INTRODUCTION

In culture, dermal papilla cells (DPCs) grow more slowly and senesce more rapidly than dermal fibroblasts and most populations of DPCs become growth-exhausted after five-ten subpassages.\(^1\) Cultured rat vibrissa DPCs induce hair follicle neogenesis upon implantation at early passage number,\(^1\) but cells with later passage number lose their hair regeneration properties.\(^2\) Cultured human DPCs also maintain their hair inductive properties before passage four.\(^3\) Many variations in DPC culture methods have been studied in an attempt to enhance their follicle inductive properties when DPCs are implanted.\(^4\) The ability to regenerate new hair \textit{in vivo} is associated with aggregative behavior \textit{in vitro}.\(^9\) Jahoda and Oliver\(^10\) were the first to observe that DPCs tended to align. Groups of DPCs start to pull apart and form aggregates before or at the same time as forming a confluent monolayer configuration. These cell groupings then become isolated clumps which consist of a...
Sari, et al.: Ovine factors effect on human DPC aggregation

combination of rounded cell clusters and more elaborate ridge-shape structures. While ovine DPCs are known to have superior aggregative and inductive properties to murine or human DPCs, the effects of ovine DPC on the aggregation or hair inductive activity of human DPC have not previously been investigated.

DPC aggregation is controlled by secreted soluble factors, such as transgelin, porin 31 HM, and heat shock protein 70. Culture medium containing these secreted soluble factors can be created to restore aggregation in long-term cultures of human DPCs that have lost their aggregative behavior. Interaction of epithelial-mesenchymal cells in different species has been observed previously since hair regenerated after rat and human DPC implantation in mice. Ovine DPCs maintain robust aggregative behavior after extensive culture in vitro, therefore it seems that their expression of key secreted soluble factors for maintaining aggregation is relatively stable. Hence, we hypothesized that soluble factors secreted from ovine DPCs could interact with human DPCs to increase their abilities in inducing aggregation and potential for producing new hair follicles. In this study, optimal culture conditions were first established because ovine and human DPCs are normally grown in different medium. Then, direct co-culture between ovine and human DPCs was tested to determine whether the human cells could incorporate into the aggregates made by ovine DPCs. Finally, ovine and human DPC co-culture separated with a semipermeable membrane was performed to determine whether soluble factors secreted by ovine had effects on human DPCs.

SUBJECTS AND METHODS

Animal and human samples

Collection of human specimen was approved by the Human Ethics Committee of Epworth Hospital, Melbourne. Scalp skin specimens were obtained from patients undergoing elective removal of pilar cysts. The skin overlying a pilar cyst was the surgical discard material following removal of the cyst. This skin contained normal hair follicles, and so was well-suited for initiating human DPC cultures. Roswell park memorial institute (RPMI) and 20% fetal calf serum (FCS) (v/v) with 2 ng/mL fibroblast growth factor-7, 200 units/mL penicillin, 0.2 mg/mL streptomycin, 25 ng/mL amphotericin B, and 1000 µg/mL fungizone (components from Invitrogen, Carlsbad, CA, and Sigma, St. Louis, MO, USA) was used as the transfer medium of the specimen. The specimen was then briefly washed with this medium three times. The lower follicle was dissected from subcutaneous fat using microscissors and scalpels. Individual papillae were isolated using needles and fine forceps, and transferred to a well of a four-well Multidish (Nunc, 1.9 cm²). Cultures were maintained at 37°C/5% CO₂ for 1 week without being disturbed. Once cell outgrowth from the explants had begun, the medium was renewed twice weekly with RPMI and 10% (v/v) FCS supplemented with 100 units/mL penicillin, 0.1 mg/mL streptomycin, 12.5 ng/mL amphotericin B, 250 µg/mL fungizone (components from Invitrogen, Carlsbad, CA, and Sigma, St. Louis, MO, USA).

