Hair Follicle as a Source of Pigment-Producing Cells for Treatment of Vitiligo: An Alternative to Epidermis?

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Abstract To discuss the advantages and limitations of hair follicle-derived cell transplantation (FCT) in vitiligo, compared to the epidermal cell transplantation (ECT), and the knowledge gap which is required to be bridged. The papers relevant to the purpose was reviewed. Surgical approaches for treating vitiligo are based on the idea of replenishing lost melanocytes. Skin and hair follicles as the main sources of melanocytes have been applied for this purpose transferring the whole tissue or tissue-derived cell suspension to the vitiligo lesions. Considering the differences between hair follicle and epidermis in terms of the constituting cell populations, phenotype and function of melanocytes, and micro-environmental factors, different response of vitiligo patients to treatment with FCT or ECT would be expected theoretically. However, there is currently a lack of evidence on such a difference. However, ECT appears to be a more feasible, less time-consuming, and more comfortable treatment for both physicians and patients. Although the current evidence has not shown a significant difference between ECT and FCT in terms of efficacy, ECT appears to be more feasible specifically in the treatment of large lesions. However, further randomized controlled clinical trials with larger sample sizes and longer follow-up durations are required to be conducted to draw a definite conclusion on comparing FCT with ECT in terms of the safety, efficacy, durability of the therapeutic effects, and indications in vitiligo patients.

Keywords Vitiligo · Regenerative medicine · Cell therapy · Hair follicle · Melanocyte

1 Background

Various surgical approaches have been introduced in recent decades for treatment of vitiligo [1]. Skin autografting for treatment of vitiligo was initially reported in 1964 when split-thickness grafting technique was employed. Later, blister roof grafting (in 1971) and punch grafting (in 1978) were introduced as alternative skin transplantation techniques in vitiligo patients [2]. In 1987, autologous epidermal cell transplantation (ECT) was introduced as a novel surgical treatment for vitiligo. Since then, numerous studies have reported safety and efficacy of ECT in vitiligo [3–17].

Researchers came up with the idea of using hair follicle in the treatment of vitiligo once clinical evidence indicated the probable role of hair follicle in skin repigmentation. Such evidence indicated resistance of glabrous vitiligo

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patches to medical therapies [18], perifollicular repigmentation in vitiligo lesions healing spontaneously or under medical/light-based treatments, and perifollicular repigmentation of vitiliginous skin following hair follicle grafting as a treatment for alopecia [19]. Meanwhile, histochemical and ultrastructural studies on vitiligo lesions showing repigmentation following photochemotherapy, confirmed this hypothesis by revealing the existence of a melanocytic reservoir in the hair follicle which cells proliferate, migrate through the infundibulum toward depigmented epidermis, and radially spread to repigment the vitiliginous skin [20–22].

In 1994, the idea was examined clinically by transplanting scalp hair bulbs on vitiliginous lesions where repigmentation around grafted hair bulbs was reported in 40% of treated patients [19]. In 1998, for the first time, Na et al., reported transferring complete single hair follicles to vitiligo lesions where peri-follicular repigmentation around the grafted hair follicles was reported in 71% of vitiligo patients [23]. Since then, several studies reported safety and efficacy of grafting autologous scalp or body hair follicles in vitiligo patients [24–28]. However, this treatment was found to be a tedious, time-consuming and delicate procedure which becomes even more difficult to perform in case of large lesions. In addition, repigmentation following hair follicle grafting was a slow process due to the time required for hair melanocytes spread [23]. To overcome these obstacles, hair follicle-derived cell transplantation (FCT) was introduced in 2009 [29]. Later, further clinical studies showed safety and efficacy of FCT in vitiligo patients. Nevertheless, FCT has not been widely used in clinical practice compared to ECT. In this review, we aim to discuss advantages and limitations of FCT in comparison with ECT in vitiligo treatment as well as the research gap that needs to be bridged.

2 Histochemical characteristics of hair follicle and epidermis

Hair follicle and epidermis as sources of pigment-producing cells in the skin tissue, are theoretically regarded as favorable cell sources for vitiligo treatment. However, these two sources differ in the composition of cell populations, melanocytes phenotype and function, and cells microenvironment which may affect their therapeutic potential for vitiligo.

The outer layer of hair follicle, called outer root sheath (ORS), has been shown to contain a melanocyte reservoir in the middle and lower parts consisting of poorly differentiated amelanotic or light pigmented melanocytes which are small, slender shaped, and nondendritic. These amelanotic melanocytes could be detected *in vivo* by antibodies

against premelanosomes, but are not recognizable by mature melanosomal antigens such as the melanocyte lineage-specific antigen named glycoprotein 100 (gp100), functional proteins such as tyrosinase and tyrosinase-related proteins (TRP) 1, 2, nor 3,4 dihydroxyphenylalanine (DOPA) which is an intermediate product in melanogenesis [30, 31]. However, some amelanotic melanocytes might be weakly positive for gp100 and tyrosinase [32]. These DOPA-negative melanocytes are currently known as melanocyte stem cells (MeSCs), a distinct population of hair melanocytes differing from active melanocytes in terms of location, size, morphology, dendricity, pigmentation degree, antigen expression, and growth characteristics in culture [30]. MeSCs are the dominant type of melanocytes in the hair follicle, mostly existing in the mid-to-lower outer root sheath, and to a lower extent, in the periphery of the hair bulb [30, 32–34]. Amelanotic melanocytes in the hair bulb are also called “transient” melanocytes that migrate from upper outer root sheath [32].

