PRESS RELEASE

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Hair Regeneration *in vitro* Using Three Types of Adult Hair Follicle Stem Cells

Identifying the "Minimal Stem Cell Set" Required for Hair Follicle Regeneration, with Potential Applications in Alopecia Treatment

A collaborative research team led by Koh-ei Toyoshima (General Manager of Research and Development, OrganTech Inc.), Miho Ogawa (Director and Chief Technology Officer, OrganTech Inc.), and Takashi Tsuji (then Team Leader, Organ Induction Research Team, RIKEN Center for Biosystems Dynamics Research) has identified a previously unrecognized accessory mesenchymal cell population that is indispensable for functional hair follicle [1] regeneration under *in vitro* conditions. Specifically, the investigators describe a "third cell type," termed hair follicle regeneration–supporting cells, which enables adult hair follicle–derived epithelial stem cells [2] and dermal papilla cells [3] to progress beyond hair bulb [4] formation to sustained downgrowth [5] and hair shaft production.

By incorporating this newly identified cell population into reconstructed hair follicle organ germs [6], the team generated organ germs composed of three adult-derived stem cell [7] types. Under defined culture conditions, these organ germs regenerated fully functional hair follicles *in vitro*, including hair shaft formation (Fig. 1).

These findings advance understanding of hair follicle development, regeneration, and hair cycle [8] regulation, and provide a defined cellular framework that may inform future translational strategies in hair follicle regenerative medicine [9], including potential applications in alopecia. In addition, the work illustrates a broader principle relevant to three-dimensional organ regenerative approaches: organ-level maturation may require accessory cell populations beyond canonical epithelial–mesenchymal pairs.

Fig. 1. *In vitro*–regenerated murine hair follicles

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1. Background

Organs arise during embryonic development from organ germs, which are induced through coordinated interactions between epithelial and mesenchymal stem cell populations possessing organ-forming potential. Following organogenesis, these organ-forming stem cells give rise to tissue-specific (somatic) stem cells that maintain tissue homeostasis and repair but typically lack the capacity to regenerate an entire organ. Consequently, in many settings of organ dysfunction caused by injury or disease, transplantation remains the only definitive therapeutic option. This limitation has motivated the development of organ regenerative therapy, which aims to reconstruct whole organs with appropriate three-dimensional organization and cellular composition for functional replacement.

Hair follicles are the organs responsible for hair shaft production and develop from hair follicle germs during embryogenesis. A mature hair follicle comprises a permanent region, including the bulge area that harbors epithelial stem cells, and a cyclic (regenerative) region containing the hair bulb, where hair shafts are produced. The cyclic region undergoes repeated phases of growth, regression, and rest through the hair cycle, resulting in periodic hair replacement (Fig. 2). Notably, the hair follicle is often regarded as a distinctive organ in that it retains a stem cell system capable of repeatedly executing a genetically programmed regenerative process throughout life.

