A model for evaluating the capability of dermal papilla cells to induce hair follicles morphogenesis

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INTRODUCTION

The hair follicle (HF) morphogenesis is governed by the inductive interactions between dermal and epidermal tissue. The studies from a number of investigators have shown that dermal papilla cells (DPs) play pivotal roles in hair formation, growth, and cycling (Stenn K.S. 2001). However, it has been proved that cultured DPs retain their original ability to elicit hair growth during early passages only (Horne K.A. 1986, Messenger A.G. 1984, and 1986). How to evaluate the capability of DPs is a challenge. In this study, we report a model for estimating the potency of DPs, by encapsulating the DPs with alginate-polylysine-alginate (APA) and implanting the DPs microcapsules into the hairless region of rat ears which lack follicles and sebaceous glands.

MATERIAL AND METHODS

Intact dermal papillae were obtained from human scalp follicles. Briefly, the connective tissue was sliced with scissors until the hair-bulb regions were visible, then mixed with collagenase I (2mg/ml, Gibco) and incubated at 37°C for 2–3 h. Next, the mixture was sucked repeatedly into a pipette to free most of the dermal papillae, which were then collected using a pipette gun under a binocular dissecting microscope. The dermal papillae were cultured in Dulbecco's modified Eagle's medium (Gibco) at 37°C in a humidified atmosphere containing 5% CO2.

Confluence cultures of third passage DP cells were microencapsulated using a specially designed high-voltage electric droplet generator, by a modification of O'Shea method (O'Shea G.M.1986). Briefly, the cultured DPs (5×10^5 cells ml^{-1}) were suspended in a mixture of 2% (w/v) sodium alginate (Sigma), the spherical droplets were formed by the high-voltage electric droplet generator with the optimized operation parameters. After washing with 0.9% saline, the droplets were suspended in 1% (w/v) poly-L-lysine (MW 15,000–22,000, Sigma) for 10 min and suspended in 0.2% (w/v) sodium alginate for 4 min. Finally, they were treated with 0.05 M sodium citrate for 6 min to liquefy the interior gel of the capsules. As a result, the high-voltage electric system produced uniform DPs microcapsules with the features of spherical shape, smooth surface, hollow, and diameters ranging from 0.3～0.5mm. The viability of the microencapsulated DPs was determined by the MTT [3-(4, 5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide] assay.

Sprague-Dawley rats (SD rat, 3–6 months old, equal numbers of males and females, $n = 10$) were obtained from the Center of Research Animals, Shantou University Medical College. All experiments were approved by the Ethical Committee on Research Animal Care from the First Affiliated Hospital, Shantou University Medical College. Three days prior to each operation, the culture medium was removed from the DPs microcapsules and replaced with serum-free medium. The animals were anesthetized with ketamine and the implantation sites (the hairless region of rat

ear) were swabbed with 75% ethanol and then 0.9% saline . In each ear designated for implanting manipulation, 1 ml of air was injected subcutaneously to isolate the epidermis and a 2 mm incision was made with the tip of scalpel blade. Then 0.1 ml (approximately 30～50 microcapsules) of DPs microcapsules solution was injected into each incision, using a 16# injection needle, which prevented the fragmentation of microcapsules. The needle were controlled in the subcutaneously all along. At the same time, the microcapsules were injected slowly and uniformly. Finally, remove the needle and apply gentle pressure to the site and sealed the incision with biogel. In each rat, the other ear was injected with empty microcapsules as control. Experimental animals were killed every week between 4 and 12 weeks. Postoperative the implantation sites were biopsied for histologic observation.

RESULTS AND DISCUSSION

Within 4～8 weeks postoperatively, hair follicle structures were found in 8 of 10 rat ears implanted with DPs microcapsules (Fig. 1).

