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(54) Title: METHOD FOR PRODUCING NEW HAIR GROWTH

(57) Abstract

The present invention is directed to a method for producing new hair growth in humans by implanting human dermal papilla cells into human scalp skin, wherein the human dermal papilla cells are cultured in a medium supplemented with a conditioned medium. The conditioned medium is formed from a culture of normal human keratinocytes.

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Method For Producing New Hair Growth

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is directed to a method for producing new hair growth in humans by implanting human dermal papilla cells into human scalp skin, wherein the human dermal papilla cells are cultured in a medium supplemented with a conditioned medium. The conditioned medium is formed from a culture of normal human keratinocytes.

2. Description of the Related Art

Hair loss is a very common human condition and many people desire treatment or correction for this problem. By far, the leading cause for hair loss is the influence of androgens on genetically susceptible follicles (i.e. "androgenetic alopecia"). Numerous treatments are available but all have significant drawbacks. Pharmacologic treatments attempt to revive and stimulate existing hair follicles. Because androgenetic alopecia may lead to the complete disappearance of hair follicles, pharmacologic treatments may always be of limited value. Hair restoration surgery involves the redistribution of permanent hair follicles to hairless areas but because the amount of hair follicles available for redistribution is limited, the ability of this teaching and to completely correct baldness is also limited.

The purpose of the present invention is to overcome the limitations of the known treatments. A method of creating new follicles from existing follicles is needed, thus providing a virtually unlimited supply of hair for treating baldness. Previous research in rats has shown that hair

follicles can actually be regenerated when the critical cellular component of the follicle, the papilla, is expanded using cell culture and transplanted into the skin (Jahoda CAB, Reynolds AJ, Oliver RF. Induction Of Hair Growth In Ear Wounds By Cultured Dermal Papilla Cells. J Invest Dermatol 101:584-590, 1993). One major limitation of this technique is that when the papilla cells are expanded in culture through serial passage, they lose their ability to regenerate hair. However, other researchers have recently shown that when rat papilla cells are cultured with the media taken from cultured keratinocytes, they retain their hair inducing capabilities through 56 passages (Takashi M, Mutsumi I, Katsutoshi Y. Hair Induction By Dermal Papilla Cells Cultured With Conditioned Medium Of Keratinocytes. Abstract from the First Tricontinental Meeting of Hair Research Societies. Brussels, Belgium. 1995).

SUMMARY OF THE INVENTION

An object of the present invention is to provide a method of inducing hair growth in humans by implanting human dermal papilla cells into human skin. The method comprises the steps of removing a papilla from a human hair follicle, isolating the dermal papilla cells from the follicle to form a cell suspension, culturing such cells in a medium supplemented with a conditioned medium taken from human keratinocyte culture, and implanting the papilla cells so cultured into the epidermis layer of the skin in contact with an epidermal cell.

Another object of the present invention is to provide a method for growing human dermal papilla cells in a medium supplemented with a conditioned medium that is formed by culturing human keratinocytes, taken from the epidermis of the follicle, for example, in the keratinocyte culture medium. The papilla cells so cultured can expand rapidly for many passages in vitro while maintaining their hair inducing properties.

The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of the disclosure. For a better understanding of the invention, its operating advantages, and specific objects attained by its use, reference should be had to the drawing and descriptive matter in which there are illustrated and described preferred embodiments of the invention.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

The implantation technique of the present invention is initiated by isolating and removing a papilla from the base of hair follicles from an area of a patient's scalp where hair loss is not expected to occur (e.g. the "donor area" used in hair transplantation comprising the mid temporo-parieto-occipital region of scalp). Such papilla samples are obtained by a punch biopsy or an excisional technique similar to that used in hair transplantation in which tumescent anesthesia is used and the tissue is removed with strip or elliptical harvesting.

The papilla is dissected free from the remaining part of the follicle, which is attached to and taken out together with the papilla, under microscopy. The dissected papilla is then initiated into a culture medium, either by simply floating the papilla in a medium containing a buffered salt solution with bovine serum to produce a cell suspension, or preferably, using a technique known to enhance the initiation of the papilla into culture (Warren R, Chestnut MH, Wong TK, et al. Improved Method For The Isolation And Cultivation Of Human Scalp Dermal Papilla Cells. J Invest Dermatol 98:693-699, 1992), in which the papilla is treated with collagenase or a similar enzymatic treatment to produce a cell suspension. This suspension is added to culture dishes containing Chang's media

(Chang, H et al. Proc Natl Acad Sci 79:4795-4799) and incubated under standard cell culture conditions.

