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Transplantation of melanocyte stem cells in vitiliginous skin

Yohanes Widodo Wirohadidjojo* and Flandiana Yogianti*
*Department of Dermatology and Venereology, Faculty of Medicine, Universitas Gadjah Mada/ Dr. Sardjito General Hospital, Yogyakarta, Indonesia

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ABSTRACT

Depigmentation in vitiligo occurs as a result of progressive loss of functioning epidermal melanocytes, and currently various modalities have been developed to re-functioning these cells. However, in area with poor melanocytes reservoir, such as old-persistent lesions or lesions on bony prominence, the modalities are hardly to achieve repigmentation. Since spontaneous repigmentation of vitiliginous skins begin mostly in follicular areas, reactivation of melanocyte precursors along the outer root sheath of hair follicle is expected to have better on this pigmentation. Melanocyte precursor came from melanocyte stem cells that originally located on bulge area of hair follicles. The latest surgical intervention in vitiligo is transplantation of melanocyte stem cells. Clinical experiments indicated that the transplantation can be performed either by transplantation of extracted follicular units or single cell suspension harvested from this area. By single cell suspension treatment, a 50 cm^2 of vitiliginous skin can be handled by 15 autologous hair follicular units. These procedures are easy and can be performed by any dermatologist especially who has been trained in dermatologic surgery as well as in cellular based therapies.

ABSTRAK

Depigmentasi pada vitiligo terjadi akibat kehilangan secara progesif fungsi melanosit epidermal dan saat ini berbagai modalitas telah dikembangkan untuk mengembalikan fungsi sel. Namun demikian, daerah dengan sumber melanosit yang rendah seperti lesi persisten yang lama atau lesi pada tonjolan tulang, modalitas tersebut sulit mengalami repigmentasi. Sejak repigmentasi spontan kulit vitiliginus mulai terutama di daerah folikel, reaktivasi pekusor melanosit sepanjang selubung akar luar folikel rambut diharapan proses pigmentasi berjalan lebih baik. Prekusor melanosit berasal dari sel punca melanosit yang terletak di area tonjolan folikel rambut. Intervensi bedah paling akir pada vitiligo adalah transplantasi sel punca melanosit. Pengalaman klinik menunjukkan bahwa transplantasi dapat dilakukan baik dengan transplantasi unit folikel yang diekstrak atau suspensi sel tunggal yang diambil dari area ini. Dengan pengobatan suspensi sel tunggal, 50 cm^2 kulit vitiligo dapat dilakukan oleh 15 unit folikel rambut autolog. Prosedur ini mudah dan dapat dilakukan oleh dokter spesialis kulit dan kelamin yang telah dilatih dalam bedah kulit juga pengobatan berbasi sel.

Keywords: Vitiligo, Melanocyte stem cells, extracted hair follicular, single cell suspensions

Corresponding author: widiokarsono@yahoo.com

INTRODUCTION

As, vitiligo is an acquired pigmentation disorder manifested with development of white macules or patches on affected skin due to a progressive loss of functioning of epidermal melanocytes. The basic mechanism has not yet been clearly identified, however, various mechanisms have been proposed, including genetic association,[1,2] autoimmunity and oxidative stress,[3-5] neurohormonal imbalance,[6] abnormalities in the microenvironment surrounding the melanocytes[7-9] and intrinsic defects of melanocytes and periphery T cell regulator which promote innate immune attack.[10,11]

Various modalities have been developed to treat vitiliginous skins, among others : a) anti-oxidant agents such as: Polypodium leucotomos as adjunct therapy with PUVA,[12] or fish oil[13]; b) immunosuppressive agents such as: topical corticosteroid,[14] tacrolismus,[15-17] or calcipotriol,[18,19] either used as single modality or in combination with NB-UVB or excimer laser; c) UVA light combined with photosensitizer agent such as: psoralen,[20,21]; d) oral minocycline[22,23] which nevertheless less effective than NB-UVB.[24] Previous study showed that minocycline could protect melanocytes from H_2O_2-induced cell death.[25] Systematic review revealed that NB-UVB showed equivalent therapeutic efficacies to UVA, PUVA or 308-nm Excimer Laser, in > 50% and > 75% of repigmentation.[26] However, these modalities have limitations, especially in achieving total repigmentation. In facts, failure of repigmentation is usually found among old-persistent lesions or lesions on skin over the bony prominences with poor melanocytes reservoir. Cui *et al.,*[27] reported that spontaneous repigmentation of vitiligo usually begins as multiple dots of pigmentation around of hair follicle areas where they are rich with melanocytes reservoir. Since human epidermal melanocytes stem cells can be found along bulge area of hair follicles, and follicle unit extractions method has been developed by trichologists where part of the bulge area is also involved, transplantation of melanocyte stem cells is now become possible. Here, the basic technique of this modality, and method of transplantation is described.