MeSCs have been shown to have a high proliferative potential *in vitro*, and act not only as the progenitors of pigmented hair bulb melanocytes (during early anagen phase of hair cycle) and epidermal melanocytes [30], but also as a melanocyte reservoir during hair regrowth in alopecia areata, repigmentation of the vitiligo lesions [31], and skin wound healing [35, 36].

Indeed, MeSCs are inactive *in situ*, and while migrating to the upper layers of epidermis, gradually alter to DOPA-positive melanocytes which actively produce melanin and repigment the vitiliginous skin [18, 37]. Dermabrasion or ultraviolet (UV) irradiation was also shown to make these cells identifiable by DOPA staining [38].

Hair follicle is also a rich source of DOPA-positive melanocytes [33] which exist in the basal layer of the infundibulum and in the hair bulb [31]. These cells constitute a minor percentage of hair follicle-derived melanocytes (2–5%), and in contrast to MeSCs, are large, polydendritic, differentiated, and intensely pigmented with limited proliferative potential in culture condition [30, 31, 37, 39]. Moderately differentiated melanocytes also exist in the basal layer of the sebaceous gland [32].

The hair follicle MeSCs appear to be at an earlier stage of differentiation compared to the epidermal melanocytes, as they are less melanogenic and express lower levels of melanosomal antigens [30]. Besides, while hair MeSCs have the potential to migrate to the epidermis to repigment the vitiliginous lesions, the epidermal perilesional melanocytes rarely migrate to the lesional area [40]. However, it is not clear whether after being transferred to the recipient site niche, epidermal and follicular melanocytes differ in their potential in repigmenting vitiligo lesions, and if MeSCs would remain undifferentiated or they differentiate to active melanocytes and produce melanin.

It is hypothesized that in vitiligo patients, melanocyte stem cells in the ORS remain undamaged as they lack melanocytic antigen on their surface and consequently evade T cell-mediated apoptosis [18, 40]. Besides, epidermal and hair melanocytes express different antigenic profiles, and as a result, they are preferentially targeted by different circulating antibodies in vitiligo and alopecia areata. It is suggested that contrary to epidermal melanocytes, hair melanocytes do not express vitiligo-related antigens; while alopecia areata-related antigens are expressed by hair melanocytes, but not by epidermal melanocytes. Therefore, follicular melanocytes are often not damaged in vitiligo, whereas epidermal melanocytes remain protected in alopecia areata [30, 37, 41]. This difference might theoretically make hair follicle a potentially superior source for replenishing lost melanocytes compared to skin epidermis since hair follicle-derived melanocytes might be less vulnerable to the pathologic microenvironment in the recipient niche. However, the probable effects of the recipient pathologic micro-environment on the protective phenotype of hair follicle melanocytes have not been investigated.

Higher density of melanocytes in the follicular melanin unit (on average, one melanocyte for every 5 keratinocytes in the hair bulb and one to one in the basal layer of hair bulb matrix) compared to epidermal melanin unit (where there is one melanocyte for every 36 keratinocytes) appears to be the other theoretical advantage of hair follicle over epidermis in treatment of vitiligo [33]. However, this difference has not appeared to cause any superiority for FCT over ECT in terms of donor tissue harvesting (considering the duration and feasibility of harvesting procedure) or the final repigmentation rate [42–44]. Besides, discrepancies exist among reports on the ratio of melanocytes to keratinocytes in the hair follicle-derived and epidermal cell suspension [7, 43, 45].

The other theoretical advantage of hair follicle-derived melanocytes is that they are larger and more dendritic. They also possess a higher synthetic capacity as they have more extensive Golgi apparatus and rough endoplasmic reticulum and larger melanosomes [33]. However, due to difficulties in hair harvesting in large scale, these characteristics does not appear to cause a significant superiority in clinical practice. Besides, while melanogenesis in epidermis is a continuous process, melanogenesis in hair follicle is influenced by the hair growth cycle. This influence is exerted through micro-environmental factors including growth factors and cytokines secreted by keratinocytes and immune cells which affect melanocyte proliferation, mobility, and dendricity [30, 33]. However, it was demonstrated that photochemotherapy induces differentiation and migration of amelanotic melanocytes regardless of hair growth cycle [39].