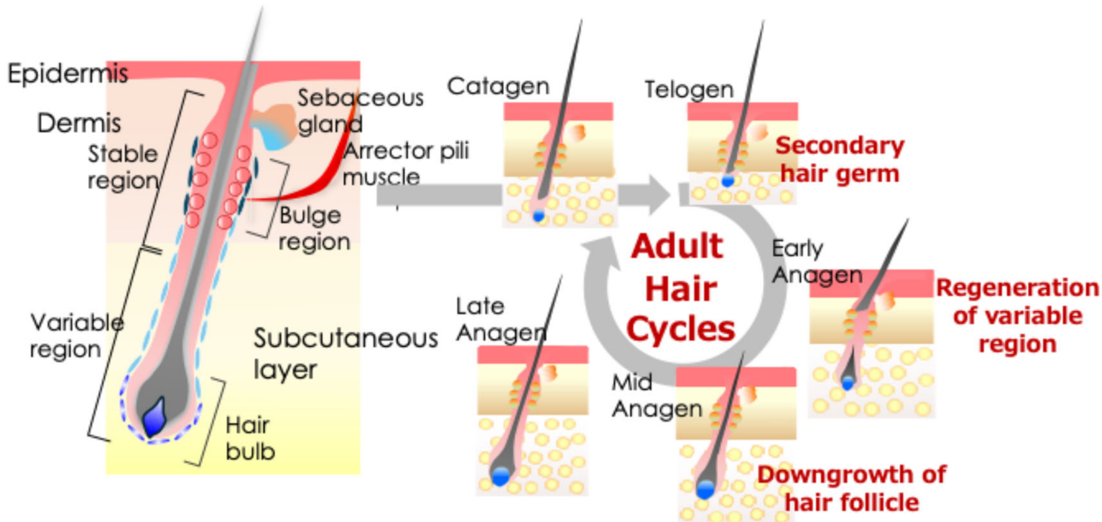

Fig. 2. Structure of the hair follicle and the hair cycle

For many years, hair follicle regeneration has been pursued using embryonic or neonatal hair follicle germs and skin cells containing hair follicles. These approaches largely recapitulated aspects of skin development with follicles rather than achieving reconstruction of the hair follicle organ itself from adult-derived stem cells. Although prior studies demonstrated regeneration of skin containing hair follicles from pluripotent stem cells [10] or from embryonic hair follicle organ germs *in vitro*, the regeneration of fully functional hair follicles capable of producing hair shafts using adult-derived stem cells had not been achieved under *in vitro* conditions.

In 2007, Tsuji and colleagues pioneered the organ germ method [11], enabling three-dimensional reconstitution of organs by high-density, compartmentalized reconstruction of epithelial and mesenchymal stem cells [12] with organ-inductive potential. Using this approach, the group demonstrated functional *in vivo* regeneration of multiple organs, including teeth, hair follicles, and secretory glands.

In 2012, the same group further showed that fully functional hair follicles could be regenerated by orthotopic transplantation [13] of hair follicle germs reconstructed from adult hair follicle–derived epithelial stem cells and dermal papilla cells. However, despite these advances, it remained difficult to achieve *in vitro* functional maturation of regenerated follicles derived from adult cells, particularly the transition into the anagen (growth) phase accompanied by downgrowth and hair shaft elongation. This gap suggested that additional hair follicle–associated cellular elements and/or microenvironmental functions required for maturation were present *in vivo* but were not replicated in prior *in vitro* systems.

Accordingly, the present study sought to identify adult hair follicle–associated stem cell populations essential for regenerating fully functional hair follicles *in vitro* and, in doing so, to define the minimal stem cell set required for complete hair follicle organ regeneration.

2. Research Methods and Results

1) Development of an *in vitro* organ culture system for hair follicle regeneration

To establish culture conditions permissive for maturation of regenerated hair follicles *in vitro*, the investigators first enzymatically dissociated epidermal and dermal tissues containing fetal skin–derived hair follicle germs into single cells. Hair follicle germs were reconstructed using the organ germ method and subsequently cultured *in vitro*. A two-step culture system was applied, involving sequential medium switching from a stem cell maintenance phase to a maturation-inducing phase containing selected cytokines [14]. Under these conditions, regenerated hair follicles entered anagen, exhibited hair bulb downgrowth, and formed hair shafts.

In contrast, when hair follicle germs were reconstructed solely from adult mouse whisker–derived epithelial stem cells and dermal papilla cells and then cultured using the same two-step system, hair bulbs formed but did not transition into the growth phase, and neither downgrowth nor hair shaft formation was observed (Fig. 3).

