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Review

An induced pluripotent stem cell-based approach for hair follicle development and regeneration

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ABSTRACT

Because hair loss is a common concern for many individuals, potential regenerative therapies of hair follicles have been extensively researched. Induced pluripotent stem cells (iPSCs) are a promising avenue for hair follicle regeneration. This review explores current iPSC-based approaches and highlights their potential applications and challenges in hair restoration. The principles of iPSC technology, iPSC differentiation into hair follicle precursor cells, and potential clinical implications for hair follicle regeneration are also discussed. This overview of iPSCs and their applications aims to contribute to our understanding of their role in hair restoration and potential future therapeutic applications.

Contents

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1. Introduction

Hair loss is prevalent and it can affect individuals of all ages and genders. Its most common form is androgenetic alopecia, which is more common in men, whereas women may experience diffuse hair thinning [1]. Factors that contribute to hair loss include age, genetics, medical conditions, and treatments. Although some degree of hair loss during aging is normal, persistent and/or severe hair loss may indicate underlying health issues that necessitate treatment. Hair follicles, skin tube-like structures that are crucial for hair growth, undergo cycles of growth, rest, and shedding, with phases including anagen (growth), catagen (transition), and telogen (resting) [2]. Hair growth is regulated by dermal papilla at the follicle's base [3,4]. Hair shafts, the visible part of hair, are mainly made of keratin. Different types of follicles have different hair texture. Various factors, including genetics, hormones, age, and health influence hair growth. Hair follicle disorders that cause conditions like alopecia may be caused by genetic, autoimmune, hormonal, or external factors [5]. For improved hair health and to develop hair loss treatments, it is essential to understand hair follicle biology. There are several potential hair loss treatments addressing various causes and stages. Topical solutions like minoxidil and oral medications like finasteride are widely used to treat pattern baldness [6–8]. Platelet-rich plasma therapy involves concentrated platelet injection into the scalp for hair follicle stimulation [9,10], whereas low-level laser therapy uses light to boost blood flow and cellular activity [11]. Surgical strategies, such as hair transplantation (follicular unit transplantation and follicular unit extraction), involve follicle relocation to regions of hair loss [12,13]. Other hair growth promotion strategies include hormone therapy, nutritional interventions, and anti-inflammatory medications, and research is also exploring gene therapy [14–17]. However, because these treatments have varying effectiveness, it is crucial to consult a healthcare professional to determine the best approach based on hair loss type and cause. Stem cell therapies, including induced pluripotent stem cells (iPSCs), have shown hair follicle regeneration potential, and ongoing research is exploring novel innovative treatments for various types of hair loss.

1.1. Principles of iPSCs

iPSCs are a promising resource for regenerative medicine and their discovery by Shinya Yamanaka and his team in 2006 [18] revolutionized stem cell research. Before their discovery, embryonic stem cells were the main source of pluripotent stem cells, but they were associated with ethical concerns because of their human embryo origin. For research and potential therapeutic applications, iPSCs are more ethically acceptable than embryonic stem cells because they can be generated from a patient's cells without the need for embryo destruction [19]. iPSCs are generated through the process of reprogramming, in which specific genes are introduced into adult cells. These genes, usually Oct4, Sox2, Klf4, and c-Myc induce a pluripotent state (similar to that of embryonic stem cells) in adult cells by resetting their gene expression patterns [20]. Because of their pluripotency, iPSCs can differentiate into any specialized human cell type, including neurons, myocardiocytes, and hepatocytes. Therefore, iPSCs offer a potentially unlimited source of cells for regenerative medicine and disease modeling [21]. By providing a platform for studying the development and progression of various diseases in controlled laboratory settings, iPSCs have revolutionized disease modeling. Generating iPSCs from patients with specific diseases and their differentiation into disease-relevant cell types can improve our understanding of disease mechanisms and facilitate the development of potential therapies [22]. By generating individual patients' iPSCs, researchers can create patient-specific cell lines for testing and screening drugs [23], thereby minimizing the risk of immune rejection and developing tailored and more effective treatments.

