

A New Method for the Establishment of Diploid Fibroblast Cell Cultures from Human Foreskins¹ (39528)

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Human diploid fibroblast cultures are a useful source of cells for many areas of investigation in biochemistry, virology, and genetics. These cultures have usually been established from skin biopsies according to the method of Martin (1) which involves mincing the piece of skin and allowing the fragments to adhere to the bottom of small culture vessels, after which the cells explant. An easily available source of human skin is the foreskin obtained from routine circumcisions, and cultures from these can be established from explants of the minced tissue. Since this source yields a relatively large amount of tissue, it should be convenient for digestion procedures. Methods involving trypsinization of tissue before establishing fibroblast cultures have been reported using embryonic lung and other organ sources (2, 3). A complex digestion procedure for the culture of foreskin, reported by Pious *et al.* (4), yielded a small amount of cells for clonal growth studies. The present communication presents a method for deriving a larger yield of fibroblast cultures from foreskins by a simple trypsin digestion. The new procedure circumvents the explanation of large amounts of minced tissue fragments and yields large numbers of cultured cells in a short period of time.

Methods. Foreskins were obtained with informed consent, and with approval of the Institutional Human Studies Committee, from routine circumcisions performed at the

Philadelphia General Hospital and at the Medical College of Pennsylvania. Foreskins were collected in Dulbecco's phosphate-buffered saline supplemented with glucose (1 mg/ml), gentamicin (50 μ g/ml), amphotericin B (2.5 μ g/ml) and Mycostatin (100 U/ml). Foreskins can remain chilled in this saline for at least 24 hr before processing for culture. After rinsing and removing any adherent adipose tissue, foreskins (average wet weight, 375 mg) were minced and torn into small pieces using sharp scissors and scalpel. This was accomplished in a minimal volume of saline. The minced foreskin was then transferred to 40 ml of a 0.25% trypsin solution in minimal essential medium (MEM) supplemented with amphotericin B (2.5 μ g/ml), gentamicin (50 μ g/ml), and Mycostatin (100 U/ml), and the pH was adjusted to 7.4. The trypsin mixture was agitated slowly at room temperature for 24 hr, or at 37° for 6 hr. The digest mixture was then filtered through lens paper to remove large pieces of undigested tissue. The filtrate was centrifuged at 500g for 10 min, and the resultant cell pellet was dispersed in Ham's medium F-12 supplemented with 20% fetal bovine serum and penicillin (50 U/ml) and streptomycin (50 μ g/ml). Cells were plated in 50-cm² culture vessels containing 10 ml of medium. The medium was replaced within 3 days and at weekly intervals thereafter. When cultures were confluent they were subcultivated using 0.05% trypsin solution in Ham's saline (5); the cells were then grown in a less complete medium such as MEM supplemented with 10% serum.

Results and discussion. By 24 hr, fibroblastic cells could be seen adhered to the vessel surface; in addition, there was a large number of spherical cells, which both adhered to the surface and floated in the me-

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dium. These latter cells could be injured cells or epithelial cells isolated by the trypsin digestion; they did not grow. The typical appearance of the initial culture after the medium change on Day 3 is indicated in Fig. 1a. The fibroblasts had begun to proliferate, and some rounded cells remained attached to the surface. The fibroblastic cells had morphological growth patterns typical of fibroblast cultures (2, 6), and they became confluent within 10 days to 3 weeks. A

typical confluent monolayer at that time is shown in Fig. 1b. The method can yield approximately 10^7 cells per foreskin within 2 weeks.

The storage of the foreskins in the antibiotic-containing saline allowed a delay of at least 24 hr before processing. This procedure also probably aided in decontaminating the foreskin. The majority of the fibroblasts is probably derived from the dermal layer which immediately underlies the epi-