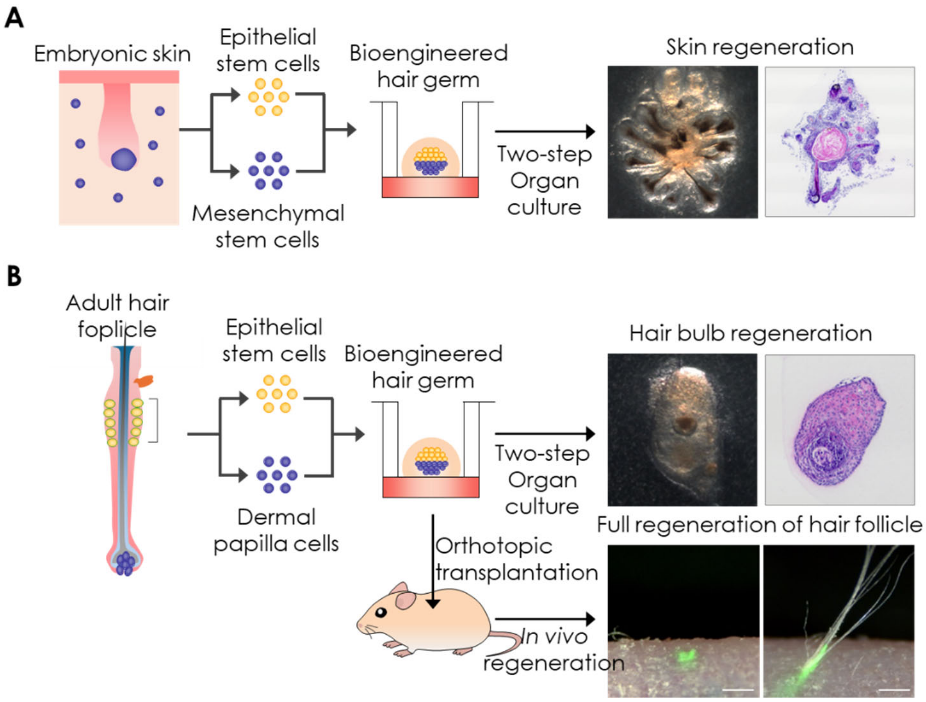

Fig. 3. *In vitro* organ culture–based hair follicle regeneration.
(A) *In vitro* hair follicle regeneration using fetal skin–derived cells.
(B) *In vitro* hair follicle regeneration and *in vivo* orthotopic hair follicle regeneration using adult whisker-derived hair follicle cells.

Notably, when these adult-cell–derived reconstructed germs were transplanted into skin *in vivo*, hair bulbs exhibited downgrowth and produced growing hair shafts. This divergence between *in vivo* and *in vitro* outcomes supported the hypothesis that adult hair follicle maturation requires an additional supportive element present in the adult tissue environment but absent from the reconstructed two-cell system.

2) Identification of hair follicle regeneration–supporting cells in the skin

To evaluate whether adult skin contains a cell population capable of supporting hair follicle maturation, adult dermal mesenchymal cells (DDMCs) were enzymatically dissociated into single cells and incorporated into reconstructed hair follicle germs under distinct spatial configurations and densities. Hair follicle development was then assessed *in vitro*.

When DDMCs were distributed around the reconstructed germ or incorporated at low density, robust hair follicle growth—assessed by hair bulb downgrowth—was not induced. In contrast, when DDMCs were incorporated at high density and spatially compartmentalized as a third cell population within the reconstructed organ germ, consistent hair follicle growth and hair shaft formation were observed *in vitro*. These experiments indicate that both the identity of the supportive population and its three-dimensional arrangement within the reconstructed architecture are critical determinants of functional regeneration (Fig. 4).

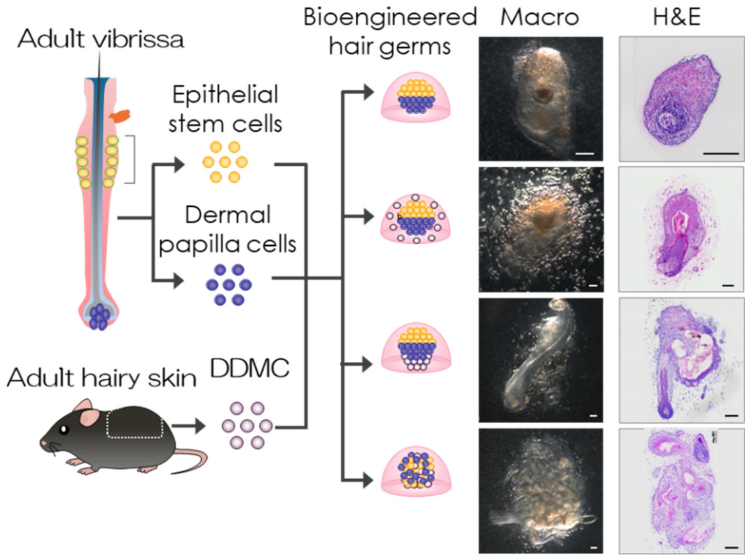

Fig. 4. *In vitro* hair follicle regeneration supported by the third cell population.