1.2. iPSC generation

iPSC generation involves reprogramming differentiated cells into a pluripotent state like the one of embryonic stem cells. The groundbreaking work for iPSC generation began with Takahashi et al., in 2007, who delivered the transcription factors, Oct4, Sox2, Klf4, and c-Myc into adult human fibroblasts, thereby reprogramming them into iPSCs that exhibited pluripotency and the differentiation potential, which opened new avenues for regenerative medicine, disease modeling, and drug discovery [24]. Shortly after, a similar feat was achieved by Yu et al. (2007), indicating that the four above-mentioned transcription factors were sufficient for human somatic cell reprogramming into iPSCs. This marked a significant advancement in stem cell research, highlighting the therapeutic potential of iPSCs [25]. Considering concerns about genomic integration and tumorigenicity, Yu et al. (2009) developed a safer approach that used synthetic mRNA to deliver reprogramming factors and successfully generated iPSCs without residual vector or transgene sequences. This non-integrating strategy, which addressed safety concerns over traditional iPSC generation methods, offered a clinically relevant strategy with potential therapeutic applications [26]. Deyle et al. addressed iPSC generation methods, including the creation of murine embryonic fibroblast-conditioned medium, murine embryonic fibroblast preparation, and iPSC passage and cryopreservation. The reprogramming procedure, which involves ectopic embryonic transcription factor expression, has been thoroughly examined for disease modeling and treatment strategy development. This resource is highly valuable for the research and practice of stem cell biology and reprogramming [27].

2. iPSCs and hair follicle precursor cells

The use of iPSCs to hair follicle precursor cells is an interesting area of research with potential regenerative medicine applications, particularly in hair regeneration and hair-associated therapy. Potential iPSC applications include using them in the study of hair follicle development and cell-based therapy, drug testing, and the enhancement of our understanding of hair disorder pathophysiology [28]. A study demonstrating iPSC generation from human hair follicular keratinocytes highlighted their potential for hair

regeneration, with the derived iPSCs expressing key pluripotency markers and differentiating into keratinocytes. By demonstrating that hair follicles are accessible for iPSC generation and that they are suitable for *in vitro* hair cloning, the study highlighted their promising potential in hair loss treatment [29]. iPSCs from individuals with hair loss offer an autologous, expandable source of cells, and their differentiation into various folliculogenic cell types enable functional hair follicle formation. iPSCs derived from individuals with hair loss were xenografted into nude mice and have demonstrated the potential advantages of iPSC-based regenerative strategies over current surgical methods, including providing a virtually unlimited source of *de novo* hair follicles. Integrating this innovative method with robotics makes it a promising strategy for routine, affordable hair restoration [30]. These observations indicate that in regenerative medicine, iPSC use is promising for hair follicle regeneration and hair disorder treatment.

2.1. iPSC differentiation into hair follicle precursor cells

iPSC differentiation into hair follicle precursor cells can lead to the development of novel treatments for hair loss and related conditions. The differentiation of iPSCs into hair follicle precursor cells involves various growth factors, signaling pathways, and genes. When compared with dermal fibroblasts, dermal papilla cells, unique regional skin stem cells, have a higher reprogramming efficiency for iPSC generation. The use of Oct4, Sox2, Klf4, and c-Myc with valproic acid and 5% oxygen achieved a reprogramming efficiency of about 0.03% in dermal papilla cells when compared with about 0.01% in dermal fibroblasts. This study highlights dermal papilla cells as a promising alternative source for iPSC generation [31]. A recent review explored hair follicle morphogenesis, cycling, and diverse cell sources, focusing on iPSCs for bioengineering. The challenges and perspectives about the therapeutic use of iPSCs for hair loss are also discussed, emphasizing their potential to meet this clinical need [28]. Although hair follicles possess regenerative capacity, *de novo* hair follicle formation is limited. Moreover, extensive skin damage or hair loss is challenging for physiological and aesthetic functions. Current regenerative strategy obstacles include the loss of trichogenic capacity in cultured cells and the lack of suitable *in vitro* testing platforms. A study that addressed these challenges highlighted the significance of cellular requirements, the hair follicle extracellular matrix, and associated signaling in regeneration of hair follicles. It also explored human hair follicle bioengineering strategies and hair-bearing skin models, thereby outlining future trends in the field [32].