It is also supposed that follicular bulbar melanocytes are vulnerable to aging. Aging results in disturbance of antioxidant system and negatively affects the mechanisms involved in neutralizing melanogenesis-induced oxidative stress. Ageing-induced increased oxidative stress disrupts melanocyte-keratinocyte interactions and leads to whitening of hair shaft. Low expression of bcl2, as an anti-apoptotic factor, as well as defect in melanosome transfer from melanotic hair bulb melanocytes to keratinocytes exacerbate the effect of age-related loss of melanogenic melanocytes in hair compared to epidermis. [33].

Melanocytes are not the only component of hair follicle-derived and epidermal cell suspensions. Keratinocytes and keratinocyte stem cells also constitute a considerable portion of hair follicle and epidermis. Interfollicular keratinocyte stem cells reside in the basal layer of epidermis, where they act as a source of keratinocytes, differentiating and moving upward to the skin surface through consecutive stages [46, 47]. Bulge area of hair follicle—a distinct part of ORS which is located between the duct of sebaceous gland and the insertion of arrector pili muscle—is also a rich source of keratinocyte stem cells [22, 48, 49]. Keratinocytes and keratinocyte stem cells not only play a prominent role in stimulating proliferation of melanocytes and melanogenesis [50], but also could potentially accelerate re-epithelialization of abraded recipient skin [49]. However, there is evidence indicating differences between epidermal and ORS keratinocytes which might affect the repigmentation potential of hair and epidermal cell suspensions in vitiligo treatment. It was shown that proliferative capacity of ORS keratinocytes is not affected by donor age while epidermal keratinocytes' ability to proliferate and produce mitogenic factors to induce proliferation of surrounding cells, reduces by aging [37].

Besides melanocytes, keratinocytes, and keratinocyte stem cells, hair follicle-derived cell suspension contains further cellular and molecular components which might exert influence on the repigmentation process. Dermal papilla, which is surrounded by the hair bulb (the enlarged part of hair follicle at the base), is a source of mesenchymal stem cells (MSCs) and essential growth factors required for hair growth and melanogenesis [51]. Additionally, since a small portion of the surrounding dermis might be extracted while harvesting hair follicles [52], dermis-resident MSCs might also exist in the hair follicle-derived cell suspension [51]. It was shown that dermal MSCs could potentially enhance the efficacy of cell transplantation via their inhibitory effect on perilesional CD8 + T-cells which negatively influence vitiligious lesions repigmentation [53]. These cells might also play role in enhancing re-epithelialization of abraded recipient skin [49, 52, 54]. Moreover, dermis-derived stem cells have been identified in the dermal layer of glabrous skin with the potential of migrating

to the epidermis and differentiating into active melanocytes [55].

3 Tissue harvesting, cell preparation and transplantation

3.1 Tissue harvesting

To treat vitiligo lesions using the ECT, the partial thickness autologous normo-pigmented skin is taken often from the thigh-buttock junction. This is usually performed using a manual or motorized dermabrader to remove the superficial layers of skin up to the dermo-epidermal junction, where pinpoint bleeding occurs. The size of the donor area has been varied from one-tenth to one-third of the recipient site between different studies [3–17].

In the FCT procedure, however, it is important to extract hair follicles in the anagen phase, when proliferation and differentiation of melanocyte progenitor cells occur and hair follicle melanocytes actively produce melanin [22, 56]. Furthermore, dermal papilla—which is a good source of mesenchymal stem cells and melanogenic growth factors [51]—is more prone to damage during the telogen or resting phase [57]. Anagen hair follicles can be clinically determined by their higher tensile strength and darker color [52]. To facilitate determination of anagen hair follicles in the anagen phase, shaving or trimming the donor area three to four days before harvesting the hair follicles is required [57].

Methods used for harvesting hair follicles include skin stripping, hair plucking, and follicular unit extraction (FUE). In Single-strip technique, an elliptical excision is made on the scalp followed by suturing [58]. After washing the obtained tissue with cold normal saline, single hairs are dissociated by a surgical blade [1]. The main disadvantage of this method is the linear scar developed on the donor site which might lead to unfavorable cosmetic appearance especially for those who want to cut their hair short [58]. Two methods used for hair follicle harvesting in FCT in vitiligo patients, include hair plucking [29] and FUE [42, 52, 59]. Unlike strip method, in these two techniques, no scar develops on the donor site, patients experience less numbness and no pain or hemorrhage following the operation, there is no need for suture removal, and the recovery time is less than that of strip technique [58]. Although hair plucking is a simple and non-invasive method, FUE results in higher cell yield with higher proportion of CD200 + cells (a marker for hair follicle bulge stem cells) [60].

FUE is based on loosening the tightest zone of the hair follicle which is point where arrector muscle attaches the follicular unit. Separating this area from the surrounding dermis leads to intact extraction of follicular unit [58]. The

FUE procedure can be performed in two different ways as follows:

SHARP method which includes two steps: First, placing the punch and pushing it over the follicular unit -not too deep—in the direction of the hair shaft. Second, gentle upward traction of follicular unit by a fine forceps until deeper connections are loosened.

