**In vitro** Culture and Histological Characterization of Extracted Human Hair Follicles

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**Abstract**

**Background:** In vitro-cultured hair, follicles can serve as an excellent model system for exploring the hair follicle biology as well as drug delivery targets. In this study, we aimed to investigate how to maintain whole human hair follicles in vitro and histologically characterize the hair follicles thereof.

**Methods:** Donated hair follicle specimens from hair transplant operation were cultured in vitro using a medium consisting of Williams’ medium E, L-glutamine, insulin, hydrocortisone, penicillin, streptomycin, and amphotericin B. The growth of hair follicles was examined daily using a digital microscope. Their histological features were studied at day 0, 7, and 14 to characterize the changes of hair follicles maintained in culture.

**Results:** Cultured hair follicles continued to grow at a rate of about 200 μm/day during the first four days. Then the growth rate declined and stopped after an average (range) of 8.4 (7-11) days in culture. Histological study showed an upward regression of the hair follicles with scattered apoptotic of outer root sheath cells, resembling the follicles in a catagen stage.

**Conclusion:** Human hair follicles can be cultured in vitro for at least 7 days before progressing to catagen.

**Keywords:** Human hair follicle; Hair culture; histological change; Hair measurement

**Introduction**

Hair transplantation surgery is indicated for the patients with male-pattern baldness that is unresponsive to medical treatments. This procedure can be performed in two different approaches. The first one is the strip harvesting technique, a strip of scalp is removed under local anesthesia and then cut into single follicular units, which are then transplanted back into the balding area in order to have re-growth hairs. The other approach is Follicular Unit Extraction (FUE), which is a novel, convenient and effective technique. Individual Hair Follicles (HF) are extracted and from the occipital scalp and then reinserted back into the recipient area [1,2].

A follicular unit is a skin appendage with complex structures containing many cell types resulting from epidermal-dermal interactions. It consists of epidermis as a matrix, inner root sheath, bulge, outer root sheath, and dermis such as connective tissues and dermal papilla. In addition to these components, follicular stem cells with highly pluripotent properties have been reported to reside in many parts of the follicle. The stem cells are not only limited to epidermal stem cells at the bulge but also include melanocyte stem cells and mesenchymal (nestin-expressing) stem cells [3-8]. These hair follicles stem cells could potentially serve as gene therapy targets for regenerative medicine [9-12].

To study the hair follicles biology or use hair follicles cells as drug delivery or gene therapy targets, in vitro culture of HFs would be of great use. There are several reports about in vitro culture of hair follicles published to date [13]. However, most of the studies focused only at the inferior portion of hair follicles because the length of the whole hair follicles usually exceeds the low power field of most microscopes. Moreover, the histological studies of cultured hair follicles are also limited. In this study, we aimed to culture whole human hair follicles in vitro and studied their morphology and growth using a novel technique based on a digital microscope, which has a wider filed, enabling a study of long hair follicles. The hair follicles were also histologically examined for any changes after cultured in media up to 14 days.

**Materials and Methods**

**Human hair follicle collection**

This study was approved by the institutional review board at Siriraj Hospital. Human HFs (77 HFs from 10 donors) were obtained with informed consents from the patients undergoing elective hair transplantation surgery for androgenic alopecia at the Department of Dermatology, Siriraj hospital, Mahidol University. All donors were more than 18 years of age. Single follicular units were obtained through either the strip harvesting or FUE techniques. Hair follicles were selected and transported to the laboratory in sterile culture plates containing normal saline solution, which were placed in an ice container.

**In vitro culture of hair follicles**

Isolated hair follicles were cultured using a method modified...
from Philpott et al. [13]. Each isolated follicle was immediately placed into a well of a 24-well plate filled with 500 mL of culture medium. The medium consists of Williams’ medium E (Gibco™), which was developed during the establishment of an enrichment technique for adult rat hepatocytes based on sequential reduction of the amount of contaminating fibroblasts during culture. Moreover, it contains higher amounts of trace elements and exhibits small variations in the supplementation. In addition, the medium was supplemented with 2 mmol/L L-glutamine (Glutamax®), hydrocortisone 10ng/ml, insulin 10 µg/ml, penicillin 100 U/ml, streptomycin 100 µg/ml, and amphotericin B 25 µg/ml. In the negative control group, 0.9% NaCl supplemented with antibiotics was added instead of the medium. All cultures were incubated at 37°C in an atmosphere of 5% CO2 and 95% air. The medium was replaced every other day.