After the initiation, the papilla cells are cultured in M199 or similar buffered salt solution with fetal calf serum in a concentration of 10 to 20% or in Chang's media, either of which may be supplemented with known papilla growth factors such as fibroblast, vascular endothelial, or platelet derived growth factors, and/or other known papilla stimulators. Preferably, these media, e.g. M199 with 10% fetal calf serum or Chang's media, are supplemented with a conditioned medium formed from the cultures of normal human keratinocytes which are taken from the epidermis, or preferably from the outer root sheath of follicles, as described by Takashi M. et al., "Hair induction by dermal papilla cells cultured with conditioned medium of keratinocytes", *Abstract from the First Tricontinental Meeting of Hair Research Societies. Brussels, Belgium. 1995,* which is hereby incorporated by reference. Most preferably, the keratinocytes are taken from the middle to lower portion of the follicles where the epithelial stem cells are thought to reside. The keratinocytes may be cultured in standard fashion but may also be treated with epithelial mitogens such as minoxidil or insulin-like growth factor. Preferably the source of these keratinocytes is from the same patient being treated (autologous source) but it may also be allogenic, such as from neonatal foreskin.

The cells may be passed in a routine fashion. The use of keratinocyte conditioned media allows for the rapid expansion of papilla cells while maintaining their hair inducing properties. Therefore cells of low or high passage may be used, the latter being used to maximize cell number from a single papilla. At the final stage, the media containing animal sera is removed and the cells are incubated with or without the patient's autologous sera mixed in M199 or a similar salt solution.

The papilla cells are harvested from the culture dish either by physically removing them or by enzymatic digestion such as with trypsin. The cells are either used directly or are subsequently aggregated such as with centrifugation. The cells may then be mixed with substances which can promote aggregation as well as easy introduction into the skin, such as fibronectin, glycosaminoglycans (e.g. dermatin sulphate, chondroitan sulfate, proteoglycans, heparan sulphate), collagen, and/or other substances known to fulfill this function.

The recipient site of the patient who is subject to the hair implantation may be pretreated with physical and/or pharmacologic methods to increase the receptiveness to the introduced cellular material. The physical methods include ultraviolet light, ultrasound, massage, or the like. Pharmacologic methods include topical minoxidil solution, or tretinoin solution or oral minoxidil, vitamins, or antioxidants, etc.

The harvested papilla cells may then be introduced into the area of the skin subject to the implantation using various techniques. For example, the papilla cells may be introduced into a blister on the skin such as that formed by a suction device; or the cells may be introduced into slit incisions in the skin made with a scalpel blade or hypodermic needle. While these incisions may be of varying width and depth, a preferable dimension for the incision is about 1 mm wide and 3 mm deep. Alternatively, the cells may be introduced into the superficial layer of the skin by injection with a syringe and hypodermic needle.

The amount of papilla cells for the implantation for each opening of the skin is preferably about 100,000, but may range from 1,000 to 1,000,000, depending on the size and depth of the opening and the overall viability and activity of the cells. The amount of the cells introduced may also be varied to vary the size of the subsequently produced hair. Preferably, the papilla cells introduced into the skin opening by the

above described implantation techniques are in physical contact with the native epidermal cells. The papilla cells may also be implanted by a co-introduction of a rigid shaft to promote the correct downward migration of the cells and proper orientation of the future follicle. This may be accomplished with the use of an absorbable suture material such as polyglycolic, polyester, or polydioxanone, preferably coated with a substance known to promote cellular adhesion and migration, such as fibronectin, collagen, and/or glycosaminoglycans. After the introduction of the cells into the desired area of the skin, the wound is left open or is covered with an occlusive dressing.

Alternatively, the dermal papilla cells may be implanted together with epidermal cells, which are either interfollicular epidermal cells or those isolated from the outer root sheath of the follicle. Preferably the epidermal cells are from the same patient being treated.

Following the implantation of the papilla cells, the patient may be treated with either topical or systemic agents known to promote hair growth, including minoxidil, tretinoin, cyclosporine, finasteride, etc. Hair growth is expected over the subsequent weeks. In patients in whom further hair loss is expected and therefore the need for further treatment is anticipated, dermal papilla cells at any stage, preferably at an earlier stage of preparation, are cryopreserved and therefore may be thawed and used at any time in the future.

The invention is not limited by the embodiments described above which are presented as examples only but can be modified in various ways within the scope of protection defined by the appended patent claims.

