PLAN OF THESIS

A COMPARATIVE STUDY BETWEEN TRANSPLANTATION OF AUTOLOGUS NON-CULTURED EPIDERMAL CELL SUSPENSION AND COMBINATION OF AUTOLOGUS NON-CULTURED EPIDERMAL CELL SUSPENSION AND NON-CULTURED DERMAL CELL SUSPENSION IN STABLE VITILIGO.

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**ABBREVIATIONS**
Vitiligo, commonly known as leukoderma or phulwari in India is a complex disease causing a selective, often progressive, loss of functioning melanocytes from epidermal basal layer resulting in white patches on the skin and occasionally mucosae. The condition is non-contagious and asymptomatic but is associated with a significant psychosocial implications leading to an exaggerated sense of humiliation, loss of self-esteem and job discrimination among patients. Worldwide prevalence of vitiligo is around 1% whereas in India it is around 3-4% ranging from 0.46% to 8.8%. Melanocytes are neural crest derived cells located mainly in the basal layer of the epidermis and in the matrix of hair follicles. Melanocytes synthesize melanin pigment, transfer mature melanosomes to basal keratinocytes thus responsible for skin color.

The clinical features of vitiligo are variable, initially starting as hypopigmented and then depigmented macules, patches and widespread involvement of skin in later stages. Vitiligo is classified in two major subtypes, segmental vitiligo (SV) and non-segmental vitiligo (NSV). SV includes focal lesions and those restricted to a segment of the integument that does not progress towards generalized disease and NSV corresponds to all generalized usually symmetrical forms, including acrofacial vitiligo. The sites of predilection for vitiligo vulgaris are the fingers and wrists, axillae and groin, and body orifices, such as the mouth, eyes, and genitals.

Etiopathogenesis of vitiligo is multifactorial and polygenic consisting of genetic, immunological and environmental factors. Environmental and genetic factors act in concert to destroy melanocytes. Reactive oxygen species (ROS) play important roles in vitiligo pathology, but the autoimmune pathogenesis has been proposed as one of the main causes of vitiligo. The histological analysis of the perilesional margin surrounding the patients’ de-pigmented skin revealed infiltration of activated T cells and other lymphocytes. Further studies confirmed that these surrounding T cells were skin-homing, and were apparently cytotoxic to nearby melanocytes.

The treatment includes both medical and surgical modalities, whose goal is to restore melanocytes to the depigmented skin so that the epidermis restores normal morphology and functions. Although medical treatment is the main stay of treatment, it is not effective in all and residual lesions require surgical treatment later for further completion of repigmentation. The
key principle of disease treatment is inducing repopulation of active melanocytes that are able to migrate, proliferate to repopulate the depigmented skin resulting in repigmentation. The proper selection of cases for surgical therapy is of paramount importance and the stability of the vitiligo is taken as the most important parameter before opting for any transplantation technique. The recommended period of stability in different studies varied widely from 4 months to 3 years, but none of these criteria were based on evidence obtained from systematic research.

Surgical methods, mainly transplantation of non cultured epidermal cell suspension are effective treatment for stable vitiligo. Transplantation of autologous noncultured epidermal cell suspension and non-cultured dermal cell suspension in combination (a mode of cellular grafting technique) is a novel surgical method for the treatment of vitiligo. Cytotoxic CD8+ T cells in vitiligo perilesions may dictate the fate of transplantation, and strategies against CD8+ T cell activation might be beneficial for patients undergoing melanocyte transplantation. Friedenstein and colleagues first characterized mesenchymal cells (MCs) from adult bone marrow, which were an adherent, fibroblast-like cell population. MCs have capacities to self-renew and differentiate into various cells with mesenchymal origins. It appeared that MCs-mediated immunosuppressive activity was major histocompatibility complex (MHC) independent, and CD4+ and CD8+ T cells were equally susceptible to MCs. MCs could inhibit T cell proliferation and induce T cell apoptosis. Bartsch first indentified and characterized dermal mesenchymal cells (DMCs). They have a multi-lineage differentiation potential into adipocytes, osteocytes and chondrocytes. Vitiligo patients’ autologous melanocytes transplantation efficiency may be predicted by perilesional skin-homing CD8+ T cell activities, and the immuneregulatory DMCs might be used as auxiliary agent to improve the efficacy.