Blunt method or surgically advanced follicular extraction (SAFE) includes three steps: First, applying a sharp punch to score the epidermis. Second, blunt dissection of the follicular unit with dull side of the punch; and third, holding the dissociated graft by a forceps and pull it out [58, 61]. Applying the blunt technique reduces the risk of transection rate and hair follicles damage [58]. However, it may lead to higher incidence of buried grafts and is a more time-consuming procedure [58].

Harvesting hair follicles using FUE—as the best approach for hair follicle harvesting—has some advantages like formation of invisible scar at the donor site and more rapid healing process, over harvesting epidermis using dermabrasion [52]. However, FUE has some limitations compared to dermabrasion which makes its feasibility questionable. First of all, the precise recognition of anagen hair follicles is time-consuming, requires high expertise and shaving or trimming scalp hairs before harvesting which might be unpleasant for some patients.

Secondly, FUE is a time-consuming, exhausting, and difficult procedure to learn. To harvest 20–25 follicular units, which is sufficient to repigment an area of just 20 cm², 20–30 min is required [52, 62]. To treat larger areas, the required number of hair follicles and consequently the harvesting time might increase. Although by a highly expert team, FUE could be performed more rapidly (in 10–15 min) even for harvesting a higher number of hair follicles, the required time for dermabrasion in ECT is often shorter regardless of the recipient surface area or the level of expertise.

Thirdly, the risk of hair follicles transection during the FUE procedure might lead to significantly reduced numbers of capable cells in the prepared cell suspension. Although applying the blunt FUE technique could reduce the transection risk, it might increase the rate of buried grafts and subsequently, the time required for hair follicle harvesting [58].

Despite all above-mentioned limitations of hair follicle harvesting compared to harvesting epidermis, it should also be considered that to treat a vitiligo lesion of certain size, the surface area of the donor scalp required in FCT is much less than that needed in ECT since healthy human's scalp contains approximately 200 hair follicles per square centimeter, out of which, 80% are in anagen phase [63]. Besides, FUE is not associated with significant scar formation at the donor site [58]; while dermabrasion might

cause hyperpigmentation, hypopigmentation, or scar formation at the donor site [10, 44, 45, 64].

3.2 Preparation of cell suspension

To prepare the epidermal cell suspension, the donor skin specimen requires to be cut into 2–3 mm² pieces and go under enzymatic treatment followed by manual separation of the epidermis from the dermis. The epidermal sheet is then treated by trypsin enzyme to obtain the epidermal single-cell suspension [3–17].

Preparation of follicular cell suspension, however, requires fewer steps. ORS cells could be easily separated by trypsinization. Unlike ECT, there is no need for separation of dermis from epidermis, and manual breaking of epidermis to small pieces for isolating cells from hair follicle, and trypsinization is enough to obtain the cell suspension [6, 10, 42, 52, 59].

The addition of the platelet rich plasma (PRP) to the epidermal cell suspension was recently shown to cause a significant enhancement of the final repigmentation following ECT [65]. However, PRP has not been applied in the preparation of transplanted follicular cell suspensions.

Most studies which have evaluated the efficacy of ECT or FCT in vitiligo patients have not mentioned either the exact number of transplanted cells per cm² or the required cell number to achieve the optimum repigmentation. The number of transplanted hair follicle-derived cells reported in previous studies with partially acceptable clinical results, varies between 1000 and 7000 cells per cm² of recipient area [29, 42, 43, 52, 59]. However, in most of these studies, it was not exactly determined if the mentioned cell numbers reflect pure melanocyte number or the total cell number present in the suspension. Concomitant treatments with cell transplantation (such as UV therapy) in some studies should also be considered a variable which might have affected the required cell number.

Only one study reported cell concentration required to achieve the optimum repigmentation (1187 melanocytes/cm²), by comparing transplanted cell number between patches with > 75% repigmentation and patches with < 75% repigmentation. However, it should be considered that the maximum concentration of transplanted cells in this study was 2747 melanocytes/cm² and the efficacy of higher cell concentrations was not evaluated. Besides, > 75% repigmentation is not considered the ideal treatment response and no statistical significant difference in the transplanted cell number was found between those with > 90% repigmentation and those with < 90% repigmentation. However, the number of hair follicle stem cells differed significantly between > 90% repigmentation and < 90% repigmentation groups [59].

Mohanty et al. also concluded that 25,000–50,000 cells—which can be obtained from 15 to 25 follicular units—were sufficient to pigment up to 25 cm² of the recipient area. However, they did not assess the exact relation between cell number and final repigmentation rate [52].