2.2. Co-culture and organoid formation

In cell biology and tissue engineering, co-culture and organoid formation involve interaction between different cell types to create three-dimensional (3D) structures called organoids. These methods are widely used in various fields, including regenerative medicine, developmental biology, and disease modeling. Organoids, which mimic organ architecture and functionality, typically form from self-organizing stem cells or fragments of tissues and they represent miniaturized, simplified versions of organs or tissues for use in *in vitro* studies. These structures can recapitulate the complexity and functionality of their tissues of origin. iPSCs are co-cultured with key cell types, including dermal papilla cells, keratinocytes, and melanocytes, which are crucial for hair follicle development. This co-culture aims to provide a supportive environment for iPSC differentiation into functional hair follicles. Additionally, iPSCs, along with the pertinent cell types, are organized into 3D organoid structures, which faithfully replicate natural hair follicle architecture and functionality. In a recent study, researchers characterized 3D hair follicle organoids that were self-assembled via neonatal mouse epidermal cell coculture with dermal cells in Matrigel. The hair follicle organoids resembled native anagen hair follicles and were made up of distinct structures, including infundibular cyst-like, lower segment-like, and bulb-like structures. Analyzing the expression of hair follicle, stem cell, and dermal papillae markers confirmed that the hair follicle organoids had specific compartments consistent with native hair follicles. The study highlighted complete hair follicle organoids in Matrigel as a powerful model for comprehensive hair follicle disease studies and precision therapy and provided valuable insights into hair follicle organoid structure and properties [33]. The potential of cord blood mononuclear cell-derived human iPSCs for skin regeneration has been described. The iPSCs were differentiated into fibroblasts and keratinocytes, forming distinct epidermal and dermal layers, and overlaying these layers generated a 3D skin organoid, which, after successful transplantation into the Severe Combined Immunodeficiency (SCID) mouse model, demonstrated effective skin lesion healing. This humanized skin model is a valuable *in vitro* and *in vivo* dermatological research tool with potential regenerative medicine applications [34]. Fig. 1 summarizes the use of iPSCs to treat hair disorders.

3. Clinical implications of iPSC-derived hair follicle regeneration therapy

Using iPSCs and hair follicle cells to treat hair loss has important clinical implications. Because they can be generated from hair follicles, iPSCs are easily accessible for treatment, and since they have a high tendency for re-differentiation into hair follicles, these pluripotent cells are well-suited for growth in the hair scalp tissue microenvironment. Moreover, hair follicle regeneration depends on the cooperation between epithelial (epidermal stem cells) and hair-inducive mesenchymal (dermal papilla) cells [35]. Autologous cell-based male and female pattern hair loss treatment using dermal sheath cup cells has also been investigated as a potential therapeutic strategy [36]. Lim et al. investigated human hair follicles as a potential source of iPSCs. This study highlighted hair follicle genetic and microenvironmental elements that may aid hair follicle re-differentiation efficiency, thereby identifying promising applications for the treatment of patients with hair loss and burns requiring grafting [29].