This pilot study is planned to compare transplantation of autologous noncultured epidermal cell suspension v/s its combination with non-cultured dermal cell suspension as a novel method in vitiligo surgery in stability of vitiligo with regards to extent of repigmentation, color matching of repigmented area, patient satisfaction and adverse events if any. This is the first study using transplantation of autologous noncultured epidermal cell suspension and non-cultured dermal cell suspension in combination as a new modality in vitiligo surgery.
Vitiligo, the most common depigmenting disorder\textsuperscript{16} is an ‘idiopathic’, acquired pigmented disorder caused by the loss of functional melanocytes from the epidermis. The term is derived from the Latin word ‘vitelius’ which means calf,\textsuperscript{17} where the characteristic lesions of skin resemble the milky white cutaneous macules of spotted calf. The course of the disease is unpredictable but is often progressive with phases of stabilized depigmentation.\textsuperscript{18} It usually begins during childhood or young adulthood. Approximately one third to one half of the patients develops the disease before the age of 20 years. A large series on childhood vitiligo reported a prevalence of 2.4\% in India.\textsuperscript{19} The presence of vitiligo on exposed areas of body leads to social embarrassment, psychological turmoil, and cosmetic disfigurement in those affected.\textsuperscript{20}

Vitiligo is known since times immemorial. The oldest information comes from the period of Aushooryan (2200 BC), in the classical Tarikh-e-Tib-e-Iran.\textsuperscript{18} In the ancient Indian sacred book ‘Atharva Veda’, dated around 1400 B.C the disease is mentioned as ‘Shweta kushtha’.\textsuperscript{18}

Its prevalence is 1\%, ranging from 0.1 to \(>\) 8.8\% in different countries of the globe. The highest incidence of the condition has been recorded in inhabitants of the Indian subcontinent.\textsuperscript{18} The reported difference in incidence between different geographical areas may be due to a higher reporting of vitiligo in certain populations, where an apparent color contrast and stigma attached to the condition may force the patients to seek early consultation. Both sexes are equally affected although the greater number of reports among females is probably due to the greater social consequences to women and girls affected by this condition.

**CLASSIFICATION**

*Classification of vitiligo according to the distribution of lesions* \textsuperscript{1}

1. Localized
   a) Focal
   b) Segmental
   c) Mucosal
2. Generalized
   a) Vulgaris
   b) Acrofacial
3. Universal

**Bordeaux VGICC classification and consensus nomenclature**

1. Vitiligo/Non Segmental Vitiligo
   a) Mucosal (more than one mucosal site)
   b) Generalized
   c) Universal
   d) Mixed (associated with SV)
   e) Rare variants

2. Segmental Vitiligo
   a) Uni-, bi-, or plurisegmental

3. Undetermined/Unclassified Vitiligo
   a) Focal
   b) Mucosal (one site in isolation)

There are two main clinical presentations of vitiligo: unilateral (segmental, asymmetric) and bilateral (nonsegmental, symmetric).

**Clinical features of segmental vitiligo**

Segmental vitiligo, a subtype of vitiligo, is characterized by its early onset, rapid stabilization and unilateral distribution. The reported prevalence of segmental vitiligo ranges from 3.5% to 20.5% of all patients with vitiligo. Associated autoimmune diseases in patients with segmental vitiligo and their family members are reported less frequently than in generalized vitiligo. Face is the most common site of segmental vitiligo regardless of the gender of the patient. The trunk, neck, extremities, and scalp are involved in descending order. So far, several hypotheses for segmental vitiligo have been put forward, including (i) neuronal mechanisms, (ii) somatic mosaicism and (iii) microvascular skin homing, whether or not leading to an autoimmune destruction of melanocytes. Most of the patterns of segmental vitiligo did not follow a dermatomal distribution. High rates of repigmentation with surgical techniques are frequently achieved.

