Hair Growth and Hair Follicle Cell Proliferation in Histocultured Mouse Skin

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Murray demonstrated that cultured vibrissa follicles from rat embryos could produce hair *in vitro*.[1] Hardy explanted skin from the trunk of mouse embryos, and this tissue produced keratinized pelage hairs.[2] This development was observed in living explants, but was demonstrated only by the comparison of stained sections of cultivated mouse skin. Frater and Whitmore showed that postembryonic mouse skin grown on rat tail collagen gels as a substrate formed hairs only when stimulated by a tryptic digest of early mouse embryos in the culture medium.[3] Hair growth was also slow, only about 0.2–0.4 mm in 4–5 days, and seemed to cease at this point. Recently Philpott *et al.* were able to grow and maintain hair follicles from a rat.[4] Vibrissae follicles of the mouse were also cultured by Buhl *et al.*[5] Very recently Philpott *et al.* have successfully maintained and grown human hair follicles *in vitro*.[6]

We report here studies, utilizing sponge-gel–supported *in vitro* three-dimensional histoculture of mouse skin, that demonstrate that histocultured mouse skin can produce hair that grows in length approximately at the *in vivo* rate.[6]

MATERIALS AND METHODS

Histoculture of Skin

Small pieces of intact normal- or athymic nude-mouse skin (about 2 × 5 mm and 2 mm thick) were cut with a scissors under a dissecting microscope and put onto various substrates, including collagen-containing sponges, cellulose sponges, or combinations of the two in medium as soon as possible after surgery.[7,8] The medium used was Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum and gentamycin. Cultures were maintained at 37 °C in a gassed incubator with a mixture of 95% atmosphere, 5% CO_2.

Fluorescent-Dye Labeling of Live and Dead Cells

Viable cells were selectively labeled with the dye BCECF-AM. Nonviable cells, whose plasma membranes are leaky, were labeled with propidium iodide (PI), a dye that enters only cells with nonintact membranes. Both dyes were used at a concentration of 5 μM.[7] A confocal microscope (Bio-Rad) was used to observe the dye-stained histocultures.

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[^{3}H]Thymidine Labeling of Proliferating Cells

Briefly, cells within the three-dimensional skin histocultures capable of proliferation were labeled by administration of [^{3}H]thymidine at 4 μCi/ml for 3 days and measured by histological autoradiography using a polarizing microscope.[8]

RESULTS AND DISCUSSION

The histocultures were compared for hair growth with respect to their supporting substrate, which includes collagen-containing sponges, cellulose-containing sponges, matrigel-coated cellulose sponges, and combined collagen and cellulose sponges. The most luxurious and extensive growth of hair occurred on the combination substrate of collagen and cellulose sponges (FIG. 1). The hair can be seen growing upward, and the roots of the hair can be seen interacting with the substrate (FIG. 2). When skin from baby mice was explanted, the hair grew extensively and over a long term, reaching over 3 mm by day 10. Importantly, the rate of growth *in*

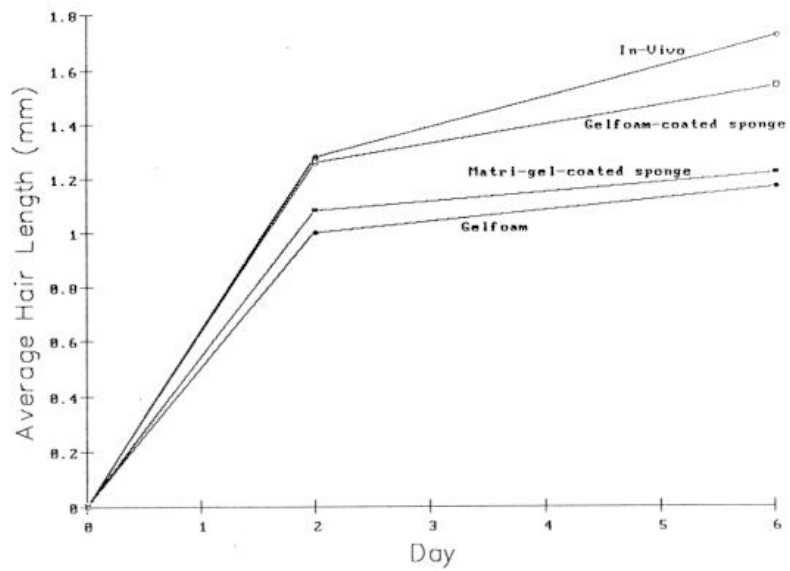

FIGURE 1. Curves demonstrating hair growth *in vitro* on gelfoam, gelfoam-coated cellulose sponge, matrigel-coated cellulose sponge, and *in vivo*. Average hair length has been measured under a dissection microscope at time 0, 2 days, and 6 days. As the curves show, hair growth was much faster in the first 2 days than in days 2–6. Comparatively, hair growth *in vitro* on gelfoam-coated cellulose sponge has the highest correlation with *in vivo* hair growth.

FIGURE 2. Shaved mouse skin histocultured on gelfoam for 2 days. Note the hair growing out from the skin (*arrow*). Confocal scanning laser microscopy projection image. Magnification: 200 ×.

vitro on the combined substrate of collagen sponge and cellulose sponge was similar to the *in vivo* hair-growth rate (FIG. 1). Doxorubicin, which causes alopecia *in vivo*, also prevents hair growth on the histocultured skin.

[^{3}H]thymidine incorporation studied by histological autoradiography was compared in histocultured hairy-mouse skin and histocultured nude-mouse skin. Both types of hair follicles extensively incorporated [^{3}H]thymidine into their cells.

The histocultured skin system described here can be used to study the stimulation of growth of hair and its inhibition in the native state and should prove to be more useful than the study of isolated hair follicles in culture.

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