3.1. Patient-specific therapy

Patient-specific therapy, a cornerstone of personalized medicine, seeks to tailor therapy to the individual's genetic makeup, lifestyle, and medical history. For hair loss, personalized therapy is invaluable for addressing the various underlying factors and effects that are unique to each patient, thereby promoting more precise and efficient treatment strategies. The innovative use of iPSCs reprogrammed from the patient's cells is at the heart of patient-specific therapy. iPSCs, which can differentiate into various cell types, are invaluable research tools that offer avenues for disease research, drug development, and regenerative medicine [37,38]. A key advantage of using iPSCs from the patient's cells is that it overcomes the challenge of immune rejection because such iPSCs are genetically the same as the patient's cells, which significantly reduces their likelihood of being recognized as foreign by the immune system. This is particularly crucial for cell transplantation therapies because transplanted cells must seamlessly integrate and function effectively in the patient's body. There are several iPSC generation methods with unique advantages and disadvantages. For instance, retroviral-mediated reprogramming, which is widely used, has the risk of reprogramming factor integration into the

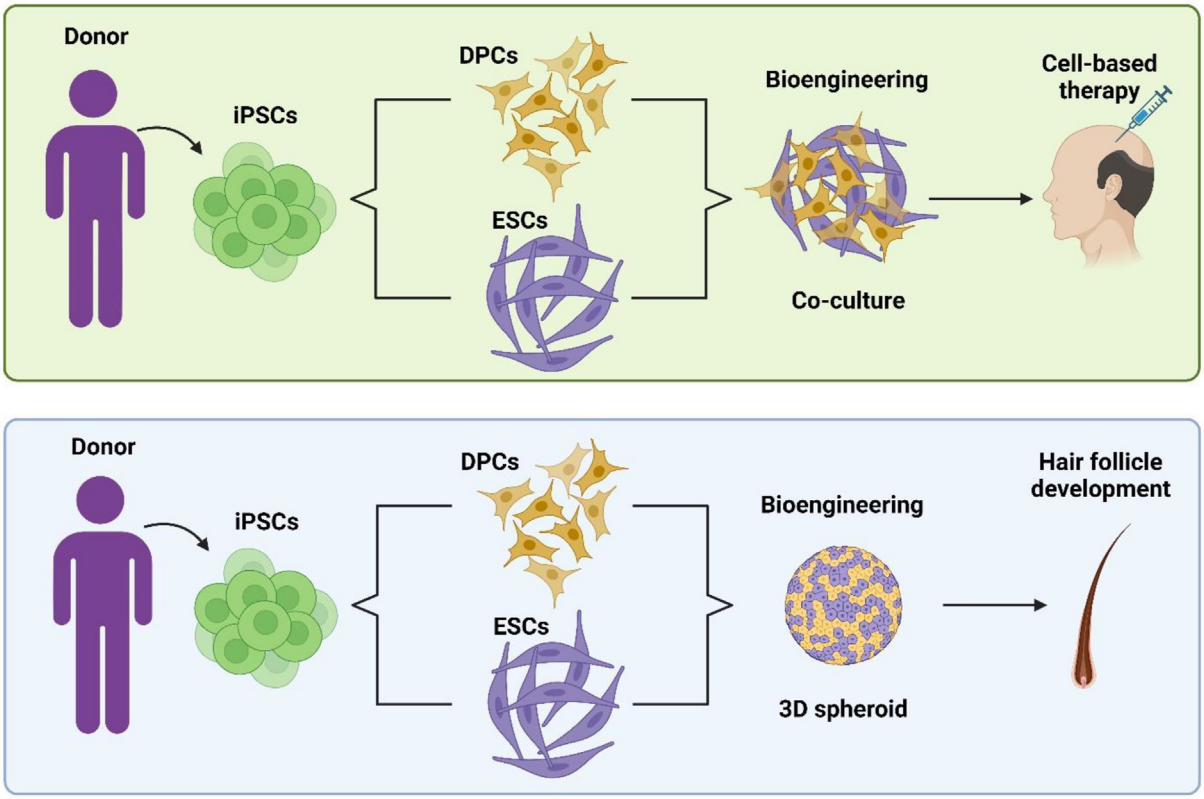

Fig. 1. iPSC use for hair disorders. Hair follicle (HF) regeneration can occur through or generating iPSC-derived dermal papilla cells (DPCs) and epithelial stem cells (ESCs), and then combining them to generate HFs through bioengineering (co-cultures or 3D spheroids). The regenerated HFs have various applications, including studying cell-based therapy and HF development. Created with BioRender.com.

