Surgical Treatment of Vitiligo

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INTRODUCTION

Vitiligo is a common acquired autoimmune depigmenting disorder, characterized by depigmented macules, whose etiopathogenesis is still under debate several pathogenic factors are included in the pathogenesis of vitiligo: biochemical/cytotoxic, neural, and autoimmune [1,2,3].

Vitiligo has an estimated prevalence of 0.5–2% of the population in both adults and children worldwide [4,5]. Vitiligo affects ethnic groups and people of all skin types with no predilection [6,7].

All patients with vitiligo should be initially treated with medical treatment. Surgical treatment is considered a valuable alternative in cases with stable vitiligo that are usually resistant to medical treatment and phototherapy [8]. Vitiligo surgery is based on melanocytes transfer from uninvolved skin to the stable vitiligo patch [9].

Following surgical treatments, repigmentation can improve by ≥ 68% with one treatment session only [10,11]. Even though surgery cannot stop disease progression, achieving a cosmetically acceptable repigmentation of the affected area is the primary goal for surgery [12]. There are many mechanisms for repigmentation after vitiligo surgery. In the injury and healing process during recipient site preparation, the melanocytes dissociate from the basal layer, proliferate, migrate, and are repositioned in the basal layer, essentially the normal physiologic process for melanocyte homeostasis. The healing process is stimulating the pro-melanogenic factors like hepatocyte growth factor, FGF, and keratinocyte growth factor to providing an appropriate environment for melanocyte stimulation [13,14].

The matrix metalloproteinases contribute to the migration of melanoblast during wound healing [15]. Besides the downregulation of adhesion molecule E-cadherin during wound healing is believed to facilitate melanocyte migration, and it has been demonstrated in pigmented skin post-punch grafting. The increase in heparanase post-grafting level and heparanase-mediated reduction in heparan sulfate at the dermo-epidermal junction is also believed to increase the pro-melanogenic factors [16].

Parameters of patient selection for surgery include [17]:

1. Duration of stability
2. Test grafting
3. Keobner’s phenomenon
4. Longevity of stability

5. Disease versus lesional stability.

Disease stability must be evaluated, according to the Indian Association of Dermatologists, Venereologists, and Leprologists suggested 1 year as an acceptable period to establish stability with the following definition: “absence of new lesions, absence of an increase in the pre-existing lesions, and absence of Koebner phenomenon for at least 1 year” [12,13].

Patient selection:

Surgical treatment is the treatment of choice for SV, as well as for stable NSV that is refractory to medical treatment. Surgery can also be recommended for areas of difficult treatment, including the hands, feet, and mucosa, lesions with leukotrichia [12].

The head and neck, which have a higher vascular supply and follicular density, have a better response to surgery than the extremities [18]. Patients with a family history of vitiligo may be more susceptible to recurrences [14]. Younger patients have a better response [19]. Acrofacial disease and areas over joints have a weak response, probably due to repetitive motion, friction, and injury in these areas [20].

Ideal donor site:

The upper lateral aspect of the thigh is the ideal donor side besides, the medial aspect of the forearm, arm, abdomen, and gluteal area can be used. For facial lesions, the post-auricular area can be considered an ideal donor site due to the good color match [17].

Recipient-site preparation:

Preparing the recipient site is a fundamental step in achieving a successful repigmentation. Regardless of the method of grafting, recipient-site preparation permits access to the underlying structures essential for melanocyte adherence and nutrition. The different methods for recipient site preparation include [21]:

Liquid nitrogen:

The recipient site is exposed after a cryo-blisters are created with liquid nitrogen and then deroofed. Peripheral hyperpigmentation or hypopigmentation, perigraft halo, and hypertrophic scarring are all risks associated with this procedure [21].

Suction blisters:

When compared to liquid nitrogen, suction blisters have a low risk of complications. Since harvesting blisters for large sites is time-consuming, it is a good method for small recipient sites [22]. Recipient sites can be prepared chemically by PUVA, phenol, and trichloroacetic acid [23]. This technique allows rapid preparation of the recipient site with a low risk of scarring as the reticular dermis is not involved, but some risk for carcinogenesis is present [24].

Dermabrasion:

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Dermabrasion is a common and cost-effective approach for preparing the recipient site, with pinpoint bleeding as the clinical endpoint. Manual dermabrasion has the disadvantages of being time-consuming and difficult to apply on large or concave surfaces such as the eyelids, neck, axilla, and glans penis. Motorized dermabrasion is a rapid choice, but it requires skill since controlling the depth is more difficult.