Certainly, current data is not sufficient to draw a definite conclusion on the effect of cell number on the final repigmentation rate, and dose-finding trials should be conducted to find the optimum cell number required in FCT and ECT. Nonetheless, based on the available evidence, number of transplanted cells per area unit appears to positively influence the final repigmentation rate. In this regard, melanocyte culture has become an appealing approach with the aim of increasing the efficacy of melanocyte transplantation. Although the safety and efficacy of cultured ECT have been reported by numerous clinical studies [11, 12, 14–17, 66], there is no *in vivo* report on transplanting cultured FCT. This might be due to the safety concerns arising from possible risk of genomic abnormality that might be induced in cultured adult stem cells [67], as well as the tumorigenesis potential of hair follicle cells, particularly bulge stem cells considering their long life, slow cell cycle and susceptibility to carcinogenic mutations [49]. Although it was reported that most of mouse hair follicle melanocyte stem cells begin to differentiate after three passages *in vitro* [39], more investigations are required to define and if possible, minimize the risks of hair melanocyte culture using safe agents in the culture media. The relatively low numbers and low proliferative potential of ORS cells in routine culture media [37] might be considered other limitations of hair melanocyte culture. However, efforts have been made to develop and improve hair melanocytes culture methods with research (not therapeutic) purposes.

Successful culture of hair follicle melanocytes was first reported in 1995 by Tobin et al. who isolated and cultured melanocytes from both hair bulb and middle and lower parts of ORS. They removed dermal tissue from isolated hair follicles to avoid prolonged Geneticin treatment with the aim of omitting fibroblasts, which could result in melanocyte loss in parallel. After confirming separation of epidermis, they identified two distinct cell populations in the culture with undifferentiated cells originated mostly from ORS being dominant over differentiated cells originated from hair bulb which were few in primary culture and disappeared after passage 3. They attributed this phenomenon to the low proliferative capacity of differentiated cells or insufficient support for their proliferation by the culture conditions [31].

Later, more efforts were made to culture human hair follicle-derived amelanotic melanocytes by modifying culture conditions [68, 69].

In 2006, Na et al. introduced a novel culture method using ORS keratinocytes (ORSK). They cultured hair follicle-derived cell suspension in melanocyte growth medium with stem cell factor, and added ORSK from the 2nd passage to the culture media. While ORSM did not proliferate in melanocyte growth medium alone, addition of ORSK from the 2nd passage, led to initiation of ORS melanocytes (ORSM) proliferation. Besides, some DOPA-negative ORSM became DOPA-positive after passaging with ORSK [37].

While all the previous reports on human hair melanocyte culture applied scalp skin to harvest hair follicles, culturing melanocytes derived from plucked hair follicles, as a simple and non-invasive harvesting technique, was first reported by Dieckmann et al. in 2010. No enzymatic treatment, which could be harmful for cells, was applied in this method. Besides, no contamination with fibroblasts was observed probably due to the specific melanocyte growth medium applied in this new approach. Although epilation resulted in harvesting just incomplete root sheaths and subsequently a decreased number of obtained melanocytes, it was shown that decreasing oxygen level in the culture condition led to 1.5-time faster proliferation of ORS melanocytes as a result of reduction in oxidative stress. Using this culture method, $1\text{--}1.5 \times 10^6$ pure melanocytes were yielded from 60 plucked anagen hair follicles within 6–8 weeks [63].

Later in 2012, Savkovic et al. improved hair melanocyte culture method introduced by Dieckmann, by introduction of the DLM medium (low-serum content melanocyte culture medium), microdissection, collagenase treatment and antibiotic selection; such changes led to faster growth and sufficient differentiation, out-selection of competing cells, and finally, higher purity of yielded differentiated melanocytes (at least 10^6 of 95% pure differentiated human melanocytes from epilation of 60 hairs) within a 50% shorter time period (4 weeks); Reaching melanotic stage happened within 3–4 week post-epilation in the third passage of adherent culture [70].

In 2013, Wang et al. introduced a new method to culture melanocyte precursors from ORS in a way that maintain their undifferentiated state with the aim of studying mechanism(s) underlying repigmentation. They also showed that vitamin D significantly enhanced the differentiation of amelanotic melanocytes to DOPA-positive melanocytes [71].

It is not yet clear if the MeSCs in the hair follicle-derived cell suspension maintain their stemness or differentiate to DOPA-positive melanocytes in the recipient niche. Revealing the MeSCs fate and function in the recipient site is necessary to elucidate the optimum culturing conditions for ORS melanocytes to be applied in treatment of diseases like vitiligo.

3.3 Cell transplantation

Various techniques have been reported for preparation of the recipient site before transplantation of epidermal or follicular cell suspension on vitiligo lesions; such techniques include manual or motorized dermabrasion [9, 10, 42, 52, 59], CO₂ laser [6, 43], and Er:YAG laser [29]. Injection of the epidermal cell suspension into the blistered recipient skin has also been reported [72]. Although adverse events were not reported in the majority of these studies, some reports indicated pain, hypopigmentation, hyperpigmentation, scarring, allergic reaction to the applied dressings, and recipient-site infection as adverse events following transplantation of epidermal or hair follicle-derived cell transplantation in vitiligo patients [3, 4, 6, 8–10, 62, 72, 73]. However, these techniques differ not only in safety, feasibility, cost, and the transplantation procedure time, but also in the final repigmentation rate and color match [64, 74, 75].