MELANOCYTE STEM CELLS

Characteristic of Melanocyte Stem Cells

Stem cells are defined as cells that able to self-renew so they can divide and maintain their population and provide specialized and differentiated daughter cells of their specific tissue type.[28,29] In addition, the degree of plasticity and the markers expressed is different between adult and embryonic stem cells, as well as between adult stem cells of different tissue types. Stem cells are present in an environment as a special cellular organization called the niche.[30,31] The signals within niche has important factor in regulation of stem cell's self-renewing and differentiation.[32,33]

Melanocyte stem cells (MSCs) were first identified in the hair follicle and are located in the bulge region as their niche (FIGURE 1).[34] They differentiate into hair follicle melanocytes at the beginning of anagen phase and undergo apoptosis at catagen phase.[35]

MSCs are generated from melanoblasts that populate the hair follicle bulge, and they are stimulated to become inactive by their niche at the final stage of hair follicle morphogenesis.[36,37] The responsible niche for this purpose is signaling of transforming growth factor β (TGF-β) by inducing cell cycle arrest, down-regulation of MITF (Microphthalmia-Associated Transcription Factor) gene expression, and suppression of melanogenic genes in melanoblasts.

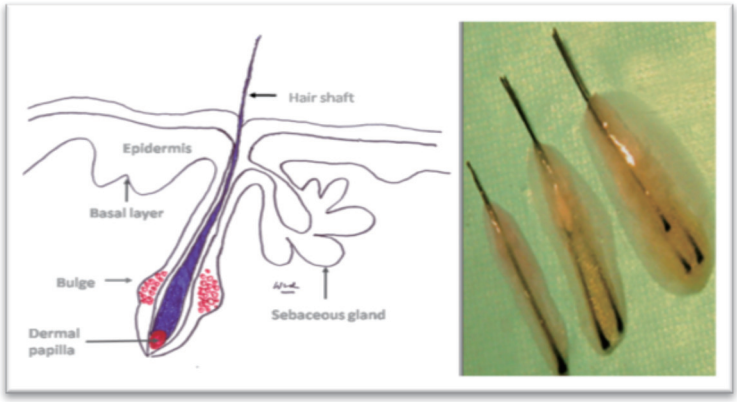

FIGURE 1. Location of MSCs along hair follicle (left), and extracted hair follicle unit (right)

Signaling TGF-β through TGF-β RII is also as key role in regulation melanocyte stem cell immaturity and quiescence.[38] Both states of immaturity and quiescent is important factor for maintenance MSCs population during growing phase of hair cycle. In this condition, *Bcl2,* an anti-apoptotic factor, is essential for survival of MSCs during this phase.[37,38] MITF gene product, namely *MITF*, is also an important transcription factor to prevent premature MSCs differentiation and pigmentation.[38] Hair graying, as one phenotype of human aging, occurs as a result of loss of hair follicle melanocytes which is caused by gradual loss of MSCs.[37,39] Loss of MSCs is caused by failure of MSCs maintenance in the bulge region of hair follicles.[37] Inappropriate maintenance of MSCs results in the failure of MSCs to proliferate and differentiate, migrate to wrong places, or death.[39] All of these events are related to TGF- β signaling. Other studies indicated that TGF-β production and TGF-β RII expression is down-regulated among aged skin.[40,41]

Isolation and cultivation of MSCs

The best source of MSCs is hair follicles, especially the outer root sheats of hair follicles. These cells can be propagated to multiply by explant method,[42] but the hair shaft must be plug out together with dermal papilla because dermal papilla is an important niche for epidermal stem cells, including MSCs.[43,44] For this purpose, follicular unit extraction (FIGURE-1) either by using mini-punch instrument or micro motorized mini punch is considered as the best method.[45] This technique allows the hair of donor sites to regrowth with the absence or minimal scarring. Furthermore, extracted follicular unit contains dermal papilla together with a part of bulge area.[46] Cultivation of extracted follicular unit can be done by using stem cell-medium.[42] In the Dermatology Department Universitas Gadjah Mada, cellular expanding with melanin production can be obtained by Dulbecco's minimal essential media (DMEM) supplemented with 10 % fetal bovine serum (FIGURE 2).