Two lines of ovine DPCs were compared for all experiments. Each was isolated from wool follicles collected from the lateral neck of a different Romney crossbred lamb, as previously described. Frozen stocks of DPCs were thawed and grown in medium depending on the serum of the freezing mix. DPCs which were in 90% (v/v) lamb serum (LS) and 10% (v/v) dimethyl sulfoxide used minimum essential medium (MEM) with 20% (v/v) LS supplemented with 100 units/mL penicillin, 0.1 mg/mL streptomycin, 12.5 ng/mL amphotericin B, glutamax (x100), 250 µg/mL fungizone (components from Invitrogen, Carlsbad, CA, and Sigma, St. Louis, MO, USA) as their medium. Cells were allowed to adhere to the base of a 100 mm petri dish or 75 cm² tissue culture flask at 37°C/5% CO₂ overnight before performing full medium change. In the co-culture experiment with human and ovine DPCs, both types of cells must have the same medium; therefore, the medium was modified into the desired medium. For this case, ovine DPCs were cultured with MEM 20% (v/v) LS, thus, MEM with 20% (v/v) LS was the old medium and was modified to RPMI 10% (v/v) FCS since this medium was used to culture human DPCs. The percentage used for adapting the cells to the new desired medium were 50% old medium: 50% new medium on day 1, 10% old medium: 90% new medium on day 2, and finally, 100% new desired medium on day 3.

Immunofluorescence and histochemical staining

Cells for immunofluorescence experiments were either grown on glass coverslips or directly in tissue culture plates (made of polystyrene or permanto). For cells on coverslips, the medium was removed, the cells were briefly washed in phosphate-buffered saline (PBS) (three times), and the specimen was fixed with ice-cold acetone for 10 min. For cells in tissue culture wells, the specimen was fixed with 90% (v/v) ethanol, 5% (v/v) acetic acid, and 5% (v/v) of water for 20 min. Then, the specimen was briefly washed in ice-cold PBS (three times). In these experiments, before the cells were incubated with
the first antibody, second antibody, or 4',6-diamidino-2-phenylindole (DAPI), the undersides of coverslips were blotted on tissue paper and then the coverslips were placed on a piece of parafilm, on the lid of a plate above the original well. For cells in tissue culture wells, the well was covered with parafilm after blocking instead of performing this step. The wells and coverslips were placed in a dark humidified chamber, followed by blocking the cells in 1% (w/v) BSA/0.025% Triton X-100 for 2 h. After 2 h, the specimen was washed three times in PBS for 5 min. The specimen was incubated first with antibodies at 4°C overnight, while the negative controls were maintained in the blocking solution. The primary antibodies used were mouse antihuman versican (1:2000 dilution, DSHB, clone 12C5), mouse antiovine major histocompatibility complex (MHC) Class I (1:2000 dilution, Novus Biologicals, NB 100-65416), and rabbit antihuman and ovine COX IV (1:2000, Cell Signaling, 3E11). The next step was to wash the cells (three times) in PBS for 5 min. The cells were protected from light as much as possible before incubating the specimen with the second antibody for 2 h. Secondary antibodies were antimouse IgG1 (1:200 dilution, Life Technologies, A-21121) for versican and MHC Class I, and antirabbit IgG (1:200 dilution, Life Technologies, A-21206). Then, the specimen was washed (three times) in PBS for 5 min. 0.2 µg/ml DAPI was added for 10 min, then the specimen was washed again (three times) in PBS for 5 min. For the cells on coverslips, the underside of coverslips was blotted on tissue paper before mounting in fluorescence mounting medium and photographing.

To measure the size of aggregates, cells grown on glass coverslips were stained with either crystal violet or van Gieson’s solution. Cells were stained with crystal violet by washing them (two times) with PBS followed by fixation and staining with 0.5% (v/v) crystal violet in 25% (v/v) methanol. After staining, the cells were briefly washed in PBS (five times) in preparation for photography.

For staining with van Gieson’s solution, the specimen was briefly washed in PBS two times, then fixed with 90% (v/v) ethanol, 5% (v/v) acetic acid, and 5% (v/v) water for 20 min. The specimen was then briefly washed three times with water before adding the van Gieson’s solution for 3 min. Another four washes with water of 2 min each were performed. Then, the specimen was washed in 50% ethanol (5 min), 70% ethanol (brief wash), 95% ethanol (brief wash), and 100% ethanol (two washes of 3 min each) and was photographed as soon as possible. For both crystal violet and van Gieson’s solution stained cells, aggregate diameters were measured with image analysis. The image analysis gave length and width of the aggregates, and the diameter was calculated from the square root of area from the length and width.

