CELLULAR GRAFTS OF SUSPENDED MELANOCYTES AND KERATINOCYTES IN THE TREATMENT OF VITILIGO

Gamal M. Hashem*,
Amany M. Abdel-Latif,**
N. Van Geel***
K.Ongenae***
J.M. Naeyaert***

Cellular grafts of suspended melanocytes and keratinocytes in the treatment of vitiligo

Abstract:

Although the treatment of vitiligo has improved during the last decade, therapy is still not satisfying for many patients. This is probably due to the fact that the etiopathogenesis is unknown. Several treatment modalities, such as PUVA, UVB and local corticosteroids are used for vitiligo. However these treatments usually induce incomplete repigmentation. Surgical methods intended to repigment leucoderma are an interesting therapeutic option if patients have a stable disease, do not exhibit a positive koebner phenomenon and have no history of keloid formation. Two types of surgical techniques are available: tissue grafts and cellular grafts. Tissue grafts are full thickness punch grafts, split thickness grafts, suction blister grafts and autologous cultured epithelial grafts. With tissue grafts, only a limited surface area can be treated but mostly with good results. However, cellular grafts cultured from autologous keratinocytes and melanocytes can be used for larger areas. The exact success rate of repigmentation with cellular grafts is still unknown, since only a small number of uncontrolled studies have been published We report the results of cellular grafting with a basal cell layer enriched suspension of melanocytes and keratinocytes.

Introduction:

For the patients with stable and refractory vitiligo, autologous transplantation methods may be considered as a therapeutic alternative [1] by the end of 1998 63[2] studies were reported of which 16 reported on minigrafting, 13 on split thickness grafting, 15 on grafting of epidermal blisters, 17 on cultured melanocytes and 2 on grafting of non cultured epidermal suspension. Punch grafts are easy to perform but complications were observed as cobblestone appearance of the treated area [3]. Using a dermatome, a split thickness epidermal grafts can be removed to treat larger areas with rapid and good results [4,5]. Epidermal blisters can be induced on normal skin by suction where the roofs can be transplanted to denuded recipient sites [4]. Gauthier and Surleve- Bazeille [6] injected a cell suspension obtained from the scalp into blisters raised by freezing on vitiliginous skin and obtained good results in focal vitiligo on areas up to 50 cm2 With the use of cultured epidermal sheets areas up to 240 cm2 were treated [7] However, by using cultures of melanocytes, areas of up to 500 cm2 can be treated [8]. More recently, Olsson & Juhlin [9] used a non-cultured melanocyte enriched cell suspension for treatment of leucoderma and obtained excellent results. They also found that by diluting the cell suspension, the recipient area could be increased to up to 10 times the size of the donor area with the same good results, as without or with less dilution. A modification of this procedure has been described by van Geel et al in 2001[15]. The aim of our work was to evaluate the usefulness of this modified technique for treatment of stable and refractory vitiligo.

*Departments of Dermatology,
AlAzhar*, Tanta** (Egypt) and
Ghent*** (Belgium) Universities
Corresponding author:
Prof. Dr. JM Naeyaert
Department of Dermatology
De Pintelaan 185
9000 GENT
FAX : 09/240 49 96 - TEL : 09/240 22 98
e-mail: jeanmarie.naeyaert@rug.ac.be*

Patients and methods:

This work has been carried out in the Dermatology Department of Saudi German hospital in Jeddah. Forty patients with stable and recalcitrant vitiligo were selected for the procedure .i.e no enlargement of the existing patches or development of new lesions for a period of one year.

They included 18 males (45%) and 22 (55%) females. Their age ranged from 12-62 years (mean ± SD; 32±11.4), and duration of their disease ranged from 3 to 15 years (mean ± SD; 5.8±3.6).

Positive family history was obtained in 16 (40%) cases. The size of the treated areas ranged from 24-260 cm3 (mean ±SD; 80.4±12.4), and involved different body areas including face, trunk and extremities. The skin type of the treated patients ranged from type III to type IV. The procedure was performed as described by van Geel et al [15] Under local infiltration anaesthesia with 2% lidocaine hydrochloride (20mg/ml), a shave biopsy specimen was taken from normally pigmented gluteal area