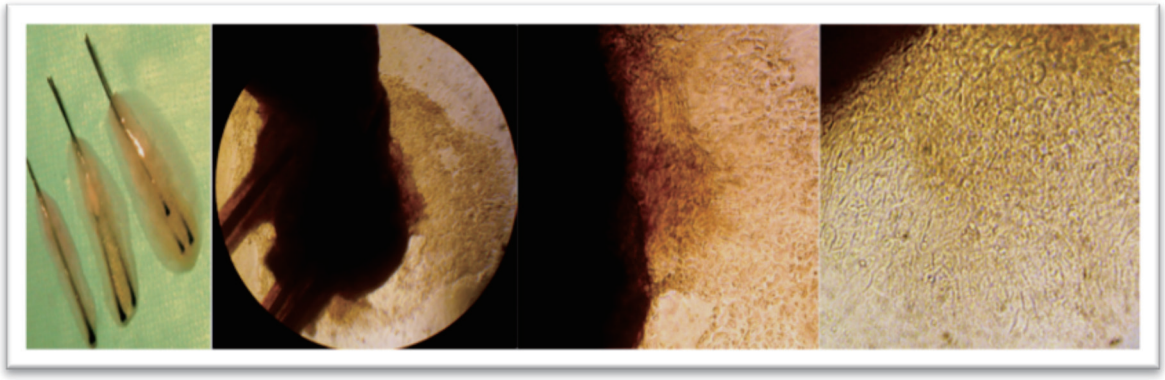

FIGURE 2. Culture expansion of melanin-producing cells from extracted hair follicles

A single cell suspension can be obtained from extracted hair follicles by simple trypsin digestion and cultured in melanocyte growth medium with stem cell factor. To maintenance their proliferation rates, a second passage of keratinocytes must be added into culture dish as feeder layer, after day 21.[47] In this method, beside of MSCs, neural crest stem cells (NCSCs) can also be alternative as concomitant culture.[48]

MSCs in vitiligo

Controversy of totally loss or inactivation state of MSCs of vitiliginous lesion has existed for a long time. In order to get certain answer for this problem, Seleit *et al*.,[49] evaluated the MSCs-lineage in follicular and inter-follicular vitiliginous epidermis through immunohistochemical localization of Human Melanoma Black-45 (HMB-45) and Tyrosinase Related Protein 2 (TRP2). They found that MSCs were detected in 44% of inter-follicular epidermis and 46.7% of follicular epidermis of vitiliginous lesions. Melanocyte precursors were detected in 54% of inter-follicular and 63.3% of follicular in skin lesions. The existence of MSCs and melanocyte precursors was higher among black hair than white hair of vitiliginous lesions. This finding indicates that killing melanocytes by innate immune attack is not directing against MSCs, but into mature melanocytes. This finding also supports previously hypothesis that failure of re-pigmentation of vitiliginous lesions is located in skin area with poor hair follicles.[50]

The presence MSCs on vitiliginous lesions may be caused by elevation of serum level of TGF-β, as it has been described on above. Serum level of TGF-β of non-segmental vitiligo is indeed elevated.[51,52] Conversely, this elevation is responsible for failure residual melanocytes in producing melanin as it has been discovered by Kim *et al*.,[53] When autologous MSCs from normal skin planned to elevate numbers of precursor melanocytes in vitiliginous lesions, those transplanted MSCs must receive special treatment so that inhibition of melanin production by TGF-β can be minimize. Blocking TGF-β RII expression can be achieved by giving ultraviolet irradiation onto vitiliginous lesions after MSCs transplantation.[54,55] Study conducted among neonatal mouse skin indicated that repeated ultraviolet B (UVB) irradiation can induce MSCs differentiation into melanocytes and melanin production.[56] Broad band UVB showed a better efficacy in awakening MSCs than narrow band UVB.[57] Similar event also happened among hair follicles and the epidermis of human vitiliginous skins.[58]

MELANOCYTES STEM CELLS TRANSPLANTATION ON VITILIGINOUS LESIONS

Principle of surgical interventions

The basic principle of surgical treatment in vitiligo is to achieve cosmetically acceptable repigmentation of the vitiliginous areas by transplantation of autologous melanocytes or MSCs from the unaffected pigmented skin to the vitiliginous skins. Surgical intervention cannot stop the progression of this disease, therefore this technique is only indicated for stable vitiligo that does not show adequate response to medical therapies.[59] Although there is no universal consensus on how to define stable vitiligo, the Indian Association of Dermatology, Venereology and Leprology (IADVL) establish a consensus recommendations to define this stability that is a patient reporting no new lesions, no progression of existing lesions, and absence of Koebner phenomenon during the past one year.[60]