**Direct co-culture**

Co-cultures were grown in 6-well plates. Both ovine and human cells were seeded in the plates with different ratios: 100:0 human:ovine, 90:10 human:ovine, 50:50 human:ovine, and 0:100 human:ovine at a total density of 10^5 cells/cm². The two cell types were stained with vital dyes to allow them to be distinguished in living cultures [Table 1]. The human DPCs were stained with DiI (red) and the ovine cells with DiO (green). The anti-MHC Class I and anti-COX IV antibodies, which have different species specificity were also evaluated for tracing the cells in co-cultures.

**DiI and DiO staining for ovine and human dermal papilla cells**

DiI and DiO staining were used to differentiate ovine and human DPCs in direct culture. After passaging the cells, the cells were resuspended at 10^6 cells/ml. About 5 µl/ml DiI was added to human cells while 5 µl/ml DiO was added to ovine cells. The cells were incubated at 37°C for 20 min, followed by centrifugation for 5 min at 368 g, then resuspended in same volume as before. This centrifugation and resuspension were repeated twice before counting the cells to be divided into the wells. After confluency was reached, the cells were fixed with 90% (v/v) ethanol, 5% (v/v) acetic acid, and 5% (v/v) of water and stained with 1 µg/ml DAPI using the same steps as in previous experiments.

**Major histocompatibility complex: Class I and COX IV double staining for ovine and human and dermal papilla cells**

After the cells were passaged, they underwent direct co-culture in a 6-well plate using coverslips coated with 0.08 mg/ml Type I collagen (from rat). After confluency was reached, the medium was removed for undertaking immunofluorescence studies with ovine MHC Class I and COX IV antibodies.

**Co-culture with permeable membrane**

Co-cultures were grown in 6-well plates containing permeable membrane inserts. The membranes allowed the

| Table 1: Staining for direct co-culture experiment |
|--------------------------------------------------|
| Human DPCs | Ovine DPCs |
| Dil-DiO experiment | Dil (red) | DiO (green) |
| Species-specific antibodies | COX IV (green) | COX IV and MHC II (yellow) |

DPCs – Dermal papilla cells
passage of secreted soluble factors in the culture medium, but kept the two cell types separate. The ovine cells were seeded into the inserts and the human cells onto the bottom of the well. The insert was removed at the end of the experiment, and the response of the remaining human cells to the co-culture was then analyzed. Control wells contained only ovine cells in the inserts. The aggregative morphology of the human cells was evaluated using immunofluorescence techniques.

After the human and ovine cells were passaged, these cells were seeded into a 6-well plate containing the insert with approximately 80,000 cells/cm² for human cells in the bottom of the well above glass coverslip and 250,000 cells/cm² for ovine cells above the insert. Cells were seeded at different densities because the size of the human DPC was greater than ovine DPC. At above densities, the cells were seeded at similar confluencies. Four treatments were compared: (1) human cells with coverslip covered with collagen I; (2) human cells with coverslip only; (3) human cells with collagen I covering the bottom of the well; and (4) human cells without coverslip or collagen I. The medium was changed twice each week. After the human cells reached confluency or contained aggregates, the versican immunofluorescence, crystal violet staining, or van Gieson’s staining procedure was then performed.

**RESULTS**

**Culture conditions**

Before beginning human-ovine co-culture experiments, the type of medium and culture conditions to be used needed to be decided. Human and ovine cells are usually cultured in different media. The standard culture for human DPCs was RPMI with 10% (v/v) FCS and for ovine cells was MEM with 20% (v/v) LS. The human DPC was used at passage eight at which time they had ceased to aggregate under the standard culture conditions.

Four different variables were compared, base medium, serum supplementation, culture substrate, and the presence of Type I collagen [Table 2]. Both human and ovine cells aggregates were observed in media containing 20% (v/v) LS. In contrast, aggregation was less common for ovine cells and not seen at all for human cells in media with 10% (v/v) FCS (passage eight). Versican expression was observed in human and ovine cells which were still in monolayer state in media with FCS, but was confined to aggregates in media with LS [Figure 1].

In Table 2, the aggregations of human and ovine DPC were less likely to be observed on polystyrene (standard tissue culture plasticware) compared to other substrates, even though they were cultured in 20% (v/v) LS. Collagen-coated substrate and the type of medium had less effect on both species aggregation. However, collagen-coated substrate was needed to increase cell adhesion to substrate after seeding.

MEM with 20% (v/v) LS was chosen as the culture condition for the co-culture experiments between ovine and human DPCs. For substrate, the glass with collagen coating was chosen. This combination allowed aggregation of both ovine and human DPC, with the collagen coating also enhancing cell attachment after seeding.