host's genome, which may cause genomic instability and tumorigenesis. However, although promising (especially in mouse iPSCs), the use of piggyBac transposon-mediated reprogramming to derive human iPSCs remains limited. Other methods, such as minicircle vectors and episomal plasmids, offer unique benefits, and research to validate their effectiveness is ongoing [39,40]. Clinical trials on iPSC use for hair follicle regeneration are also ongoing. For over two decades, Stemson Therapeutics has been at the forefront of stem cell therapy, and has witnessed first-hand, the ground-breaking impact of iPSCs in this field. In their pioneering approach, adult cells are reprogrammed into iPSCs, which are then guided to differentiate into hair follicle cells. To form a follicular unit that encourages directional hair follicle growth and hair shaft outgrowth, the specialized cells are combined with biomaterials and hydrogels [41].

The potential of iPSC-based therapy has been further reinforced by a study of the potential use of iPSCs in human hair follicle regeneration, which highlighted the multifaceted role of hair follicles in physical protection, thermoregulation, sensation, and wound healing, and underscored the potential of iPSCs as a limitless source of hair follicles for transplantation [28].

iPSC can differentiate into various cell types, including those crucial for hair follicle regeneration. When compared with traditional treatments, patient-specific iPSCs have numerous advantages for hair loss therapy. By investigating the factors underlying hair loss, rather than addressing the symptoms alone, this personalized approach offers more effective tailored treatment plans, thereby enhancing successful outcomes and patient well-being by mitigating the risk of immune rejection and other complications that are commonly associated with conventional treatments [42].

3.2. Disease modeling

iPSCs are crucial for disease modeling because they can differentiate into various cell types, including cells involved in hair follicle biology. Thus, researchers can create models that closely recapitulate the disease in patients. For instance, turning iPSCs from a patient's cells into specific hair follicle cells, such as dermal papilla cells or keratinocytes, can allow deeper research into hair follicle development, renewal, and the pathobiology of hair disorders [43].

For instance, researchers can mimic genetic disorders like alopecia areata or androgenetic alopecia using iPSCs. Through the conversion of iPSCs from people with these disorders into hair follicle cells, scientists can comprehensively investigate how genetic factors affect hair growth. In addition to understanding the disease better, this type of research can also identify more effective treatments [43,44].

A functional hair follicle regeneration study offered insights into the challenges and advances in hair follicle regeneration and highlighted the need for optimal cell reprogramming techniques and biomaterials for successful hair regeneration [35]. An *in vitro* study of the restoration of human dermal papilla cells' intrinsic properties highlighted the potential of iPSC-derived dermal papilla cells in hair follicle regeneration [45].

Using iPSCs for disease modeling has significant advantages, especially in the study of hair follicle genetic disorders. iPSCs provide a unique platform for studying disease mechanisms, screening potential therapeutics, and personalizing treatments. iPSC disease models can recapitulate genetic disorders' cellular environment, thereby improving our understanding of disease pathology and progression. By enabling drug candidate testing on patient-specific cells, iPSCs can also be used for drug screening to identify effective

treatments. Furthermore, by allowing researchers to tailor therapies to the patient's genetic and disease characteristics, iPSCs enable personalized treatment. Overall, iPSCs are a powerful disease modeling tool that offers versatile, personalized strategies for studying genetic disorders that affect hair follicle development and function [43,44].