**Clinical characteristics of non-segmental (bilateral) vitiligo**
Bilateral vitiligo is a slowly developing condition, with a tendency to progress throughout life. Arrest of the condition may occur in a small percentage of these individuals. Focal vitiligo exhibits one or few macules in one area, most commonly in the distribution of trigeminal nerve, although neck and trunk are also commonly involved. Focal vitiligo is a starting point leading to other types of vitiligo. Mucosal vitiligo affects mucosae of the mouth and genitalia. Acrofacial vitiligo encompasses depigmentation of the distal parts of the extremities (hands rather than feet) and facial orifices, the latter in a circumferential pattern. Lip-tip vitiligo is a variety in which tips of fingers, toes, nipples, penis and lips become depigmented. Vitiligo vulgaris is composed of several scattered macules and is the most common form of the disease. Depigmented patches are widely and usually symmetrically distributed. Universal vitiligo implies loss of pigment over the entire body surface area and complete or nearly complete depigmentation can be noted. More important is to differentiate between active and stable vitiligo for the initiation of appropriate therapy. Active vitiligo usually requires medical therapy. Surgical therapy is indicated when medical therapy fails and could actually be considered as the first therapeutic option for the treatment of stable vitiligo. Active vitiligo is characterized by increase in size of old lesions, development of newer lesions, and appearance of white macules after trauma (Koebnerisation).

In patients affected by segmental vitiligo, the causative factor(s) usually disappears, leaving well-defined depigmented lesions. Even generalized vitiligo can enter long phases of clinical remission in which the size and number of lesions are stationary for several years and the Koebner phenomenon is absent. This stage of the disease is referred to as stable vitiligo. Sometimes vitiligo is a slowly spreading disease or is limited to a specific anatomic region, and on other occasions it becomes aggressive dermatosis developing in a relatively short period of time. Fortunately, most patients have a slow and prolonged course over several years, but progression is the rule, especially with vitiligo vulgaris.

Various hypotheses, not mutually exclusive, have been proposed for pathogenesis of vitiligo. Of these, the most accepted theories include genetic, autoimmune, neurogenic, and the melanocyte self-destruction hypothesis. So far, no convincing model describing the interplay of these contributing factors has been formulated. A multi-factorial etiology has been proposed based on existing research.
ETIOLOGY

Though vitiligo is an ancient disease, the exact etiology still eludes us. There appears to be a combination of genetic predisposition and a number of potentially precipitating factors.

Heritability

Vitiligo is a heritable condition, up to 30% of the patients have a positive family history and 20% have an affected first degree relative. The pattern of inheritance points to a polygenic trait with the involvement of 3 or more diallelic alleles. HLA studies have shown an aggregation of HLA-DR4 in blacks and HLA-B13 in Moroccan Jews. HLA-B12 has also been shown to be associated with vitiligo. Recently HLA-A2 has been correlated with vitiligo. It has been suggested that the genetic background of these patients may render them more susceptible to melanocyte damage and hence to vitiligo.

Precipitating factors

Patients frequently attribute the onset of vitiligo to a specific life event such as an accident, crisis, physical or emotional stress. It may follow a cut or abrasion due to Koebner phenomenon.

PATHOGENESIS

The importance of the disease makes the understanding of the pathophysiological, biological and molecular events leading to melanocyte death or dysfunction, crucial to the outcome. Multiple theories have been postulated to explain the appearance of vitiligo patches. The proposed causative factors are not mutually exclusive.

Autoimmune hypothesis

It is the most popular hypothesis. Vitiligo is considered as an autoimmune disease due to the following features: an association with other autoimmune disorders; chronic relapsing and remitting course so typical of autoimmune disorders; possible response to immunosuppressive therapies like UV phototherapy, topical and oral corticosteroids, and topical calcineurin inhibitors; presence of auto-antibodies directed against melanocytes and related structures in patient’s sera, T-cell infiltrates in perilesional skin; anti melanocyte cytotoxic T-cells in the skin and circulation and proinflammatory cytokine patterns of a Th-1 type response; the detection of auto-antibodies in first degree relatives of subjects with vitiligo and the association of the disease with HLA–DR4, HLA-DR1.
In vitiligo, there is production of auto antibodies against melanocyte antigens. These anti-
melanocyte antibodies have different target antigens on the surface of melanocytes. The titer of
these antibodies correlates with the activity and extent of the disease.\textsuperscript{31} Recently anti-tyrosinase
antibodies were also detected. Baharav et al\textsuperscript{31} demonstrated these antibodies to be more in
extensive widespread vitiligo than in localized vitiligo. Whether these antibodies represent a
primary event or are secondary to the release of antigens from previously damaged melanocytes
is not yet known. In a recent study conducted in Mumbai, antibodies were detected against
tyrosinase, tyrosine hydroxylase, thyroid peroxidase, thyroglobulin and keratinocytes at
frequencies of 11\%, 22\%, 18\%, 24\% and 27\% respectively. Overall, antibodies were more
common in patients with nonsegmental vitiligo (50–67\%) than in those with segmental disease
(0–17\%), and were detected more frequently in patients with shorter disease durations (<10
years).\textsuperscript{32} Patients with vitiligo have been reported to have a reduced number of lymphocytes and
helper T-cells and an increased number of natural killer cells in the serum whereas in
inflammatory vitiligo there is an increase in T-cell infiltration, predominantly CD-8+ T-cells at
the periphery of the lesions. T-cell infiltrates were found in normal-appearing skin remote from
the site of vitiligo, with microscopic loss of melanocytes termed ‘microdepigmentation’. These
could well represent sites of future vitiligo.\textsuperscript{9} A statistically significant decrease in Helper T–
cells/Suppressor T-cells ratio was obtained in the study as compared to controls.