To define the responsible subpopulation, DDMCs were fractionated by fluorescence-activated cell sorting (FACS) using established differentiation markers. PDGFRα^{high} CD31^{-} CD45^{-} mesenchymal cells were isolated and further subdivided based on CD34 and Sca1 expression. When each subpopulation was incorporated into reconstructed hair follicle germs with appropriate positioning and cultured *in vitro*, only the PDGFRα^{+} CD34^{+} Sca1^{+} triple-positive population reproducibly supported hair bulb downgrowth and hair shaft formation (Fig. 5).

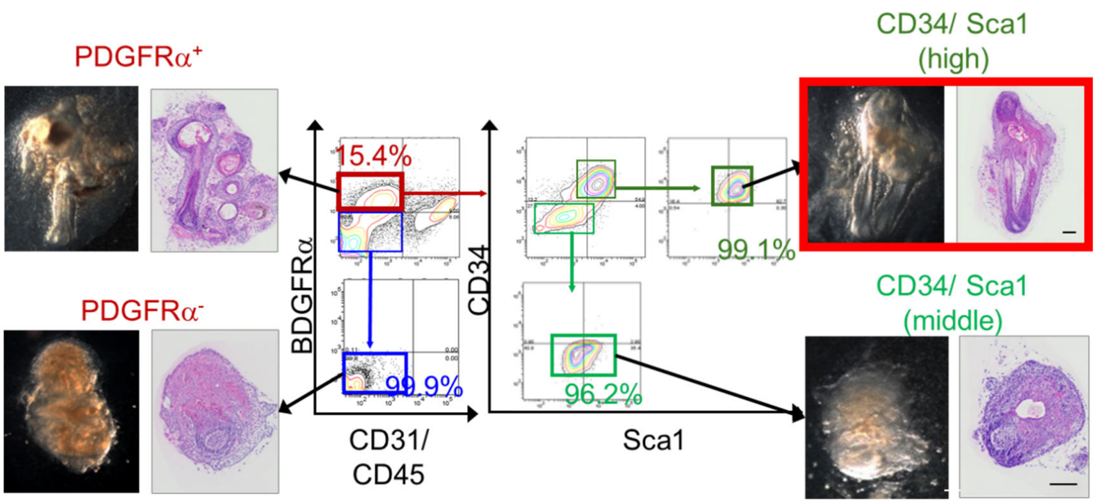

Fig. 5. Identification of a DDMC subpopulation supporting regenerated hair follicle growth.

3) Localization of hair follicle regeneration–supporting cells in adult hair follicles

To determine whether the supportive population is present within adult hair follicles, whisker follicles were used as a model. Immunohistochemical analyses [15] demonstrated that cells expressing high levels of PDGFRα and CD34 localized consistently to the mesenchymal region surrounding the bulge area (ABM) across the hair cycle.

Cells isolated from the ABM region contained a $PDGFR\alpha^{+}$ $CD34^{+}$ $Sca1^{+}$ triple-positive population similar to that identified within DDMCs. When ABM-derived cells were isolated by cell sorting and incorporated into reconstructed hair follicle germs, robust *in vitro* hair follicle growth was reproducibly observed (Fig. 6).

Collectively, these results identify a novel accessory population—termed hair follicle regeneration–supporting cells—that is essential for *in vitro* hair follicle growth and hair formation, and localize this population to a defined anatomical region within adult hair follicles. This study therefore provides experimental support for a minimal cellular requirement for inducing a fully functional hair follicle organ using adult-derived cells.