There are various methods of surgical intervention for vitiliginous skin, some of those techniques are attempted to transplant melanocytes from the donor to the recipient areas, such as: suction blister grafting, thin epidermal grafting, and mixed epidermal cell suspensions grafting,[61] whereas others may provide melanocytes, melanocyte precursors, and also MSCs, such as: hair follicular grafting and mini-punch grafting. Among hair follicular grafting which is harvested by follicular unit extractions, the grafts are composed by hair shaft, a part of hair bulge where MSCs are located, and dermal papilla as niche of MSCs and other epidermal stem cells.

Hair Follicle Unit Extractions (FUE)

Before FUE was developed in hair transplantation surgery, follicular unit transplantation was obtained from hairy skin stripping followed by stereo-microscopic dissections. This strip-harvesting technique caused donor scarring on occipital area which sometimes are difficult to be covered by surrounding hair. FUE was firstly introduced by Rasmann *et al*.,[62] who used one-millimeter punch to insulate follicular units from the surrounding tissues down to the level of the mid dermis then followed by extractions of these units with forceps. By this method, scarring due to single strip harvesting does not occur and microscopic dissections are not needed. In addition, by leaving a part of bulge area in punched holes, hair shaft can regrowth and hair density on donor area is not markedly disturbed[46] (FIGURE 3). Furthermore, in order to increase survival rate of harvested grafts, Senthi *et al*.,[63] modified FUE hair transplantation by implanting FUEs as soon as they were harvested and this technique showed a better results in hair transplantation.

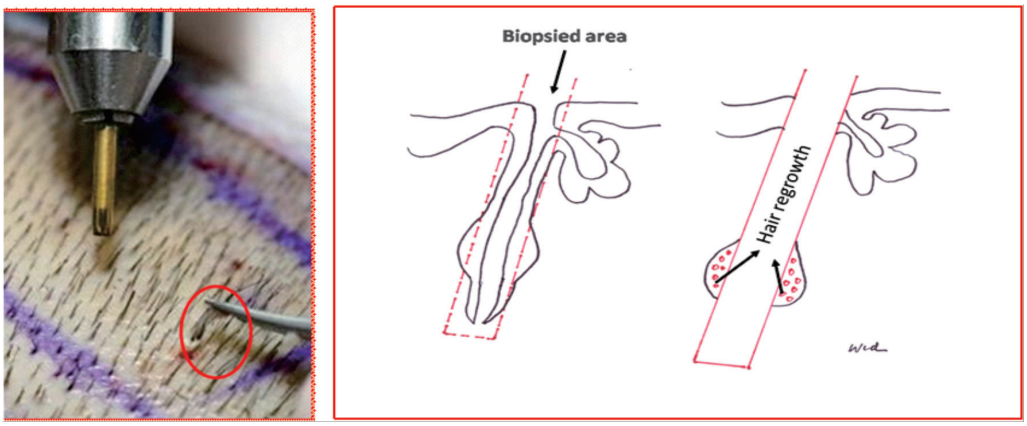

FIGURE 3. Follicular Unit Extraction Follicular unit extraction (red-circle) (left), leave a part of bulge area as a source for hair regrowth (right)

Aplication FUE onto Vitiliginous Skin

Direct application of autograft hair follicles onto vitiliginous skin has been reported by many authors. Recipient area must be non-hairy areas otherwise hair removal treatment must be performed afterward. Transplantation of FUE onto the right eyebrow of eight years duration of 12 years old female was reported by Sacchidanand *et al*.,[64] which procedure was easy, scarless and sutureless, but seemly only for small size of stable vitiligo. Aziz Jalani *et al*.,[65] reported that occipital hair follicles transplantation, harvested by punch biopsy instrument then dissected to various follicular units can induce repigmentation of persistent segmental vitiligo among 10 patients. FUE can be harvested from hair body or scalp areas and FUE from this region indicate same characteristic with FUE from scalp area, such as reported by Chouhan *et al*.,[66] when they successfully transplanted FUE from the right upper thigh to the left shin, calves and both ankles vitiliginous skin. Repigmentation rates of hair follicles transplantation is equal with those achieved by multiple mini punch grafts, however mini punch grafting is much easier and the procedure is faster than hair follicles transplantation method. Based on this finding, Mapar *et al*.,[67] suggested to perform multiple mini punch grafts for stable vitiligo. In Department of Dermatology and Venereology Sardjito General Hospital, we performed multiple mini punch grafts from the left upper thigh skin with vellus hairs and transplanted onto the left eyebrow of 17 years old female of a Sardjito Hospital's segmental vitiligo patient as additional procedure of suction blister grafting (FIGURE 4).