We previously introduced intralesional injection as a new cell delivery route in vitiligo patients with the aim of reducing the cost and complications and making such intervention on curved surfaces easier. We also showed that transplanted cells were localized above the basal layer at the recipient site following intralesional injection [7]. However, other cell delivery techniques might result in higher repigmentation rates [45] probably due to the intrinsic therapeutic effect of dermabrasion on repigmentation of vitiligo patches [76–78].

Further randomized clinical trials are required to compare different cell delivery techniques in terms of safety, tolerability, and cost-effectiveness. In clinical practice, factors such as anatomical location and size of lesions have to be considered in selecting the most appropriate cell delivery technique for each patient.

The technical differences between ECT and FCT have been summarized in table 1.

4 Clinical safety and efficacy

The findings of clinical studies on the hair follicle-derived cell transplantation in vitiligo patients have been summarized in Table 2.

FCT for vitiligo treatment was first reported by Vanscheidt et al. in 2009. They transplanted the cell suspension obtained from plucked hair follicles on leukodermic patches in 5 patients with stable vitiligo. After 3 months, almost complete repigmentation was achieved in 3 patients [29]. Later in 2011, Mohanty et al. applied FUE method to harvest hair follicles and transplanted extracted cell suspension in 14 vitiligo patients. They claimed that the obtained cell suspension was applicable to larger vitiligo

Table 1 Technical differences between ECT and FCT

Technique	FCT	ECT
Donor tissue	Pre-shaved anagen hair follicles	Normo-pigmented skin of thigh or buttock
Common Harvesting techniques	FUE (follicular unit extraction) No dressing required	1. Dermabrasion 2. Dressing the donor site
Cell suspension preparation	Enzymatic cell isolation (trypsinization)	1. Separation of dermis from epidermis using enzymatic treatment and manual separation 2. Manual breaking of epidermis into small pieces 3. Enzymatic cell isolation (trypsinization)
Cell culture	Not tested <i>in vivo</i> Safety concerns regarding tumorigenicity	Safety and efficacy was shown in repigmenting large vitiligo lesions

patches compared to plucked hair follicle-derived cell suspension. They reported significant repigmentation rate (> 75%) in the majority of treated patients (9 out of 14) at a mean follow-up time of 6.9 months (range: 1–14 months) [52]. Later, the authors showed that the cell suspension extracted using FUE, contains significantly higher percentage of CD200 + cells [60]. Since then, further trials demonstrated safety and efficacy of transplantation of hair follicle-derived cell suspension prepared using FUE in vitiligo patients [42, 44, 59, 62].

However, few studies have compared the efficacy of ECT and FCT in vitiligo patients; one of these reports was a randomized controlled trial performed by Singh et al. Results of this study that was conducted on 30 patients with 47 stable vitiligo lesions, showed higher mean repigmentation extent in the ECT group at different time points till 16 weeks post-transplantation. However, the difference was statistically significant only at week 4 post-transplantation indicating more rapid onset of repigmentation process in the ECT group compared to the FCT group. The study results also showed significantly higher patient satisfaction score in the ECT group. However, there was no significant difference in DLQI (Dermatologic Life Quality Index), color match and repigmentation pattern between the two groups [42]. In another prospective comparative study conducted by El-Zawahry et al., analysis of 85 lesions in 16 patients with stable vitiligo treated with either ECT (10 patients, 61 lesions) or FCT (6 patients, 24 lesions) showed no significant statistical difference in the clinical outcome in terms of the number of responders with > 75% repigmentation, VASI (Vitiligo Area Scoring Index), start of repigmentation and color match. This report did not mention whether treatment allocation was randomized, and although no significant statistical difference was found in patients' demographic characteristics and baseline disease parameters, there were notable differences—though significance was not statistically evaluated—in the anatomical distribution of treated lesions between the two

treatment groups. Most importantly, the differences in the total number of treated lesions on the anatomical areas resistant to treatment namely, acral areas and distal fingers (13 lesions in the FCT group versus 48 lesions in the ECT group), might have led to misinterpretation of the comparison made for clinical efficacy of the two interventions. In terms of safety, the authors reported that the donor site in the FCT group healed faster with no scar, while in the ECT group, transient dyspigmentation and scars were reported in 100 and 40% of patients, respectively [43].

In another non-randomized clinical trial performed by Donaparthi et al., 60 stable vitiligo patches in 11 patients were allocated to receive either ECT or FCT in an uncontrolled sequential manner. The two treatment groups were not comparable in gender, age, disease duration, and stability time. Although patches treated with ECT sooner showed higher repigmentation degree, no difference was shown in repigmentation stability between the two groups within the 6 month follow-up period. It should be considered that the results of the study could not be completely attributed to the transplanted cell source, as there could be an impact of uncontrolled confounding variables on treatment response. The authors suggested that poorer results obtained for FCT pertained to the higher mean age of patients in this group in addition to technical problems with FUE (due to transacted hair follicles) which caused insufficient and ineffective harvesting of hair follicles. [44].