**Lasers:**

The carbon dioxide (CO2) and Erbium-doped yttrium aluminum garnet (Er: YAG) laser are used for recipient site preparation. It depends on the wavelengths emitted by both lasers are absorbed by water leading to tissue heating and consequent destruction by vaporization. This method has the advantages of speed, low user fatigue, and a bloodless field with a uniform depth of ablation, which is essential for tendinous or concave sites.

The penetration depth of an Er: YAG laser is one-sixth that of a CO2 laser, allowing for more effective and precise tissue ablation without the risk of thermal necrosis.

**Sterile sandpaper (dermasanding):**

For manual dermabrasion, sandpaper is used. This is a very simple and cost-effective procedure for dermabrasion of the skin.

**Figure (8): Sandpaper for preparation of recipient sites in vitiligo surgeries.**

**Dermarolling Benzekri and Gauthier:**

Benzekri and Gauthier (2017) reported the use of a derma roller with a keratinocyte/melanocyte suspension in five cases. After 6 months, three patients showed excellent repigmentation while it was mild in the other two.

**Electrofulguration-assisted dermabrasion:**

Superficial electrofulguration performed including 1-2 mm of the surrounding normal skin to the vitiligo patch then followed by the rest of the patch. It results in good margin and depth control and uniform preparation of the recipient site. This method allows for the dermabrasion of concave surfaces at a low cost and with ease of access. Epidermal coagulation is also reported using radiofrequency.

**Types of vitiligo surgeries:**

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Vitiligo surgery could be classified according to the type of the graft into tissue grafts and cellular grafts:

**Tissue graft:**
It includes transferal of tissue to the recipient site without processing and they are suitable for treating small areas [31].

**Mini-Punch Grafting.**

**Suction blisters epidermal grafting.**

**Split-thickness skin grafts.**

**Smash grafting.**

**Flip-top grafts.**

**Hair follicle grafts.**

**Cellular grafts**
Epidermal cells are first harvested from autologous donor skin and then transplanted (with or without prior selective cultivation) onto vitiliginous recipient sites. Unlike tissue grafts, which are limited by lesions size or the number of grafts, cellular grafts are used to treat large, depigmented areas using a small amount of donor tissue [32].

**Cultured cellular grafting.**

**Non-Cultured cellular grafting:**

**Non-cultured epidermal Cell suspension transplantation.**

**Non-cultured outer root sheath hair follicle cell suspension (NCORSHFS) transplantation.**

**Mini-Punch Grafting**
In this technique, punch grafts were taken from normal skin and then transplanted to the vitiligo patch. Repigmentation is based on the ‘pigment spread phenomenon’ by the grafted piece of normal skin. Under local anesthesia, the grafts will be inserted into perforations previously made at the recipient site with a biopsy punch. Since pigment cells do not appear to migrate beyond 5 mm, the grafts should be spaced 4–8 mm apart [33].

In 68–82% of cases, good repigmentation was achieved. Within one month of surgery, pigment spreads gradually after grafting, and complete repigmentation can be achieved in 3–6 months [34,35]. Many complications are recognized such as scar formation at the donor site in about 40% [35], color mismatch, hyperpigmentation, and cobblestone appearance of the recipient area [36,37].

**Split-thickness skin grafts**
A dermatome can be used to obtain a split-thickness skin graft, which can then be applied directly to the dermabraded recipient region. This procedure has a high success rate of 78–91%, which is comparable to epidermal blisters [37,38]. Temporary small epithelial milia-like cysts can be observed in the recipient area during the first months [37]. Scar or keloid formation at the donor site is reported in 12% of patients treated with split-thickness grafts [35].
Suction blisters epidermal grafting

The grafts are carefully extracted with sharp scissors and forceps after the blister has been harvested manually or with specially designed equipment. This epidermal sheet is then grafted onto the denuded recipient site. Repigmentation occurs gradually within a few weeks. The success rate according to the literature is 73–88% [39; 40] .

Pre-operative PUVA radiation therapy of the donor site may improve pigment distribution after epidermal blister grafting [41] . It's an easy, safe, low-cost, and successful treatment. Since the dermis remains intact, there is no risk of scarring. Temporary hyperpigmentation and mottled pigmentation can be seen in the grafted sites in 2–65%. The technique of harvesting blisters is time-consuming [39; 40; 35] .

Smash grafting

It is a modification of the STSG where the graft is smashed into tiny pieces before being applied over the recipient site. Postoperative phototherapy is required to guarantee pigment spread as the donor site is only one-tenth the size of the recipient site [42] .