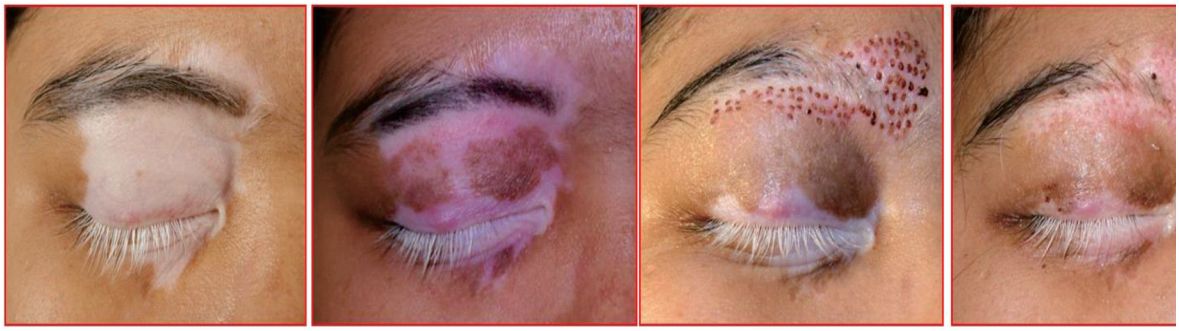

FIGURE 4. Mini punches graft from vellus hairs area onto eyebrow area as additional procedure after suction blister grafting of a Sardjito Hospital's patient (From left to right side: before procedure, a week after suction blister grafting, vellus hair mini punch grafts, a week later)

Except for hairy areas, another limitation of FUE transplantation is convenient only in small areas of skin. For larger areas, FUE transplantation must be modified to become hair follicle cell suspensions so that it can be sprayed onto large dermabraded vitiliginous skin, similar to mixed epidermal cell suspensions grafting method. The advantages of this technique are the amount of MSCs and melanocytes precursor collected in this suspension. Preparation of extracted hair follicle outer root sheath cell suspension can refer to the method of Kumar *et al*.[68] By simple warm trypsin digestion of extracted hair follicle units, a large number of mixed cells can be obtained (FIGURE 5).

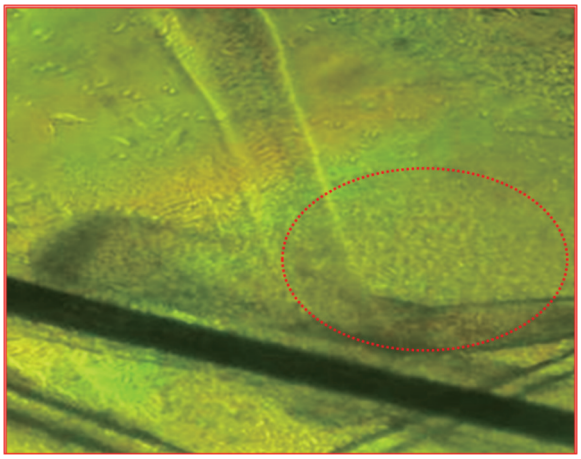

FIGURE 5. Enzymatic digestion of bulge area (red-circle) of extracted hair follicle units.

Enzymatic digestion by trypsin must be performed for at least 90 minute in 37^0C. In the next step, cell suspension collected from this procedure can be sprayed onto dermabraded vitiliginous skin after removing the residual trypsin. A number of 15 hair follicles can be used to cover 30.8 ± 26.5 cm^2 and the repigmentation rates can be around 65.7 ± 36.7%.[68] Another advantage of this procedure is resistance of transferred melanocyte stem

cells from innate immune attack as one of basic mechanism of vitiligo.[69]

CONCLUSION

Loss of functioning epidermal melanocytes which cause depigmentation of vitiliginous skin can be ameliorated by MSCs transplantation. Bulge area of hair follicle is a rich source of MSCs and transplantation of MSCs can be performed by FUE of hair transplantation especially on haired skin with limited affected areas. For larger area, spraying cell suspension of extracted follicular unit on to dermabraded area is believed to be the best choice.

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