In a non-randomized uncontrolled trial performed by our group on 18 patients with stable vitiligo (8 men and 10 women, 16 generalized and 2 segmental) with the mean age of 26.78 ± 7.94 (range: 13–45) years, 20–25 anagen hair follicles were extracted from occipital area of each patient's scalp using FUE technique as described elsewhere [42]. We injected autologous epidermal cell suspension and hair follicle-derived cell suspension intra-lesionally into 25 and 43 patches, respectively. An average of $306,840 \pm 206,960$ (median 250,000) cells were obtained from 20 to 25 hair follicles per patient and transplanted on

Table 2 Clinical studies on follicular cell transplantation in vitiligo

Study design	N. of P/L	Follow up duration (month)	Recipient site preparation method	Combination therapy	Repigmentation percentage: percentage of P/L MR	Other clinical outcomes	References
Open label uncontrolled	5 P	6	Er:YAG laser	NBUVB	> 90%: 60% of P ~ 50%: 20% of P <10%: 1: 20% of P MR: 65.7%	Repigmentation pattern: diffuse	Vanscheidt [29]
Open label uncontrolled	14 P	1–14	Motorized dermabrasion	PUVA or PUVAsoL	FCT ~ ECT [#] FCT: 90–100%: 65% of L > 75%: 78% of L ECT: 90–100%: 83% of L > 75%: 92% of L	Patient satisfaction: ECT > FCT* Early repigmentation (4 w): ECT > FCT* Color match (ECT ~ FCT [#]): Excellent: > 60% Darker: > 20% Lighter: > 10% Repigmentation pattern (ECT ~ FCT [#]): Mostly diffuse DLQI improvement: Sig* (ECT ~ FCT [#])	Mohanty [52]
Randomized clinical trial	FCT: 15 P 23 L ECT: 15 P 24 L	~ 4	Manual dermabrasion	Sunlight exposure 5–30 min/day	0%: 15% of L > 75%: 35% of L > 90%: 16.67% of L	Color match: Excellent: 43.33% Darker: 38.33% Lighter: 3.33%	Vinay [59]
Open label uncontrolled	30P 60L	~ 6	Dermabrasion	Sun exposure throughout the study	ECT > FCT* FCT: > 90%: 43.33% of L > 75%: 43.34% of L ECT: > 90%: 83.33% of L > 75%: 90% of L	Earlier repigmentation in the ECT group*	Donaparthi [44]
Comparative	11 P FCT: 30 L ECT: 30 L	6	Manual Dermabrasion	Sun exposure from 22 days post-procedure	90–100%: 60% of P <50%: 5% of P MR: 80.15%	Color match Same color: 25% Darker: 75% Repigmentation start time: 2–3 w post-procedure Repigmentation pattern: diffuse No recurrence at 6 m	Shah [62]

Table 2 continued

Study design	N. of P/L	Follow up duration (month)	Recipient site preparation method	Combination therapy	Repigmentation percentage: percentage of P/L MR	Other clinical outcomes	References
Comparative	FCT: 6 P 24 L ECT: 10 P 61 L	18	CO2 laser	NB-UVB therapy 3 weeks after the procedure	FCT ~ ECT [#] FCT: 90%–100% : 16.7% of P 75%: 16.7% of P 50%: 16.7% of P 25%: 33.2% of P 10%: 16.7% of P 0%: 0% of P ECT: 90%–100% : 0% of P 75%: 20% of P 50%: 50% of P 25%: 10% of P 10%: 0% of P 0%: 20% of P	Repigmentation start time: within 3 months post-procedure (ECT ~ FCT [#]) Color match (ECT ~ FCT [#]): FCT: 100% good match ECT: 75% good match Patient satisfaction (ECT ~ FCT [#])	El-Zawahry ^a [43]
Case series	5 P 12 L CCT ^b 7 L ECT: 5L	~ 4	Manual dermabrasion	No additional treatment apart from regular sun exposure	CCT: ≥ 75%: 100% (v and c) ≥ 90%: 100% (v), 86% (c) MR: 94% (c) ECT: ≥ 75%: 80% (v and c) ≥ 90%: 20% (v and c) MR: 78% (c)	Early repigmentation (≥ 75% at 8 w): CCT: 71.4% ECT: 0% Average patient global assessment: CCT: 26.2/30 ECT: 22.6/30	Razmi [79]

N number, *P* patients, *L* lesions, *MR* mean repigmentation, *NB-UVB* narrow-band ultraviolet B, *PUVA* psoralen + UVA, *PUVA-sol* psoralen + solar light UVA, *FCT* follicular cell transplantation, *ECT* epidermal cell transplantation, *CCT* combined (follicular and epidermal) cell transplantation, *v* visual assessment, *c* computerized image analysis

**P* value < 0.05, [#]*P* value > 0.05, ^aThe data does not include the third study group in this trial, in which ECT was done with cryoblebbing as the transplantation method. ^bEpidermal to follicular cell ratio: 1 to 5

^aWe have not included the third study group data in this trial, in which ECT was done with cryoblebbing as the transplantation method