Flip-top grafts:

They used a razor blade to shave a 2- to 4-mm donor epidermis sample containing minimal underlying dermis. Donor skin was sectioned into 1- to 2-mm grafts for transplantation. At the recipient sites, a razor blade was used to elevate a 5-mm flap of the epidermis containing minimal papillary dermis. The grafts were then placed at each recipient site with the dermal side down. The epidermal flap acts as a biological dressing and maintains the graft in place. No visible scarring occurred in any of the grafts [43] .

Hair follicle grafts:

The main idea is that the stem cell population in the bulge region will cause repigmentation by retrograde migration. This method is effective in the treatment of leukotrichia without the use of specialized equipment [44] .

Cultured Epidermal Grafts

Rheinwald and Green (1975) [45] were the first to describe this technique. A shave biopsy of normally pigmented skin is the source for epidermal cell culture. The cells are seeded in a medium that allows melanocytes and keratinocytes to co-culture after the epidermis and dermis have been separated. After a few weeks, a cultured sheet is obtained, which is then released using dispase and attached to a support of petrolatum gauze. Subsequently, the gauze will be applied onto the dermabraded recipient site and covered with an occlusive dressing (Kumagai and Uchikoshi, 1997) [46] . Epidermal skin cells can also be seeded on a supporting layer (membrane of hyaluronic acid or collagen-coated sheet) [47] .

The main benefit of this technique is the ability to expand the cells in culture, allowing for treatment of a large area with just a small amount of donor skin. The treatment is non-scarring since only superficial dermabrasion is used [48] . The Complications of this procedure are similar to other minor surgical procedures that include infection, bleeding, and graft failure. Temporary hyperpigmentation but disappear spontaneously after several months [46].
Cultured melanocyte:

Lerner et al. (1987) [48] first described the use of cultured pure autologous human melanocytes. They utilized many growth factors and chemical media to augment pigment cells from a shave biopsy from normally pigmented skin in vitro [49,50] .

12-O-tetradecanoylphorbol-13-acetate (TPA) was originally an obligatory ingredient for obtaining rapid expansion of pure melanocytes. However, this tumor promoter will increase the risk of developing malignancies. Later, it became possible to culture large numbers of normal melanocytes in a defined medium supplemented with natural melanocyte growth factors including FGF, [49; 51; 50] .

In vitro culture has the advantage of being able to expand and cryopreserve cells for further use. However, widespread application of cultured cellular grafting is limited [32] . Until the 1980s, it was impossible to develop large numbers of melanocytes in vitro. This was due to keratinocytes preferentially overgrowing melanocytes in culture. The discovery of melanocyte mitogens in 1982 was recognized as a turning point [52] .

Eisinger and Marko (1982) [52] were able to selectively cultivate human melanocytes from neonatal foreskin and adult skin by adding TPA into the culture medium. Halaban et al. (1988) [53] was found that FGF is an important keratinocyte-derived factor influencing melanocyte survival and proliferation, a natural mitogen for melanocytes and it became possible to cultivate human melanocytes in vitro without TPA and fetal calf serum

Cultured epidermal grafts appear similar to cultured melanocyte grafts, but the involvement of both cell types causes them to form a sheet that resembles the skin’s basal layer [54,31] .

Non-Cultured cellular grafting

Non-cultured epidermal Cell suspension transplantation (NCES):

Gauthier and Surleve-Bazeille (1992) [55] defined the transplantation technique by using a suspension of non-cultured keratinocytes and melanocytes in the treatment of lesions. It is considered the standard vitiligo surgery [11] . Donor skin was taken from the occipital region and immersed in a 0.25 percent trypsin solution for 18 hours. The epidermis of the donor skin can be extracted from the dermis in vitro the next day with fine forceps. A cellular suspension is formed after many procedures[55] .

Blisters were developed in the recipient region using liquid nitrogen. After aspiration of the viscous blister fluid, the cellular suspension is injected into each blister at the recipient site. The transplanted cells are kept in place by the intact blister top, which acts as a natural dressing. In 8 of 12 patients treated, a repigmentation of more than 70% was obtained [55] .

The high costs, the need for advanced equipment and a trained team, and the risk of mitogenesis by using the melanocyte medium are all drawbacks. The run-off of the suspension from the recipient site makes it difficult-to-treat uneven areas [56; 11] . No adverse effects at the donor or recipient site have been reported for non-cultured epidermal suspensions. A disadvantage is long-lasting erythema (up to 6 months) at the recipient site due to dermabrasion [57] .