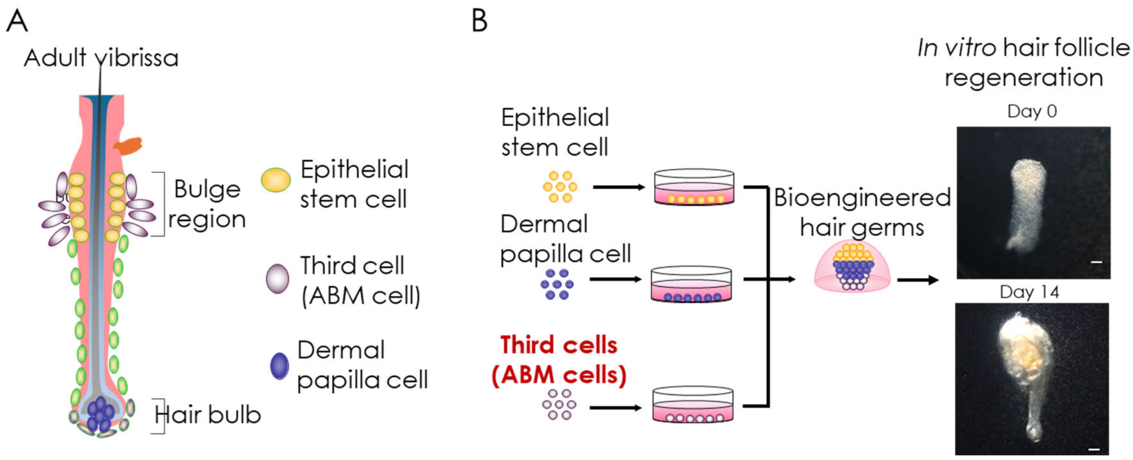

Fig. 6. *In vitro* hair follicle regeneration using adult-derived cells.
(A) Localization of the third cell population in adult hair follicles.
(B) Hair follicle regeneration supported by ABM cells derived from adult whisker follicles.

4) Analysis of the hair cycle in regenerated hair follicles *in vitro*

To assess functional competence beyond hair shaft formation, reconstructed hair follicle germs incorporating regeneration-supporting cells were transplanted into an engineered three-dimensional skin equivalent constructed *in vitro*. The regenerated follicles within the artificial skin [16] were cultured for 23 days, during which they matured, exhibited hair bulb downgrowth, and produced emergent and elongating hair shafts.

With continued culture, regenerated hair follicles repeatedly transitioned between anagen (growth) and catagen (regression) phases, thereby recapitulating core features of the hair cycle *in vitro* (Fig. 7). The observed average durations of anagen and catagen were approximately 9 days and 6 days, respectively, comparable to those observed in regenerated follicles formed *in vivo*. These findings support the conclusion that regenerated follicles derived from adult cells can reproduce cyclical behavior under *in vitro* conditions when reconstructed with the appropriate cellular components.

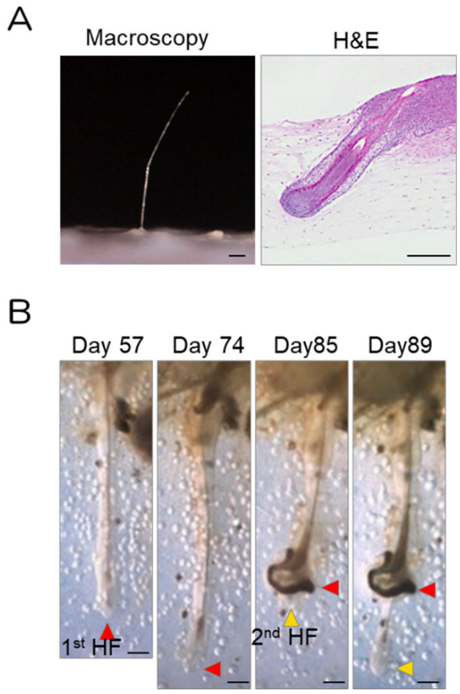

Fig. 7. Hair cycle of regenerated hair follicles.
(A) Hair shaft growth and histological features of regenerated hair follicles in artificial skin.
(B) Recapitulation of the hair cycle in artificial skin.

5) Functional role of hair follicle regeneration–supporting cells

To investigate how regeneration-supporting cells promote hair follicle growth—particularly downgrowth—the investigators examined the fate and dynamics of the third cell population during regeneration. During downgrowth, regeneration-supporting cells differentiated into connective tissue sheath (CTS) cells [17] and surrounded the regenerating follicle (Fig. 8). Three-dimensional live imaging [18] showed that outer root sheath cells, supported by CTS derived from regeneration-supporting cells, migrated downward in coordination with follicle development (Fig. 8), suggesting a mechanistic role in enabling or stabilizing downgrowth.