**Examination of hair follicles using a digital microscope and image analysis**

A handheld digital microscope (Dino-Lite™ Polarized microscope) which has a field of view of 9 × 7 mm was used to examine and photograph the hair follicles instead of the inverted microscope due to its wider study field. The images of each follicle were taken daily for 2 weeks. DinoXcope program (Dino-Lite™) was used to analyze the length of hair follicles from digital images. The length measured from the top end of the cutted follicle to the bottom end of the bulb is defined as Total Hair Length (THL) and the length of hair shaft above the epidermis is defined as External Hair Length (EHL). The diameter (D) of hair shaft was measured at the infundibular opening. The endpoint of the follicle growth is defined as the time when there is no increase in total hair length for 3 days.

**Histological changes of in vitro hair follicles**

One to two longitudinal sections of hair follicles at day 0, 7, 14 were bisected and processed in a routine manner that included 10% neutral-buffered formalin fixation, embedding in paraffin, sectioning at 4 µ staining with hematoxylin and eosin and examined under a microscope.

**Statistical analysis**

The data were shown as means and standard deviation. Statistical analysis using one way repeated ANOVA was used to show significant increase of follicle length during in vitro culture. Pearson’s correlation coefficient was used for correlation analysis. The difference was considered significant when P value is less than 0.05.

**Results**

**Study of whole human hair follicle in-vitro**

The whole hair follicles including the epidermal part, the isthmus part and the lower portion could be isolated using FUE techniques. The images of a whole hair follicles could be clearly and conveniently taken by a handheld digital microscope (Dino-Lite™ Polarized microscope), which allows a wider field of study up to 9 × 7 mm; an example is shown in Figure 1.

**Growth of human hair follicles in vitro**

Cultured hair follicles continued to grow in vitro, particularly during the first few days; sequential images of a follicle at various days after culture clearly demonstrate an elongation of hair shaft and follicle as shown in Figure 2. When the lengths of hair follicles were measured and plotted against the time after culture, an increase of hair length seems to be in linear fashion in the first four days with an average growth rate of 0.24mm per day, then slowly declined and eventually stopped growing after an average of 8.4 days after culture (Figure 3). Accumulative measurement data from 10 subjects, 77 hairs were maintained in modified Williams’ medium E and 42 hairs were maintained in NSS which is the standard solution using in transplantation operation (Table 1). The hair follicles in NSS were not growing at all because there are separation of outer root sheath and basement membrane in histological section (data not shown). The hair follicle in modified Williams medium E could be growth average 1.86mm (0.98 – 2.52) with mean growing period of 8.4 days (7-11).
The average growth rate of all hairs was 0.21mm per day (1.4-2.7). The average hair diameter of occipital terminal hair of this study was 75.7 μm.

**Histological change**

The histological character of *in vitro* hair at day 0, 7 and 14 shows normal hair follicle at the beginning (Figure 4). At day 7 in culture medium, bulb remains intact, but decrease of matrix cell and 1-2 necrotic cells in outer root sheath can be observed. At day 14, changing of bulb shape, separations of matrix cell and scatter apoptosis of outer root sheath cell can be seen. In some instances upward regression of and hair follicle may also be observed (data not shown), which resemble catagen stage.