of the patients. A hand dermatome (E.WECK&CO) was used to shave 25-I00cm^2 of donor epidermis containing minimal underlying dermis. The donor site was covered with an adhesive surgical dressing and secured with gauze adhesive tape for one week. The thin donor skin was immediately put in a test tube containing Dulbecco's modified Eagle medium supplemented with 40ul fetal bovine serum and 2ul Raid solution (penicillin / streptomycin 10,000 u/ml, fungisone 5mg/ml and gentamycin 40 mg/ml). In the laboratory it was transferred to a Petri dish where it was washed with phosphate buffered saline (PBS) then once with 0.25% trypsin and 0.08% W/V EDTA and torn into pieces of 2Cm^2. The skin pieces were incubated in trypsin/EDTA for 50 minutes at 37°C in 10% Co^2. After incubation the trypsin/EDTA solution was removed and about 2ml of trypsin inhibitor (fetal calf serum) 0.5mg/ml in PBS was added. The epidermis was removed from the dermis with fine forceps. The dermis was vortex mixed in a tube containing the melanocyte medium [low calcium (0.03mM) M199 medium supplemented with insulin (I0microgeam/ml), hydrocortisone (0.4microgeam /ml), cholera toxin(10^{-9}M), and penicillin/streptomycin (10,000U/ml) and T/T (5microgram/ml transferrin and 2x10^{-9}M triiodothyronine)] for l5 seconds. The epidermal pieces were transferred together with the trypsin inhibitor to the tube with the dermal suspension. This was vortex mixed for 45 seconds and centrifuged for 6 min. at 150g. After removing the supernatant, the pellet was resuspended in 5ml melanocyte medium and centrifuged once more. The obtained pellet was resuspended in a total volume of 0.8ml melanocyte medium. The only growth hormone added to the medium is 5ng/ml basic fibroblast growth factor(bFGF). The recipient areas were cleaned by 70% alcohol. Using Erbium YAG laser at fluence of 600 mJ/pulse, the superficial epidermis was removed. This was done I hour after application of a topical anaesthetic cream (lidocaine 25mg, prilocaine 25mg/gm) under occlusion . The suspended cells were applied with a pipette on laser abraded recipient vitiligenous areas and covered with a transparent occlusive dressing (tegaderm). This was secured with dry gauze and adhesive tape and left in place for one week. The patient was immobilized in bed for 4-5 hours after the procedure and rested at home one week afterwards. Clarithromycin 250mg tablets twice daily was given for 7 days. The bandage was removed after one week. Three weeks after procedure all patients started with ultraviolet radiation (narrow band - UVB or psoralen plus UVA[PUVA]) two to three times a week. Photographs of the recipient areas were taken before and three and six months after transplantation.

Results:

4-6 weeks following the procedure, 24 (60%) patients showed start of repigmentation. 16 patients were lost for follow-up. 6 months later, 6 (15%) cases showed moderate improvement (defined as pigmentation f>25-50% of the treated area), 6 (15%) of cases showed good improvement (defined as repigmentation of >50-75% of the treated area) and 12 (30%) patients showed excellent improvement (defined as epigmentation of>75% of the treated area). Figure I show a patient before and 6 months after the procedure. In the first few months following the procedure, the treated areas were often hypo or hyperpigmentated but after 6-8 months, they had acquired the same color as the surrounding skin. The donor site healed in 1-2 weeks with no side effects or complication occurred except for scarring occuring in one patient.

Discussion:

Vitiligo is a disease characterized by idiopathic asymptomatic leucoderma. Since the aetiology of the disease is based on multiple theories, no definite single treatment exists for all patients and in many cases, repigmentation of the skin does not occur in response to the available medical treatment. In those conditions, treatment by autologous transplantation methods may be valuable [10].

On reviewing the results of surgical methods for treating leucoderma, Njoo et al (1998)[2] reported that the highest mean success rates (87%) were achieved with split thickness and epidermal blister grafting. The mean success rate of the culturing techniques varied from 13 to 53 %.

In the present study, non-cultured melanocyte enriched cell suspension was used to treat selected cases of refractory and stable vitiligo. The technique was modified from that described by Olsson and Juhlin (1998)[9&15]. The modification done is that Erbium Yag laser instead of dermabrasion using Goulian biopsy knife was used to obtain a depth-controlled and precise dermabrasion. Similarly Yang and Kye (1998)[11] used Erbium Yag laser in treatment of vitiligo with autologous epidermal graft-

ing. In addition, we used PUVA or narrow band-UVB therapy to stimulate repigmentation three weeks after grafting.

The procedure was easy, almost no side effects occurred. Start of repigmentation was obtained in 60% of cases 4-6 weeks following the procedure.

6 months later, 15 % of patients showed moderate improvement, 15% showed good improvement and 30% showed excellent improvement. Olsson and Juhlin [9] reported excellent repigmentation in 24/28 cases of leucoderma treated where 18/28 patients showed 100% repigmentation. The latter were cases of segmental vitiligo, piebaldism and halo naevus.

The use of non-cultured melanocytes has the advantage that cell culture is not needed, so the procedure is simple and less time consuming and it is more suitable than epidermal sheet grafts when several small areas are to be treated . Moreover extensive achromic areas can be treated [9 & 10].

