate buffer, treated with the antibody (10 mg dry matter/ml phosphate buffer), washed three times in phosphate buffer, and incubated with anti-mouse IgG conjugated with peroxidase (1:10, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences). Incubation was carried out for 60 min at 37°C in a humidified atmosphere. The cultures were then washed three times with neutral phosphate buffer and treated with freshly prepared substrate solution: 3,3'-diaminobenzidine (Sigma) in Tris-HCl buffer (0.5 mg/ml, pH 7.3) with 3% hydrogen peroxide. Preparations treated with nonimmune serum or with one of the monoclonal antisera served as the controls.

The FN content in the culture medium and in FB was determined by the immunoturbidimetric

A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow

0007-4888/96/0005-0520$15.00

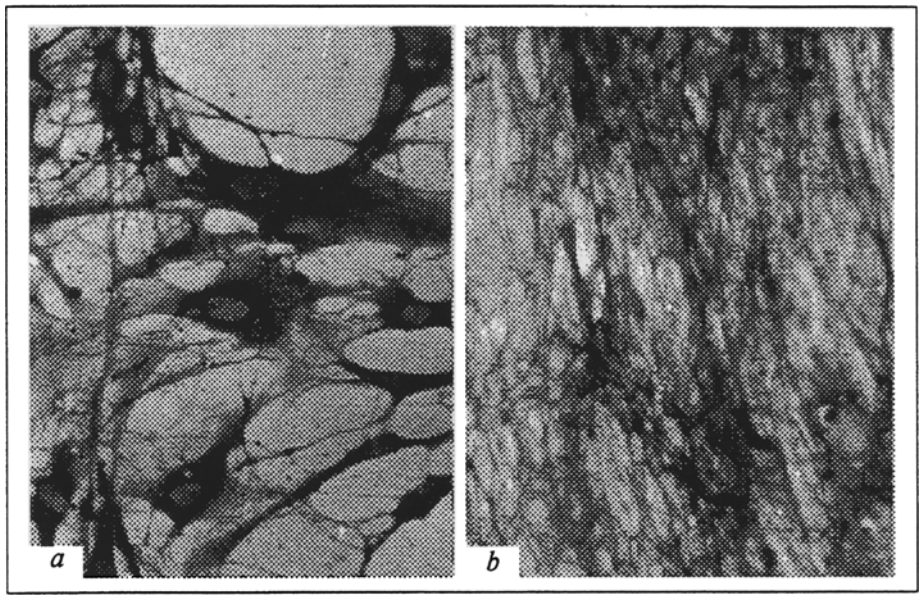

Fig. 1. Accumulation of fibronectin (FN) in the cytoplasm of human fibroblasts on days 1 (*a*) and 3 (*b*) in a confluent culture. Immunoperoxidase method. ×400. *b*) FN is identified in the extracellular matrix as fibrous structures.

method [7] with the use of Boehringer kits. For the determination of the intracellular FN content the culture medium was discarded, FB were lysed with Triton X-100 (final concentration 0.5%), and the FN content was measured in the phosphate buffer (pH 7.4) supplied with the kit.

The number of FB remained constant (10^6 cells) throughout the study, allowing for statistical analysis of data.

In a separate study it was shown that the culture medium contains no fibronectin.

RESULTS

On day 1 in a confluent culture, FN was immunomorphologically identified only in the cytoplasm of FB as small brownish red granules (Fig. 1, *a*). Immunochemical analysis also revealed only intracellularly accumulated FN (Table 1). The culture medium contained no FN.

On day 3, the FN content in the cytoplasm of FB significantly decreased from 12±2 to 7.9±0.5 μg/10^6 cells. The cytoplasm was stained less intensely. Fibronectin was revealed as oriented fibrous structures not associated with the cells (Fig. 1, *b*). The content of FN in the culture medium was many times higher than in the FB cytoplasm (Table 1).

TABLE 1. Dynamics of Fibronectin Synthesis in a Culture of Human Fibroblasts (μg/10^6 cells)

FN content	Day of culturing		
	1	2	3
In cells	12±2	7.9±0.5	5.3±1.5
In culture medium	Not detected	49.9±5.0	40.1±1.2

On day 6, the FN content tended to decrease both in the cells and in the culture medium, remaining considerably higher in the latter. The intensity of FB staining was reduced.

Thus, the synthesis of FN in a confluent culture of human FB proceeds in two stages. In the first stage (24 h in culture), FN is accumulated solely in the cytoplasm of FB. In the second stage (days 3-6), FN is excreted in the culture medium. The FN content in the medium is considerably higher compared with the intracellular content, which is significantly lower than during the first stage. These parameters remained stable during 6 days of observation.

The production of FN in a confluent culture of human FB reached the maximum on day 3. Therefore, the use of 3-day cultures is preferable for wound treatment. It can be hypothesized that intensive synthesis of FN and its secretion are necessary for the realization of its adhesive properties and its ability to stimulate proliferative processes in a wound, where the FN concentration will be maximal at these times.

REFERENCES

1. E. V. Glushchenko, A. A. Alekseev, S. S. Morozov, *et al.*, *Khirurgiya*, No. 11, 26-29 (1993).
2. E. V. Glushchenko, V. P. Tumanov, G. G. Serov, and A. A. Pal'tsyn, *Byull. Eksp. Biol. Med.*, **116**, No. 11, 541-544 (1993).
3. K. N. Grinberg, V. I. Kukharenko, V. N. Leshko, *et al.*, *in: Methods of Cell Culturing* [in Russian], Leningrad (1987), pp. 250-257.
4. D. S. Sarkisov, A. A. Alekseev, V. P. Tumanov, *et al.*, *Khirurgiya*, No. 3, 22-26 (1993).
5. D. S. Sarkisov, E. V. Glushchenko, V. P. Tumanov, *et al.*, *Byull. Eksp. Biol. Med.*, **111**, No. 5, 542-544 (1991).
6. D. S. Sarkisov, V. P. Tumanov, E. V. Glushchenko, *et al.*, *Ibid.*, **109**, No. 3, 400-402 (1990).
7. T. M. Saba, W. Albert, F. Blumenstoen, and G. Evanega, *J. Lab. Clin. Med.*, **98**, 482-491 (1981).