Consistent with this interpretation, regeneration-supporting cells exhibited elevated expression of genes associated with the cytoskeleton and smooth muscle contraction, supporting a model in which CTS contributes active, tension-generating or contractile properties rather than functioning solely as a passive scaffold.

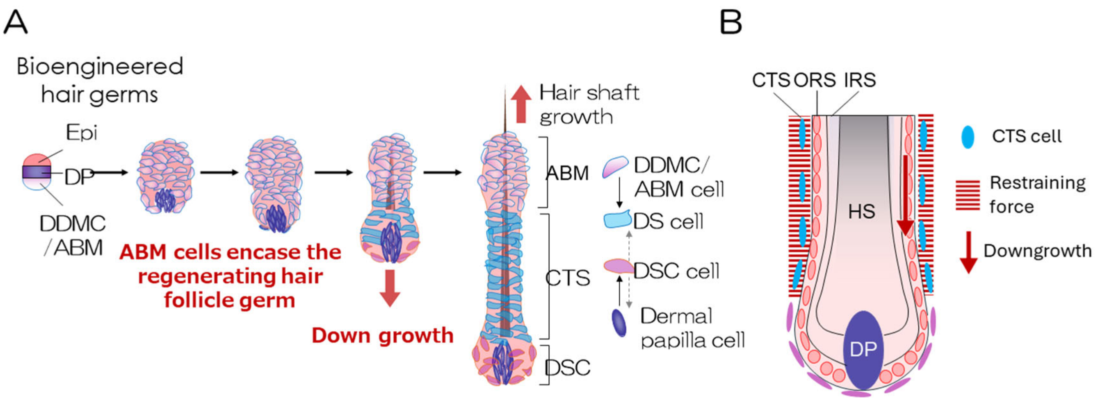

Fig. 8. Cellular dynamics of hair follicle regeneration–supporting cells and the proposed mechanism underlying support of hair follicle downgrowth.
(A) Changes in the third cell population during *in vitro* hair follicle regeneration.
(B) Supportive role of hair follicle downgrowth mediated by connective tissue sheath (CTS) formation.

3. Future Perspectives

This study identifies, for the first time, the minimum essential set of adult hair follicle–derived stem cells required for *in vitro* regeneration of a functional hair follicle organ: (i) epithelial stem cells in the bulge region [19], (ii) dermal papilla cells, and (iii) hair follicle regeneration–supporting cells. Beyond advancing fundamental understanding of stem cell biology and the cellular mechanisms underlying hair follicle development and cycling, the work establishes a defined reconstruction framework that may facilitate future translational research in hair follicle organ regenerative medicine [20].

Building on this foundation, further investigations will be required to refine culture systems, characterize molecular mediators, and evaluate robustness, scalability, and applicability across hair types and conditions. The platform may also support drug discovery programs that interrogate hair follicle growth and cycling using organ-level readouts.