3.3. Challenges and future directions

3.3.1. Challenges

The use of iPSCs for hair follicle regrowth faces some challenges. A key challenge is how to convert iPSCs into the right type of hair follicle cells, which would require ensuring their reliable and effective transformation into specific cell types needed for hair regrowth [41,46]. Indeed, getting human hair follicles to grow back consistently and naturally is hard, and further research is needed to generate hair follicles that behave as expected, with regular growth cycles [28,46]. It is essential to create the right conditions for cells to grow into hair follicles, and an environment that supports the various cell types involved in hair follicle building and rebuilding is necessary [28]. In the field of autologous iPSCs, the timelines required to create iPSC banks are challenging since their lengthy culture times using strategies like the Sendai virus delivery system can impact iPSC-based therapy efficiency and timelines [41], and it takes a long time to generate enough cells. The time some methods take to generate iPSCs can slow down the entire therapy development process [46], and it is vital to ensure that every batch of cells meets the required standards. Therefore, robust methods that consistently generate the right cell types for hair regrowth, irrespective of the donor, are needed [46]. Addressing these challenges via advanced differentiation protocols and optimized culture conditions and manufacturing processes is crucial for the successful use of iPSCs for hair follicle regeneration, which would offer promising strategies for hair loss disorder treatment and regenerative medicine.

3.3.2. Future directions

As research in regenerative medicine continues to advance, the use of induced pluripotent stem cells (iPSCs) holds tremendous promise for addressing the challenges associated with hair loss and hair regeneration. iPSCs, derived from adult cells and reprogrammed to a pluripotent state, offer a versatile platform for generating various cell types relevant to hair follicle formation and growth. In recent years, significant progress has been made in utilizing iPSCs for hair regeneration therapies, but there are still several areas that require further exploration and refinement. In this section, we will outline key future directions in iPSC-based hair regeneration research, highlighting avenues for enhancing differentiation protocols, leveraging genome editing technologies, integrating biomaterial scaffolds, advancing personalized medicine approaches, and conducting clinical trials to pave the way for the translation of iPSC-based therapies into clinical practice.

- Enhanced differentiation protocols: continued refinement of differentiation protocols for highly efficient generation of pure hair follicle progenitor cell populations.
- Genome editing technologies: use of precise genome editing tools like CRISPR-Cas9 for iPSC modification for better safety and efficacy.
- Biomaterial scaffolds: integrating iPSC-derived cells into biomaterial scaffolds for enhanced *in vivo* engraftment, survival, and functionality.
- Personalized medicine: advanced personalized medicine approaches, including patient-specific iPSC generation for reduced immune rejection and optimal treatment outcomes.
- Clinical trials and regulatory approval: conducting well-designed clinical trials to determine the safety and efficacy of iPSC-based hair regeneration therapies.

4. Conclusion

iPSCs are a promising strategy for hair loss treatment and advancing regenerative medicine. By overcoming ethical concerns over the use of embryonic stem cells, adult cell-derived iPSCs can support extensive research into personalized therapies. Adult cell reprogramming into pluripotency offers a versatile source of cells for hair follicle regeneration. Clinically, iPSC-based treatments offer tailored therapies, which circumvent immune rejection. However, challenges remain, including for efficient human hair follicle differentiation and stable reconstitution. These challenges can be overcome through optimized protocols, genome editing, and biomaterial integration.

Finally, iPSC-based therapies represent a paradigm shift in the treatment of hair loss and offer personalized solutions for advanced regenerative medicine. They have the potential to revolutionize treatment and offer hope to millions, worldwide.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

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Authors' contributions

Poornima Sivamani, Ramya Lakshmi Rajendran, Prakash Gangadaran and Byeong-Cheol Ahn contributed to the conception, writing, and discussion of this manuscript. Poornima Sivamani and Ramya Lakshmi Rajendran contributed equally for the manuscript. All authors have approved the final version of the manuscript.

Declaration of competing interest

The authors have declared that no competing interest exists.

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