**Direct co-culture**

Next, human and ovine DPCs were combined in co-culture to investigate whether human cells were able to incorporate into ovine DPC aggregates. Cells were mixed at different percentages: 100:0 human:ovine, 90:10 human:ovine, 50:50 human:ovine.

---

**Table 2: Effect of culture conditions on human and ovine cells**

| Medium | Serum  | Substrate | Coating | Ovine aggregation | Human aggregation |
|--------|--------|-----------|---------|-------------------|-------------------|
| RPMI   | 10% (v/v) FCS | Glass – | Yes | No |
|        |        | Collagen | Yes | No |
|        |        | Permanox | – | No |
|        |        | Collagen | No | No |
|        |        | Polystyrene | – | No |
|        |        | Collagen | No | No |
|        | 20% (v/v) LS | Glass – | Yes | Yes |
|        |        | Collagen | Yes | Yes |
|        |        | Permanox | – | Yes |
|        |        | Collagen | Yes | Yes |
|        |        | Polystyrene | – | No |
|        |        | Collagen | No | No |
| MEM    | 10% (v/v) FCS | Glass – | No | No |
|        |        | Collagen | Yes | No |
|        |        | Permanox | – | No |
|        |        | Collagen | No | No |
|        |        | Polystyrene | – | No |
|        |        | Collagen | No | No |
|        | 20% (v/v) LS | Glass – | Yes | Yes |
|        |        | Collagen | Yes | Yes |
|        |        | Permanox | – | Yes |
|        |        | Collagen | Yes | Yes |
|        |        | Polystyrene | – | No |
|        |        | Collagen | No | Yes |

FCS – Fetal calf serum; MEM – Minimum essential medium; LS – Lamb serum; RPMI – Roswell park memorial institute
human:ovine, and 0:100 human:ovine. Two different types of staining were applied to differentiate between species. Firstly, human cells were stained with DiI (red), while ovine cells were dyed with DiO (green). Second, a sheep-specific antibody to MHC Class I was used as a specific marker for ovine DPCs. A study done by Thangapazham et al.[16] showed that a COX IV antibody stained only human DPCs, not mouse cells. However, we found that this antibody stained sheep as well as human cells [Figure 2d and e].

Aggregation was beginning by day 2 [Figure 2]. Well-formed aggregates could be seen in the 90:10 human:ovine and the 50:50 human:ovine wells at days 2 and 3. DiI-labelled human cells were seen in these aggregates. In the 90:10 human: ovine cultures, some aggregates appeared to contain predominately human cells. In contrast, the 100:0 human: ovine cells were still in the monolayer state after 18 days. Similar results were seen in the second method. Human and ovine cells were often incorporated into the same aggregate.

Co-culture with semi-permeable membrane

The ovine cells were cultured above a permeable membrane suspended in a tissue culture vessel, and the human cells were grown on their usual substrate at the bottom of the vessel, below the membrane. The membrane allowed soluble factors secreted by ovine cells to be transferred to
human cells, but it prevented any direct contact between the two cell types.

Aggregation could be seen in human cells with the inserts above a glass substrate. Among four experiments performed with three different types of human DPC, two experiments showed that the addition of ovine cells above the insert made the human cell aggregates bigger in size [Figure 3, note that images were taken at different magnifications – see scale bars]. From experiment one and three, the average of aggregate diameter without insert was $98.4 \pm 2.4 \mu m$ ($n = 100$ aggregates) and with the insert was $165.3 \pm 9.1 \mu m$ ($n = 64$ aggregates) and this difference was statistically significant ($t$-test with unpaired samples and unequal variance, $P = 7 \times 10^{-10}$). Two experiments showed that ovine DPCs above the insert enhanced aggregation in other respects. In experiment two, human cells were observed to aggregate over $6.3 \text{ cm}^2$ of the substrate with the insert, while aggregates were only seen over $4.2 \text{ cm}^2$ of the substrate without an insert. Thus, aggregation was more extensive, with a greater number of aggregates formed in the presence of ovine DPCs [Table 3]. In experiment four, the cells started to make aggregates earlier, from day 3, and they usually stopped to grow after day 21, when they were lysed. In matched cells without an insert, aggregation did not start until day 8.