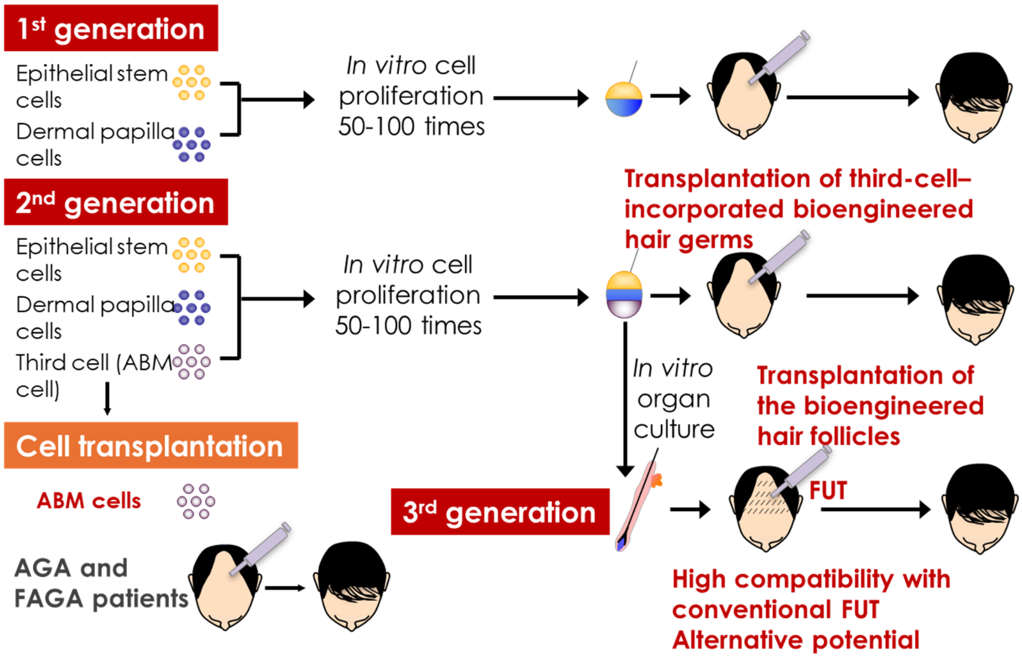

Fig. 9. Pipeline for hair follicle organ regenerative medicine.

Supplementary Explanations

[1] Hair follicle

The hair follicle is a microscopic organ of the skin organ system. It produces hair shafts and secretes sebum onto the body surface, contributing to functions including barrier protection, thermoregulation, sensory perception, and social signaling.

[2] Epithelial stem cells

Epithelial stem cells give rise to hair matrix cells and contribute to epidermal and sebaceous lineages; they reside in the permanent region of the follicle.

[3] Dermal papilla cells

Dermal papilla cells are mesenchymal cells within the hair bulb that regulate hair properties and cycling through instructive signals.

[4] Hair bulb

The hair bulb is the follicle base where matrix cells proliferate and differentiate to produce the hair shaft.

[5] Downgrowth

During development and anagen, the follicle elongates downward toward subcutaneous tissue; this process is termed downgrowth.

[6] Organ germ

An organ germ is a primordial structure formed during embryonic development from epithelial and mesenchymal stem cells and serves as the foundation for organ formation through coordinated signaling, proliferation, and differentiation.

[7] Stem cells

Stem cells possess the capacity for self-renewal and differentiation. Adult tissues contain somatic (tissue-specific) stem cells that support homeostasis and repair.

[8] Hair cycle

Hair follicles undergo cyclic phases of growth (anagen), regression (catagen), and rest (telogen), producing periodic hair replacement.

[9] Regenerative medicine

Regenerative medicine aims to restore tissue or organ function using stem cells or related approaches. Autologous strategies may reduce immune rejection risk.

[10] Pluripotent stem cells

Pluripotent stem cells (e.g., ES and iPS cells) can differentiate into many cell types and are widely used in regenerative research.

[11] Organ germ method

A technique for reconstructing organ germs by dissociating and reassembling epithelial and mesenchymal stem cells at high density in compartmentalized configurations, enabling functional organ regeneration *in vivo*.

[12] Mesenchymal stem cells

Mesenchymal stem cells can differentiate into multiple mesenchymal lineages. In broader contexts, they include organ-specific mesenchymal progenitors.

[13] Orthotopic transplantation

Transplantation into the original anatomical site, enabling interaction with the native microenvironment to support physiological maturation.

[14] Cytokines

Low-molecular-weight proteins that mediate intercellular communication by binding specific receptors and modulating cellular behavior.

[15] Immunohistochemical analysis

A method using antigen–antibody binding to localize proteins within preserved tissue architecture.

[16] Artificial skin

Engineered tissue models composed of dermal and epidermal layers used for evaluation and mechanistic study.

[17] Connective tissue sheath (CTS) cells

Cells forming the outer mesenchymal layer surrounding the follicle, comprising mesenchymal cells, extracellular matrix, and vessels; CTS may regulate follicle morphology and dynamics.

[18] Three-dimensional live imaging

Confocal-based approaches enabling time-resolved observation of living tissues in three dimensions to analyze cellular dynamics.

[19] Bulge region

The bulge region is a stem cell niche in the permanent region of the follicle near the arrector pili muscle attachment site.

[20] Organ regenerative medicine

Organ regenerative medicine seeks to regenerate entire organs and replace those that have lost function.

Publication Information

Title

Fully Functional Hair Follicle Organ Regeneration Using Organ-Inductive Potential Stem Cells with an Accessory Mesenchymal Cell Population in an *In Vitro* Culture System

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