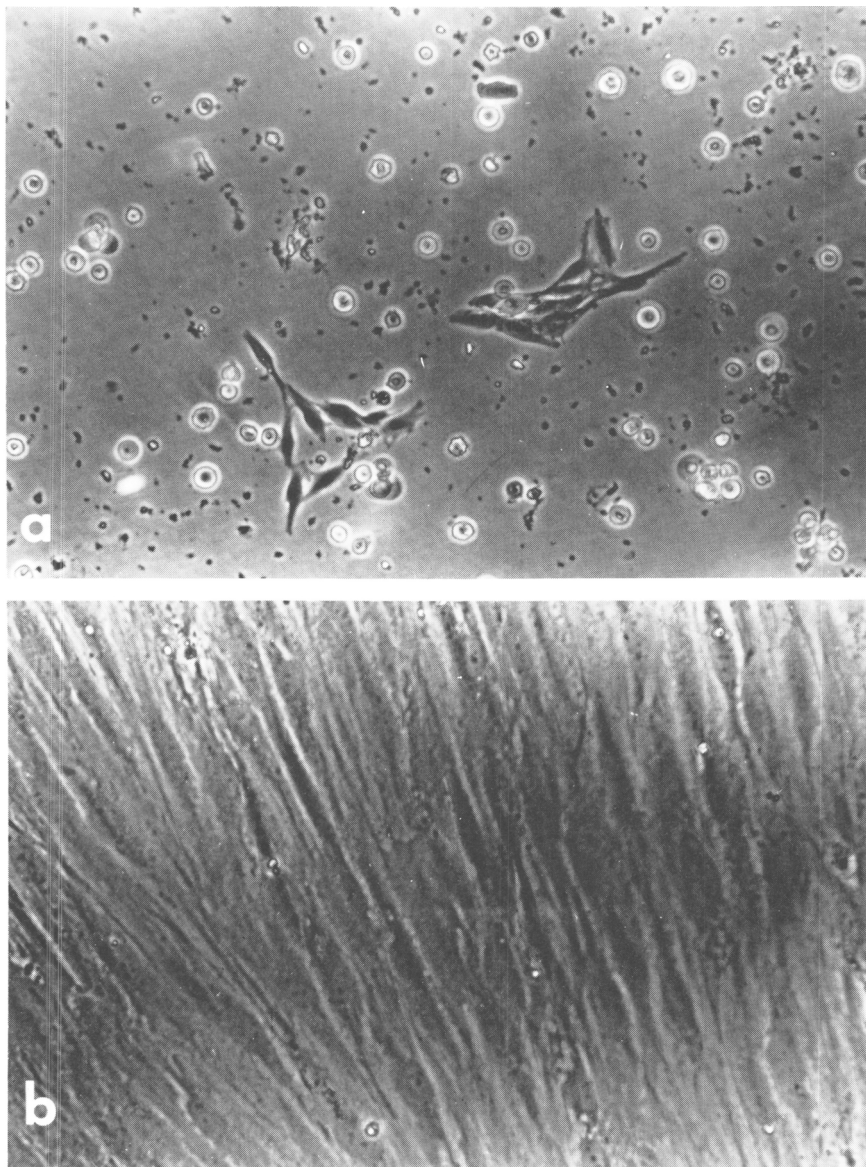


FIG. 1. (a) Phase photograph of human foreskin culture on third day after digestion ($\times 224$). (b) Phase photograph of confluent monolayer of human foreskin fibroblasts ($\times 224$).

dermis. However, it did not seem necessary, and in fact it was technically very difficult, to separate the epidermal layer by dissection before incubation. The careful mincing in very small pieces appeared to ensure adequate digestion of the dermal layer.

A number of different enzymes and media was tried. Neither collagenase nor Pronase resulted in as efficient a cell dissociation as did the standard cell culture with trypsin. The inclusion of antibiotic and antimycotic agents at the stated levels did not seem to inhibit cell yield or growth. Digestion in complete medium at constant pH resulted in an increased cell yield, as compared to a digestion in saline. This method required a much longer period of digestion than that described for other tissues, since skin is more difficult to dissociate than embryonic tissues or most other organs. A number of different digestion conditions was explored (Table I). Digestion could be conducted at room temperature for 24 hr, or at 37° for 6 hr. Digestion at room temperature for only 6 hr resulted in a lower cell yield, whereas cells were probably killed by digestion at 37° for 24 hr. The volumes of the digestion mixtures and the numbers of vessels used for the initial cell cultures were also important (Table II). Separation of the minced foreskin into three or four aliquots of trypsin mix before digestion resulted in lower cell yields. In addition the cells obtained after digestion grew best if initially dispersed into one or two culture vessels; further dilution of the cells led to a lower plating efficiency. The use of the more com-

TABLE I. TRYPSIN DIGESTION OF HUMAN FORESKINS FOR ESTABLISHMENT OF FIBROBLAST CULTURES.^a

Time (hr)	Temperature (°C)	Cell yield (No./foreskin)
6	22	3.4×10^5
24	22	1.0×10^6
6	37	2.2×10^6
24	37	$<1 \times 10^4$

^a The minced tissues from four foreskins was pooled and divided into four parts. Each part was digested for the indicated time and at the indicated temperature in 40 ml of trypsin solution and cultures established as described in Methods. Cultures were harvested when the fullest culture was half confluent, and cell number determined using a hemacytometer.

TABLE II. INFLUENCE OF DIGESTION AND MEDIUM VOLUMES ON CELL YIELDS OF FIBROBLAST CULTURES ESTABLISHED FROM FORESKINS.^a

	A Volume of trypsin mix used per foreskin (ml)	B Number of culture vessels used for initial plating	Cell yield (No./foreskin)
A.	40	4	7.6×10^5
	40	2	5.4×10^6
	40	1	4.2×10^6
B.	40	2	3.4×10^6
	4×10	2	3.0×10^5
C.	40	2	1.8×10^6
	2×20	2	1.7×10^6
	4×10	2	4.7×10^4

^a For each experiment minced tissues from the foreskins were pooled and aliquots dispensed into the trypsin mix as indicated in column A. Cells were collected from each digestion as indicated in the methods section and initially plated in varying numbers of vessels as indicated in column B. Culture vessels were 50 cm² and contained 10 ml of medium. Cultures were harvested when the fullest was half confluent, and cell number determined in a hemacytometer.

plete F-12 medium for the initial plating also appeared to increase the cell yield.

This method for establishing human foreskin cultures has consistently yielded viable cultures. The method has the advantage of yielding a large number of cells with one simple digestion, and it eliminates the difficult requirement of adherence of the minced pieces of tissue to the culture vessel. The cultures that result from multiple digestion appear similar in growth characteristics; however, like all fibroblasts cultures, there is undoubtedly a mixture of cell types present. Cultures obtained by this method have been used successfully for studies of lipid (7) and collagen (8) metabolism, and the method should be applicable for any system requiring human diploid fibroblast cell cultures.

Summary. A method is described for the establishment of diploid fibroblast cell cultures from human foreskins. Foreskins are minced and digested for a relatively long period of time in standard tissue culture medium containing trypsin and antibiotics. Undigested tissue is removed by filtration, and the cells are isolated by centrifugation

and dispensed in culture vessels. This procedure circumvents the explanation of large amounts of minced tissue fragments and yields larger numbers of cells in a short period of time.

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