CLAIMS

1. A method for producing new hair growth in a first area of human skin where hair growth is desired, said human skin including an outer epidermis layer and an inner dermis layer, comprising the steps of:

a. removing a papilla from a hair follicle from a second area of human skin where hair loss is relatively not expected to occur, said papilla residing within said follicle and comprising dermal papilla cells and connective tissue, said follicle including an epithelial component which comprises a matrix and an outer root sheath;

b. isolating said papilla so as to free said papilla from substantially any remaining parts of said follicle;

treating said papilla with an agent capable of separating said dermal papilla cells from said connective tissue to form a dermal papilla cell suspension;

forming a conditioned medium from a culture of normal human keratinocytes;

e. culturing said dermal papilla cells in a dermal papilla cell culture medium supplemented with said conditioned medium to allow a rapid expansion of said dermal papilla cells while maintaining the hair inducing properties;

f. harvesting said cultured dermal papilla cells;

g. implanting said cultured dermal papilla cells into said epidermis layer of said first area of said human skin in contact with a native epidermal cell within said epidermis layer.

2. The method of claim 1, wherein said conditioned medium is formed from a culture of normal human keratinocytes of human epidermis of said follicle.

3. The method of claim 1, wherein said conditioned medium is formed from a culture of normal human keratinocytes of said outer root sheath of said follicle.

4. The method of claim 3, wherein said keratinocytes is formed from a culture of normal human keratinocytes of a middle to lower portion of said outer root sheath of said follicle.

5. The method of claim 1, wherein said implantation of said dermal papilla cells further comprising the step of introducing said papilla cells into a blister on the skin.

6. The method of claim 1, wherein said implantation of said dermal papilla cells further comprising the step of introducing said papilla cells into a slit incision in the skin.

7. The method of claim 1, wherein said implantation of said dermal papilla cells further comprising the step of introducing said papilla cells into the superficial layer of the skin by injection.

8. The method of claim 1, wherein said implantation further comprises the step of introducing said dermal papilla cells together with a plurality of epidermal cells.

9. The method of claim 1, wherein said cultured dermal papilla cells are aggregated whereby to enhance interaction with said epidermal cell.

10. The method of claim 1, further comprising the step of treating said human after said implantation with a hair growth stimulating promoter so as to enhance growth of new hair induced by said implanted cultured dermal papilla cells.

11. A method for culturing human dermal papilla cells for producing new hair growth, comprising the steps of:

a. culturing human keratinocytes in a medium suitable for human keratinocytes growth to form a keratinocyte conditioned medium;

b. collecting said keratinocyte conditioned medium, absent said human keratinocytes;

c. combining said keratinocyte conditioned medium with a medium suitable for human dermal papilla cell growth to form a supplemented human papilla cell growth medium; and

d. culturing said human dermal papilla cells in said supplemented human papilla cell growth medium.

12. The method of claim 11, wherein said keratinocyte conditioned medium is formed from a culture of normal human keratinocytes of human epidermis.

13. The method of claim 11, wherein said keratinocyte conditioned medium is formed from a culture of normal human keratinocytes of said outer root sheath of said follicle.

14. The method of claim 13, wherein said keratinocytes is formed from a culture of normal human keratinocytes of a middle to lower portion of said outer root sheath of said follicle.

INTERNATIONAL SEARCH REPORT

International application No. PCT/US98/13754

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 63/00; A61F 2/10; C12N 5/08

US CL :424/93.7; 623/15; 435/366

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.7; 623/15; 435/366

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, WPIDS

search terms: dermal papilla, keratinocyte conditioned medium, implant, hair

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WARREN et al. Stimulation of Human Scalp Papilla Cells by Epithelial Cells. Archives of Dermatological Research. 1994, Vol.286, No. 1, pages 1-5, especially abstract and page 2.	11, 12 ---------- 1-4,13,14
Y	US 4,919,664 A (OLIVER et al.) 24 April 1990, col. 1, lines 44-54; col. 2, lines 1-3, 29-31, 54; col. 3, lines 3, 11, 16, 67; col.4, lines 13-15; example 3.	1, 5-10
Y	US 5,130,142 A (WONG et al.) 14 July 1992, abstract; col. 2, lines 39-59; examples I, II and III.	11-14
Y	EP 0 682 107 A2 (RESEARCH DEVELOPMENT CORPORATION OF JAPAN) 15 November 1995, abstract; col. 1, lines 5-12.	1, 11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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International application No. PCT/US98/13754

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MATSUZAKI et al. The Upper Dermal Sheath Has a Potential to Regenerate the Hair in the Rat Follicular Epidermis. Differentiation, September 1996, Vol. 60, No. 5, pages 287-297, especially abstract.	3,4,13,14
Y	JAHODA et al. Dermal-Epidermal Interactions: Adult Follicle-Derived Cell Populations and Hair Growth. Dermatologic Clinics. October 1996, Vol. 14, No. 4, pages 573-583, see entire document.	1-14