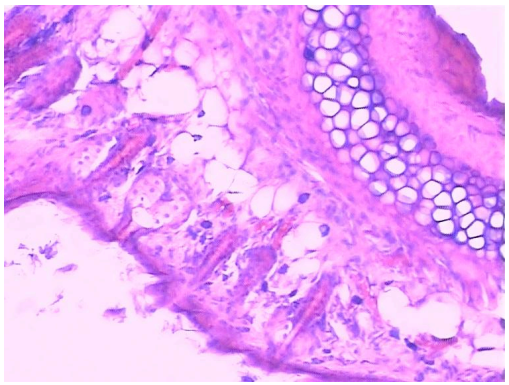
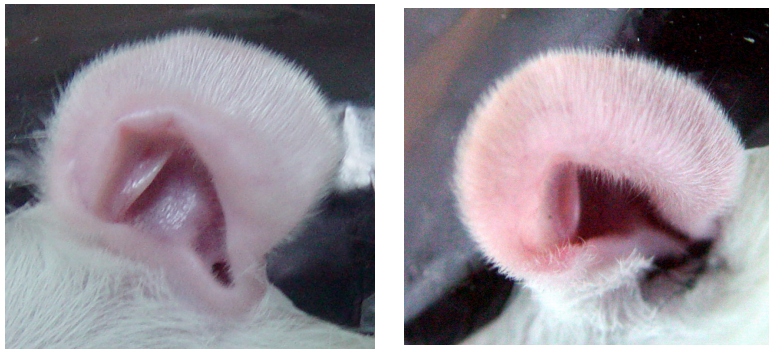


Figure 1: The regenerated hair follicles： Many follicles were formed in the hairless region of rat ear. HE stain ×200

Histologic examination of these specimens showed that a number of hair follicles and sebaceous gland-like structures were formed. Sudan IV proved the structures around the regenerated follicles were sebaceous gland. The most interesting finding was that the total number of regenerated follicles was greater than the number of injected DPs microcapsules. At 6 weeks following transplantation, white (unpigmented), high density, and regular size of hair fibers were visible in the implantation site (Fig. 2).



A B

Figure 2: The hairs distribution before and after implanting the DPs microcapsules in the hairless region of rat ear: (A) before implanting. (B) 6 weeks following transplantation, white, high density, and regular size of hair fibers were visible.

In the control series, there were no significant changes and no evidence of accumulation of inflammatory cells in the implantation site. These findings demonstrated that inductive signals from the microencapsulated DP cells were sufficient to drive follicle formation in competent epidermis. Based on these data, we believe that implanting the DPs microcapsules into the hairless region of rat ear could be used as a model for evaluating the capability of dermal papilla cells to induce hair follicles morphogenesis and regeneration. There are some advantages in this model: first, APA microcapsules are permeable to small molecules secreted by dermal papilla cells, impermeable to large molecules such as immunoglobulins and albumin, that is, DPs microcapsules avoid graft rejection after xenotransplantation. As a result, the epithelial-mesenchymal interaction in the first steps of hair development could be initiated effectively and stably by implanting exotic DP cells. Second, the inductive signals from DP cells could be standardized by encapsulating the DP cells uniformly, which was very helpful to evaluate the dermal influence on the follicle morphogenesis and regeneration quantitatively. Third, the hairless region of rat ear was chosen as the receptor since it lacks follicles and sebaceous glands and the influence of host DPs on follicle morphogenesis could be excluded. Moreover, the hairless region of rat ear was a relatively small area which was easily scrutinized.

CONCLUSIONS

The microencapsulated DP cells retained the dermal influence on the follicle morphogenesis and regeneration. The epithelial-mesenchymal interaction could be initiated and new fiber-producing follicles could be formed in the hairless region of rat ear, by implantation of the microencapsulated exotic DPs. These data demonstrated that implanting the DPs microcapsules into the hairless region of rat ear could be used as a model for evaluating the capability of dermal papilla cells to induce hair follicles morphogenesis and regeneration. Moreover, the animal model reported here provided a practical method to study the molecular mechanism by which the follicle development.

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