**DISCUSSION**

A series of experiments were undertaken to determine whether ovine secreted soluble factors are able to affect the aggregative behavior of human DPCs. First, the effect of LS was evaluated because subsequent co-culture experiments would preferably be done in LS, not in FCS which is commonly used for human DPCs. Then, the ability of ovine cells to promote the aggregation of human DPCs in direct co-culture was investigated. Third, aggregation in human cells was observed in co-culture with ovine cells separated by a semi-permeable membrane.

**Culture conditions**

The behavior of cells in cultures can be affected by many factors, including exogenous supplementation, enhancement of endogenous production of biomolecules, culture substrates, and media with different serum types. In this study, comparisons between the media with serum types and cultures substrates were performed. RPMI base medium with $10\%$ (v/v) FCS is a common medium and serum for human DPCs cultures, while MEM with $20\%$ (v/v) LS is used in cultures of ovine DPCs. The results showed that the serum had a greater effect on the behavior of the cells compared to the base media because aggregation of human cells was observed in both RPMI and MEM with $20\%$ (v/v) LS but not with $10\%$ (v/v) FCS. Thus, it could be proposed that LS in medium was sufficient to promote aggregation both in human and ovine cells. Human serum had been used in medium to culture human DPCs from balding and nonbalding scalps. The addition of human serum caused the cells to grow faster and show more aggregative behavior compared to FCS but it was fairly laborious to use human serum to expand the cells. Serum supplies numerous growth factors that play essential roles for cell interaction, proliferation, migration, and differentiation, and different sera may contain different growth factors. It is not clear whether the age (postnatal versus fetal) or species (lamb or human versus bovine) is important for promoting human DPC aggregation.

Aggregation on permanox, polystyrene, and glass was observed to evaluate the effect of culture substrates. Serum

---

**Table 3: Summary of human and ovine dermal papilla cells co-cultured with semi-permeable membrane**

| Cells used          | Culture substrate | Effect of ovine cells on human cell aggregation |
|---------------------|-------------------|-----------------------------------------------|
| Experiment one      | Hs DPC-1 (p8)     | Glass+collagen                                |
|                     | Oa DPC-9 (p8)     | Larger aggregates                             |
| Experiment two      | Hs DPC-1 (p13)    | Glass+collagen                                |
|                     | Oa DPC-9 (p9)     | Extensive aggregation                         |
| Experiment three    | Hs DPC-2 (p7)     | Glass+collagen                                |
|                     | Oa DPC-15 (p10)   | Larger aggregates                             |
| Experiment four     | Hs DPC-3 (p7)     | Glass+collagen                                |
|                     | Oa DPC-15 (p10)   | Shorter time to start aggregation             |

DPC – Dermal papilla cell
had a greater effect on human cell aggregation compared to culture dish substrate. Nevertheless, permanox and glass substrates supported DPC aggregation better than polystyrene. Human DPCs had difficulty making aggregates when they were cultured on polystyrene, although LS was added to the media. Previous studies performed various experiments to increase the aggregative behavior of human DPCs\cite{6,7,20,21} but none of them compared various substrates and serum to determine which caused greater aggregation. In contrast, collagen-coated substrates did not yield much difference in the aggregation. Collagen Type I is often added to the surface of the substrate to resemble extracellular matrix (ECM) condition in vivo and to increase the attachment of the cells after seeding\cite{11,22,23}. On the other hand, once the cells attached to the substrate, they might be able to secrete their own ECM molecules.

This experiment was also consistent with the previous study done by Young et al\cite{6} who investigated the effects of the balance of cell-cell adhesivity and cell-substrate adhesivity on vibrissa DPC aggregation by comparing substrates with differing adhesivity. They found that cell adhesivity to polystyrene was greater than to poly (ethylene-co-vinyl alcohol) (EVAL). They showed that aggregates were not found on the polystyrene surface, even though the cells were seeded at higher densities (160 × 10^3 cells/well). In contrast, aggregation was seen 3 days after seeding above EVAL (at 80 × 10^3 cells/well). They proposed that the adhesivity between DPC and polystyrene substrate was stronger than cell-cell adhesivity, whereas the adhesivity between DPC and EVAL was weaker. By the time-lapse recording, DPCs above EVAL were quite motile, thus, intercellular collision or contact was occurring frequently, leading to initial aggregation formation. They concluded that to facilitate aggregation, the adhesivity between cell and substrate should be reduced to allow cell movement and migration.\cite{6} We found that permanox and glass substrate experiments suggested that the adhesivity to these substrates supported aggregation better than polystyrene. It would be interesting to determine whether DPC adhere to permanox and glass less strongly than to polystyrene.