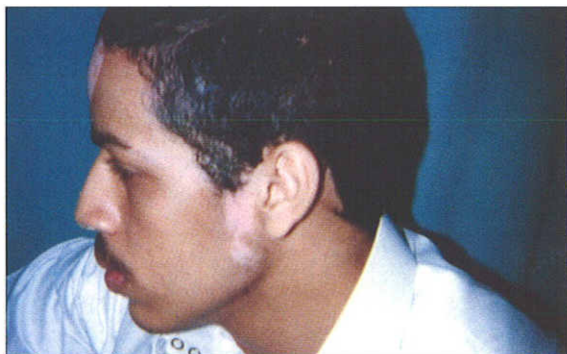
However, the procedure can usually be repeated which for the patient is more comfortable than transplanting a larger area in one session. The use of culture medium when preparing the cells and when leaving them under occlusive bandage together with the release of growth promoting factors during the healing process might be of importance. [9&14]

However, the disadvantage of this technique is the application of melanocytes in a suspension (liquid)

form making extreme immobilization mandatory in the first five hours following the procedure other wise the suspension will drop off and no implantation of melanocytes is expected.

Van Geel et al (2001)[15] added hyaluronic acid to a similar melanocyte keratinocyte suspension to obtain higher viscosity. This viscosity agent must be neither toxic to the melanocytes nor irritant or sensitizing to the skin.

A remarkable repigmentation greater than 85% was achieved in all (4) cases of vitiligo treated. The procedure of using non -cultured melanocytes can be valuable in motivated patients when vitiligo is resistant to the conventional treatment and when the areas to be treated are not actively extending.



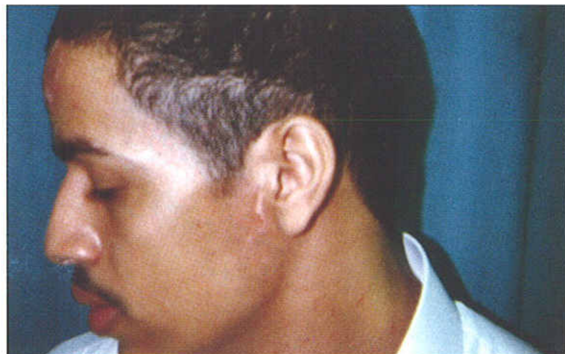


Fig 1 : vitiligo patches in the face before and 6 months after tranplantation of a noncultured melanocyte/keratincyte suspension

References:

(1) Falabella R, Arrunategui A, Baronami and Alzatea A (1995). The minigrafting test for vitiligo:

Detection of stable lesions for melanocyte transplantation.

J. Am. Acad Dermatol 32, 228-32.

(2) Njoo MD, Westerhof W, Bos JD and Bossuyt PM (1998). A systematic review of autologous transplantation methods in vitiligo.

Arch Derm., 134; 1543-9.

(3) Malakar s, Dhar(1999). Treatment of stable and recalcitrant vitiligo by autologous miniature punch grafting: A prospective study of 1000 patients. Dermatology198:133-9

(4) 01sson MJ and Juhlin L (1997). Epidermal sheet grafts for repigmentation of vitiligo and

piebaldism: With review of surgical techniques. Acta Derm Venereol (Stockh.) 77:463-6.

(5) Kahn AM and Cohen CJ: Vitiligo (1995). Treatment by dermabrasian and epithelial sheet grafting.

J. Am. AcadDermatol 33:646-8.

(6) Gauthier Y and Surleve- Bazeille JE (1992). Autologous grafting with non-cultured melanocytes:

A simplified method for treatment of depigmented lesions. J. Am. Acad Dermatol 26:191-4

(7) Falabella R, Escobar C and Borrerot (1992). Treatment of refractory and stable vitiligo by transplantation of in vitro cultured epidermal auto grafts bearing melanocytes.

J. Am. Acad Dermatol 26:230-6.

(8) Olsson MJ and Juhlin L (1995). transplantation of melanocytes in vitiligo.

Br. J. Dermatol 132: 587-91.

(9) Olsson MJ and Juhlin L (1998). Leucoderma treated by transplantation of a basal cell layer

Enriched suspension. Br. J. Dermatol. 138:644-648.

(10) Mutalik S,GmzburgA(2000). Surgical management of stable vitiligo: a review with personal experience.Dermatologic Surgery26:248-54.

(11) Yang J and Kye Y (1998). Treatment of vitiligo with autologous epidermal grafting by means of pulsed erbium-YAG laser. J. Am. Acad Dermatol 38:280-282.

(12) Gauthier Y (1995). Les techniques de greffe melanocytaire.

Ann. Dermatol Venereol 122:627-631

(13) Kumagia N and Uchikoshi T (1997). Treatment of extensive hypomelanosis with autologous cultured epithelium. Ann Plastic Surg. 39:68-73.

(14) Lemer AB, Halaban R, Klauss N and Moellmami GE (1987). Transplantation of human melanocytes. J Investigative Dermatol 89: 219-24.

(15) van Geel N, Ongenae K, De Mil M and Naeyaert JM (2001). Modified technique of autologous non cultured epidermal cell transplantation for repigmenting vitiligo: A pilot study.

Dermatol Surg. 27:873-876.