^bEpidermal to follicular cell ratio: 1–5

a mean recipient surface area of $100.77 \pm 197.43 \text{ cm}^2$ (median = 130). The mean numbers of injected hair follicle-derived cells and skin-derived cells per cm^2 were 7.93 ± 21.06 (median: 1.47×10^3) and 87.63 ± 90.84 (median: 65.36×10^3), respectively. Evaluating repigmentation of treated patches 6 months post-transplantation showed repigmentation in all the patches treated with epidermal cell suspension; while 17.4% of patches treated with hair follicle-derived cell suspension showed no repigmentation. None of the patches in the FCT group reached > 50% repigmentation, whereas 15.8% of patches

in the ECT group showed > 50% repigmentation. We did not observe any significant statistical difference in the mean repigmentation between the two treatment groups [unpublished data]. However, the non-randomized design of the study decreases the validity of our results for deducing a definite conclusion to compare the efficacies of FCT and ECT.

Hyperpigmentation of the recipient site, due to higher synthesis capacity of hair melanocytes, was suggested as one of the limitations of FCT by Vinay et al. who reported hyperpigmentation in 38.33% of patches underwent FCT

Table 3 Pros and Cons of FCT compared to ECT

Pros	Cons
✓ No need to apply dressing on the donor site	✗ Difficult procedure to learn
✓ More rapid healing of the donor site	✗ Time consuming and exhausting
✓ Invisible scar on the donor site	✗ Requires pre-shaving
✓ Fewer steps of cell suspension preparation	✗ Requires harvesting large volume of hair for treating large lesions

without significant difference in the number of transplanted melanocytes or hair follicle stem cells with other patches [59]. However, there is not sufficient clinical evidence to confirm such a claim, considering that in the only randomized trial that compared FCT and ECT, no significant difference was found in the percentage of hyperpigmentation between the two approaches [42].

Repigmentation of vitiligo lesions by transplanting combined autologous follicular and epidermal cell transplantation (E: F = 1:5) was also reported by Razmi et al. in 2017 in 5 vitiligo patients with 12 symmetric lesions. Sixteen weeks post-transplantation, they observed sooner and higher repigmentation, better color match and higher patient satisfaction in patches treated with combined cell suspension compared to symmetric patches treated with epidermal cells alone. The achieved repigmentation degree in patches treated with combined cell suspension was also higher than that achieved following FCT before the study. The superiority of combined cell transplantation (CCT) was attributed to the presence of stem cell factor and basic fibroblast growth factor in the epidermal cell suspension which probably stimulate the proliferation of stem cells existing in follicular cell suspension. The authors suggested CCT for repigmentation of acral patches which are more resistant to therapy as they lack melanocyte stem cells [79].

To summarize, due to insufficient evidence, it is not possible to fully compare the efficacy of FCT and ECT in repigmentation of vitiligo lesions. Results of clinical studies published to date on autologous follicular or epidermal cell transplantation in vitiligo patients, have shown no significant difference in the efficacy of ECT and FCT. To be precise, repigmentation of > 75% was reported in 33–78% of treated patients/lesions, 4–6 months post-transplantation [42, 44, 59, 62], while after the same time period, ECT produced > 65–75% repigmentation in 48–80% of patients [6, 9, 10, 80]. However, the effect of the concomitant light-based treatment regimens is also required to be considered as an effective factor on the final reported clinical outcomes, as applying excimer laser was shown to enhance the final repigmentation rate following ECT [81]. In this regard, further randomized controlled clinical trials are required to compare the efficacy and cost-effectiveness of ECT, FCT, and CCT, and determine the

clinical indications for each treatment in combination with and without light-based therapies as well as other treatment modalities.

5 Conclusion

Hair follicle and epidermis are both sources of pigment-producing cells which could be used in cell-based treatment of vitiligo to replenish lost melanocytes. Despite the differences between epidermal and hair follicle-derived cells with respect to phenotypic, functional, and microenvironmental characteristics, clinical studies have not shown any significant difference in efficacy between FCT and ECT. However, considering the limited number of high-quality studies, sufficient scientific evidence has not been provided for drawing a definite conclusion on FCT efficacy compared to ECT. Therefore, further controlled randomized trials with larger sample sizes and longer follow-ups are required to investigate the differences between ECT and FCT in terms of safety, efficacy, and durability of achieved repigmentation.

Although compared with ECT, FCT is associated with less donor site morbidity, and preparation of hair follicle-derived suspension takes fewer steps, FCT is an operator-dependent, time consuming, laborious and difficult procedure which is associated with more patient discomfort particularly for treating large vitiligo lesions (Table 3).

In conclusion, based on the current evidence, FCT does not appear to be a superior therapeutic choice over ECT. However, for specific indications such as small lesions, FCT might be considered an alternative treatment to ECT. Investigating the effect of patients' characteristics on the treatment outcome might reveal further indications. Besides, improving the technique of tissue harvesting, cell preparation (including cell culture) and transplantation procedure as well as combination therapy might enhance the efficacy and reduce the limitations of FCT.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical statement There are no animal experiments carried out for this article.

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