<table>
<thead>
<tr>
<th>No.of follicles (Media/NSS)</th>
<th>Case1</th>
<th>Case2</th>
<th>Case3</th>
<th>Case4</th>
<th>Case5</th>
<th>Case6</th>
<th>Case7</th>
<th>Case8</th>
<th>Case9</th>
<th>Case10</th>
<th>x/S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (μ)</td>
<td>74.5</td>
<td>75.0</td>
<td>77.7</td>
<td>78.5</td>
<td>76.5</td>
<td>72.8</td>
<td>79.9</td>
<td>69.4</td>
<td>78.8</td>
<td>773.9</td>
<td>75.7/8.5</td>
</tr>
<tr>
<td>Growth period (days)</td>
<td>6.8</td>
<td>7.5</td>
<td>7.8</td>
<td>8.2</td>
<td>8.2</td>
<td>8.3</td>
<td>10.9</td>
<td>9.4</td>
<td>9.5</td>
<td>7.3</td>
<td>8.4/1.2</td>
</tr>
<tr>
<td>Growth rate (μ/day) (Media/NSS)</td>
<td>140/0</td>
<td>140/0</td>
<td>216/0</td>
<td>187/0</td>
<td>199/0</td>
<td>252/0</td>
<td>229/0</td>
<td>270/0</td>
<td>260/0</td>
<td>216/0</td>
<td>210/45 and 0/0</td>
</tr>
<tr>
<td>Growth (μ)</td>
<td>980/0</td>
<td>922/0</td>
<td>1649/0</td>
<td>1973/0</td>
<td>2081/0</td>
<td>2518/0</td>
<td>2518/0</td>
<td>2365/0</td>
<td>2173/0</td>
<td>1419/0</td>
<td>1862/7/917 and 0/0</td>
</tr>
<tr>
<td>No growth hair in Williams’ media E</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation between hair diameter, follicle length and growth rate**

Statistical analysis of growing data showing both growth rate and total growth are synthesized with Pearson’s correlation coefficient 0.836 (p<0.001) (Figure 5). Sizes of hair (all terminal anagen hair) are not correlated with hair growth while follicle length and hair growth showed significant negative relation with Pearson’s correlation coefficient, 0.017 and -0.358 (p<0.05), respectively.

**Discussion**

Previous studies, focused most on the inferior portion of hair follicles [13]. A few studies showed growth of whole hair follicles,
including up to the epidermis, but no histological profile. One of the obstacles is that the field of study of the inverted light microscope is limited, at 5mm (4x lens), while average length of human scalp terminal anagen hair follicle is 5-6 mm. One study used a digital camera and a measurement program to study whole hair follicle, but it must take the follicle out of the medium [14]. We developed a study system using Dino-Lite™ Polarized microscope, which has the measurable study field of 9 x 6.7 millimeter. This camera has also been used in dentistry research. The digital microscope comes with DinoXcope program (Dino-Lite™), the digital measurement program. This microscope could be used under the culture plate to observe hair growth without removing the medium. We checked digital measurement vs. length reliability by using the standard scale in chamber under culture media which indicated that length from digital program and true length are reliable with standard error less than 20 µm per 1000 µm. Our system shows the clear image of a hair follicle and can be used to observe a follicle in a culture plate without taking it out of media.

The human hair follicle is source of stem cell reserve [3-8]. Gene therapy targeted hair follicle might be a novel technique to treat difficult dermatologic and non-dermatologic diseases [15]. Gene transduction targeted hair follicular cell can be done by using both viral vector and non-viral vector [16,17]. Transduction of Green Fluorescent Protein (GFP) gene to hair follicle can be detected as visible green fluorescence by fluorescent microscope within 48 hours after transduction. We show that a whole hair follicle, including sebaceous and epidermis, could maintain growth in fetal cow serum free media formula for 7-10 day. This 1-week period is enough as a model to study in-vitro hair follicular gene therapy.

In-vitro maintenance of hair follicles has shown that hairs stop growing after about 1 week. Philpott et al. shows the histology of in vitro hair culture at day 9, in the same medium, shows intact morphology and is seen to remain in the anagen phase [13]. This work was done with the lower portion of the hair follicle without epidermis. In our work, histological study at day 7 shows intact morphology. But a few necrotic cells at the outer root sheath and decreased cell number of the hair matrix were observed. At day 14, disintegration of hair matrix and epithelium column was seen; there were also groups of necrotic cells at outer root sheath, which resemble turning from anagen to telogen hair [18-20].

In-vivo human scalp anagen hairs grow 0.3-0.4mm per day and the growth rate increasing in the larger diameter [21]. In-vitro correlation between the grow rate and the hair diameter had not been defined. In our work, statistical analysis shows total growth of hair was dependent on the growth rate, but we could find the relation between hair size and growth rate.

In summary, we developed a system that can observe in vitro hair follicle growth using digital dermoscope. The hair follicle can maintain growth in modified Williams’ medium E about 7-10 days, before turning to catagen and stop growing. The total growth of hair was dependent on the growth rate but has no relation to the diameter size.

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References