Modification of non-cultured epidermal suspension surgery
From the time Gauthier and Surleve-Bazeille pioneered NCES until now, there have been numerous modifications. These continuous improvements are aimed at overcoming disadvantages such as grafting time-wasting and high costs [55].

Olsson and Juhlin (1998) [57] introduced a similar technique in which they used a basal-cell-layer enriched suspension. The rapid (hot) trypsinization was developed that consumed 60 min under 37°C. The melanocyte culture medium was used to stimulate cell growth. However, they applied the cellular suspension directly on vitiligo lesions that had been dermabraded, then it was covered with a thin collagen film, moistened gauze, and Tegaderm. After 1 week they removed the bandages. The success rate was 85 percent in 20 vitiligo patients. Larger areas can be treated, which is the most important advantage.

The culture medium was then replaced with phosphate-buffered saline (PBS). It helps the melanocyte survive by avoiding osmosis-induced cell death. It was also used to get rid of extra trypsin [56].

Hyaluronic acid was added to increase the viscosity to reduce the run of the cell suspension [58]. Adding the patients’ serum to the cell suspension increased the repigmentation percentage [58]. The battery-operated ReCell kit’s automated mechanism demonstrated successful repigmentation [59]. The cell suspension was prepared by the ‘6-well plate’ technique later with the use of trypsin inhibitor and three times wash by PBS. The cell separation and filtration were done in the fifth and sixth wells [60].

**Figure (9): Non-cultured epidermal Cell suspension transplantation technique:**

(A) Using a silver dermatome to obtain an ultrathin epidermal graft. (B) An epidermal graft in a petri dish containing trypsin-EDTA. (C) The graft is cut into smaller pieces and incubated at 37 °C in 0.25% trypsin-EDTA. (D) Centrifugation for cellular extraction: a dense cell pellet at the base of the Falcon tube (E) Application of cell suspension onto laser-ablated vitiliginous
recipient site. (F) Placing collagen sheets to hold cell suspension in place and prevent “run-off”. (G) Securing suspension and collagen sheets using Hypafix dressings [32].

Kumar et al. (2014) [61] was first published the four-compartment (4C) method to simplify the steps of NCES and to decrease its cost (fig.10). It formed a four-part petri dish in which the graft was processed. The graft was incubated for one hour at 37°C in the first partition. The graft was immersed in PBS in partitions 2 and 3 to remove excess trypsin. Cells were detached into PBS in the final partition. The separated melanocytes and keratinocytes were re-suspended in the PBS to prepare homogenous cell suspension.

Figure (10): The FC method for noncultured epidermal cell suspension preparation. (a) Schematic representation of the four-compartment method for non-cultured epidermal single-cell suspension. (b) Compartment 1: trypsin is added to the skin graft; (c) compartment 2: after trypsinization, the first washing with phosphate-buffered saline (PBS); (d) compartment 3: second washing; (e, f) compartment 4: separation of the epidermis from dermis, dislodging the cells into PBS, providing the final epidermal suspension [61].

Non-cultured outer root sheath hair follicle cell suspension transplantation

Melanocytes and melanoblasts are found in abundance in the hair follicle [62]. The perifollicular connective tissue and hair papilla contains mesenchymal stem cells that can develop into melanocyte stem cells. The follicular-melanin unit ratio of 1:5 is substantially larger than the epidermal-melanin unit which has 1 melanocyte for every 36 keratinocytes [63]. Another benefit of follicular melanocytes over epidermal melanocytes is that they are less susceptible to autoimmune destruction, but they are more susceptible to aging and exhibit cyclical activity [22].
Non-cultured outer root sheath hair follicle cell suspension transplantation needs to treat large areas with a few follicles\textsuperscript{[64]} . Hair follicles are collected from the occipital scalp using small punches (1 mm) and processed in the same way as NCES, except that the retrieved follicles are washed three times in phosphate-buffered saline and transferred to a fresh tube of trypsin every 30 minutes for a total of three tubes\textsuperscript{[65,18]}.

**Postoperative Management**

To avoid graft loss and unequal distribution of the transplanted cells, adequate immobilization is required after all transplantation techniques for several days. Prophylactic antibiotics are advisable for 1 week after treatment. To enhance the spreading of the pigmentation, PUVA or UVB therapy 3 weeks after transplantation is recommended\textsuperscript{[58]}.

**References:**


