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Enhancement by Cultured Fibroblasts of Reparative Collagen Synthesis in Rats (38032)

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(Introduced by J. Seifter)

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The critical role of fibroblasts in wound healing has long been recognized. These cells are the sole manufacturers of collagen (1–3), and the synthesizers of essential components of the ground substance such as glycosaminoglycans (3–5). The rates at which the entrance and multiplication of fibroblasts occur in wounds under normal circumstances are well known. These rates are slow enough to have encouraged investigators to attempt to accelerate them, with the expectation that wound healing would be speeded up thereby. Prudden and Wolarsky reported limited success in this regard with cartilage powder and *N*-acetyl-glucosamine polymers applied locally to the wound and with parenterally applied cartilage extracts (6). In the past, fibroblasts were generally considered to be capable of collagen synthesis only after cessation of cell proliferation (7–9) but more recent work shows that fibroblasts growing in culture synthesize collagen rapidly during the logarithmic proliferation phase (10, 11). It appeared possible, therefore, that collagen synthesis, one of the critical steps of wound repair, might be accelerated at early stages by the addition of exogenous collagen-producing cells to healing wounds.

In the present investigation, the effect of the reimplantation of rat fibroblasts grown in culture on the rate of collagen synthesis in subcutaneously implanted "Ivalon" polyvinyl alcohol sponges (12) was studied in rats.

Material and Methods. Materials. All tissue culture supplies were obtained from Grand Island Biological Co., Grand Island, N. Y. Highly inbred male rats (Fischer CDF) were purchased from Charles River Farms, Waltham, Mass. "Ivalon" polyvinyl sponges were obtained from Unipoint Laboratories, High Point, N. C.

Cell Cultures. Rat embryo skin fibroblasts were derived from the dorsal skin of 15–17 day rat embryos by trypsinization (0.25% trypsin, 0.004 *M* Na_2 EDTA) at 37°C for 30–60 min. Fibroblasts from reparative tissue were obtained from polyvinyl alcohol sponges subcutaneously implanted into rats (13) and removed after 4–5 days. The sponges were minced with scissors into fragments approx. 20–60 mm^3 in size and the cells were recovered by trypsinization as described above. The cells were grown in 100 × 20 mm Falcon Petri dishes with 8–10 ml of Dulbecco–Vogt medium (14) containing 0.025 *M* of HEPES buffer (pH 7.35) and supplemented with 10% fetal

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calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml fungizone (amphotericin B) and 50 μg/ml aureomycin, at 36°–37°C in 10% CO_2/air. Subcultures (1:1) were carried out once or twice weekly by dispersion with 0.25% trypsin containing 0.004 *M* Na_2 EDTA. Cell counts were performed by trypsinization and hemocytometer counting. At saturation density, the cell number per plate was 1.5–2 × 10^6 cells.

Reimplantation of Cultured Fibroblasts. The cells were harvested by trypsinization as described. They were washed twice by centrifugation in cold serum-free culture medium and were then resuspended in serum-free medium (1–2 × 10^7 cells/ml). Polyvinyl alcohol sponges (20–25 mg dry wt) were sterilized by boiling for 15 min in 0.85% NaCl and freed of excess liquid by squeezing between sterile 10 × 10 cm gauze pads. 0.1–0.2 ml of the cell suspension was instilled into each sponge. Control sponges contained an equal volume of serum-free tissue culture medium only. The sponges (4 per animal) were inserted aseptically into rats weighing 250–275 g under pentobarbital anesthesia (30–35 mg/kg) in subcutaneous pockets as described previously (13).

Determination of Collagen. At the end of the experiment the animals were killed, the sponges were removed and their collagen content was estimated by hydroxyproline determination according to Woessner (15).

Results and Discussion. Table I compares the hydroxyproline content of sponges inoculated with fibroblasts at the time of subcutaneous implantation and removed 4 and 7 days later, respectively, with sponges inoculated with tissue culture medium only. In sponges removed four days after implantation, a significant increase in collagen content after inoculation with repair tissue fibroblasts was observed although even in the fibroblast-inoculated sponges the collagen content was very low at this time. The difference between the collagen content of sponges inoculated with skin fibroblasts at the time of implantation and removed after four days, while mathematically significant at the $P = 0.02$ level, is of doubtful biological significance. After seven days the collagen content of the fibroblast-inoculated sponges was more than two times higher that that of the control sponges. The amount of collagen present after 7 days in fibroblast-inoculated sponges was approximately the same as that in noninoculated sponges after 14 days. The increase was about equal when skin fibroblasts and when fibroblasts from repair tissue were inoculated.

Since even 4–7 days after implantation the sponge granuloma tissue is highly vascular and filled with a mixed population of various cell types, of which fibroblasts constitute only one component, a reliable comparison of the number of viable fibroblasts in the cell-inoculated and the control sponges was not possible. Preliminary experiments, in which rapidly proliferating fibroblasts were tagged by pulse-labelling

TABLE I. Hydroxyproline Content of Polyvinyl Alcohol Sponges Containing Cultured Fibroblasts Inserted Subcutaneously into Rats.

Source of cells	Days from implantation to removal from animal	Number of animals	Hydroxyproline/Sponge μg ± S.E.[a]		*P*
			Cell-containing sponges	Medium-containing sponges	
Sponge granuloma	4	7	40 ± 9	13 ± 1	0.01
Sponge granuloma	7	17	784 ± 73	296 ± 83	<0.001
Embryo skin	4	10	19 ± 1	16 ± 1	0.02
Embryo skin	7	12	860 ± 58	398 ± 82	<0.001
None	14	8	—	649 ± 26	—

[a] Standard error.

and were then instilled into sponges prior to implantation into rats, showed that 10%–15% of the radioactive label was present in the sponges at the time of removal one week later. This radioactivity was presumably present in viable cells since dead cells and cell debris would likely have been removed from the tissue within this time period. The fibroblasts in the inoculum, although only a fraction of them may remain viable, are thus sufficient to provide a significant acceleration of collagen synthesis during early stages of wound repair.

While further studies are clearly required, our findings suggest the possibility of accelerating the healing process in patients by introducing collagen-forming fibroblasts into wounds. At first, such aid may be limited to elective surgery in patients in whom some impairment of healing might be expected because of their clinical condition, e.g., protein deficiency and other forms of malnutrition or steroid therapy. In such cases, autologous cell cultures could be established prior to surgical intervention. Summerlin *et al.* (16) have recently shown apparent loss of tissue antigens in organ cultures of skin from several species, including human. If these observations are confirmed, the establishment of fibroblast banks for use in cases where the patient's own healing capabilities are limited would be feasible.

Summary. The formation of reparative collagen in polyvinyl alcohol sponges implanted subcutaneously in rats was accelerated when cultured fibroblasts derived from rats of the same highly inbred strain were inoculated into the sponges at implantation. The enhancing effect was comparable whether the fibroblasts were derived from embryonic skin or from repair tissue.

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