### Direct co-culture

In the direct co-culture between human and ovine DPCs, aggregates containing ovine and human cells were observed. On the other hand, when human cells were cultured without ovine cells, there was no aggregation. These results indicate that ovine cells were required to initiate aggregation but the human cells were then able to incorporate into the aggregates, and so that the addition of ovine DPCs enabled human cells to participate in aggregation formation. This experiment was done above a polystyrene substratum. Our results might be consistent with human cell-cell adhesivity increasing in the presence of ovine cells, or alternatively, with human cells adhering to ovine DPC more strongly than they can to each other. Currently, there has been no published paper investigating ovine-human cell adhesivity versus human-human cell adhesivity.

It could be observed that the ovine cells were not grouped together with other ovine cells within an aggregate, and neither were human DPCs. Hence, ovine-human cell adhesion might have taken place. However further, higher-resolution three-dimensional imaging would be needed to observe the exact location of ovine and human DPCs within the aggregate. Nevertheless, this result was encouraging because it suggested that human DPCs regained the ability to form aggregates if they received the correct stimulus, and that ovine DPCs might be capable of providing that stimulus.

### Co-culture with semi-permeable membrane

In the co-culture with semi-permeable membrane experiments, aggregation was compared between human cells in LS with ovine cells above the insert, human cells in LS without the insert, and human DPCs in FCS. Previous studies showed that DPCs from later passage number (six-15) tended to lose their aggregative behavior.\cite{29}

Higher passage numbers of human cells (seven-13) were used in this experiment [Table 3], so that it could be expected that they had lost their aggregative property, hence their hair-inducing ability. In this study, aggregation of human cells was observed in LS with and without ovine cells above the insert, but the addition of ovine cells above the insert made the size of aggregates bigger and/or the field of aggregation more extensive. The average for aggregate diameter without the insert was significantly smaller than with the insert (98.4 ± 2.4 µm vs. 165.3 ± 9.1 µm). Hence, it appears that soluble factors secreted by ovine cells have effects on human cell aggregation in addition to LS. However, LS seemed to give a bigger effect on human DPC aggregation because the addition LS to the culture medium was sufficient to facilitate aggregation without soluble factors secreted from ovine DPCs.

### CONCLUSION

The aggregative behavior of cultured DPCs is correlated with the ability of these cells to regenerate hair follicles. Human DPCs in later passage number tend to lose this...
behavior. Our results show that LS in the medium promotes the aggregation of higher passage human DPCs. The addition of soluble factors secreted from ovine DPCs above the semi-permeable membrane further enhances human cell aggregation, allowing the human cells to make larger aggregates or aggregate more extensively. Hence, ovine soluble factors, either present in LS or secreted by DPC, may have the potential to enhance or stabilize the hair inducing activity of human DPC, and may bring us one step closer to therapeutic hair induction as a treatment for hair loss.

Acknowledgment

The first author was supported by an Australia Awards Scholarship. Our thanks to Prof Robert Kapsa and colleagues, in whose Laboratory at St. Vincent Hospital, Melbourne, the majority of these experiments were done.

Financial support and sponsorship

This work was funded by Epworth Hospital, Dermatology Department. The first author was funded by Australia Awards Scholarship.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Messenger AG, Senior HJ, Bleehen SS. The in vitro properties of dermal papilla cell lines established from human hair follicles. Br J Dermatol 1986;114:425-30.
2. Horne KA, Jahoda CA, Oliver RF. Whisker growth induced by implantation of cultured vibrissa dermal papilla cells in the adult rat. J Embryol Exp Morphol 1986;97:111-24.
3. Inoue K, Kato H, Sato T, Osada A, Aoi N, Suga H, et al. Evaluation of animal models for the hair-inducing capacity of cultured human dermal papilla cells. Cells Tissues Organs 2009;190:102-10.
4. Higgins CA, Richardson GD, Ferdinando D, Westgate GE, Jahoda CA. Modelling the hair follicle dermal papilla using spheroid cell cultures. Exp Dermatol 2010;19:546-8.
5. Inamatsu M, Matsuzaki T, Iwayanagi H, Yoshizato K. Establishment of rat dermal papilla cell lines that sustain the potency to induce hair follicles from alopecic skin. J Invest Dermatol 1998;111:767-75.