\textit{Neural hypothesis}

Proposed by Lerner\textsuperscript{33} about 40 years ago, this theory states that there is liberation of a
neurochemical mediator that is toxic to the melanocytes from nearby nerve endings. Support for
this hypothesis comes from a number of observations namely: vitiligo in neurologically
compromised skin, vitiligo sparing paralyzed limbs, onset of vitiligo following peripheral nerve
injury and vitiligo limited to a single dermatome, though strictly not following a particular nerve
course.\textsuperscript{34} The neural hypothesis is based on the presence of segmental vitiligo. An ultra structural
study of normal dermal nerves was performed recently. Subtle ultra structural differences were
observed between biopsies taken from marginal and central parts of vitiliginous skin and non
vitiliginous skin. The most consistent feature seen in all the biopsies from vitiliginous skin was
an increase in thickness of basement membrane of Schwann cells. This change was seen in
approximately \(\frac{3}{4}\) of dermal nerves in vitiligo biopsies and \(\frac{1}{4}\) of dermal nerves of normal control
biopsies. About half of the dermal nerves showed minor axonal damage, whereas indicators of
regeneration predominated in others. In addition, relation between the nervous stem cell and epidermal melanocyte has recently been provided. Abnormalities reflecting possible nerve mediated aberrations in beta-endorphins and met-encephalin secretion in vitiligo patients and increased immunoreactivity to neuropeptide Y and vasoactive intestinal polypeptide in vitiligo skin have been reported. Although little is known about the effects of neuropeptides on human melanocytes, the nervous system may exert a tonic effect on melanocytes in normal or diseased human skin, especially through calcitonin related peptide secretion, yet the role of the nervous system in the pathogenesis is yet to be elucidated.

Biochemical support for this hypothesis arises from the observation that acetylcholine has been shown to have an inhibitory effect on DOPA oxidase activity in marginal melanocytes in vitiligo and acetylcholinesterase activity has been shown to be absent in depigmenting skin and present in repigmenting skin.

*Self destruct hypothesis* or *autocytotoxic hypothesis*

This postulates that an intermediate metabolite of melanin synthesis, particularly quinine is toxic to melanocytes. Melanin repigmentation is produced in the melanocytes through the tyrosinase activity. Tyrosinase gene family consists of tyrosinase enzyme, TRP-1&2, Calnexin and LAMP-1. It has been postulated that mutation in TRP-1 protein is involved in cell degeneration or death, associated with faulty scavenging of intermediates of melanin pigmentation leading to apoptosis of melanocytes.

Compartmentalization of melanosomes normally protects melanocytes from destruction by such substances. However, it is thought that leaky melanosomes or high quinine: melanin ratio could damage the pigment cell. The free radical scavenging function of melanin may be insufficient to prevent damage by highly toxic quinine.

*Biochemical theory*

It has also been shown that both lesional and non-lesional epidermis has decreased catalase activity that leads to an increase in peroxidase concentration in it. Hydrogen peroxide functions as a reversible inhibitor of human tyrosinase.

*Role of Liver X receptor (LXR) expression in vitiligo*

LXR regulate a variety of cellular functions, they have robust anti-inflammatory activity in skin, but they also modulate epidermal proliferation, carcinogenesis, differentiation and permeability barrier function. Kumar et al demonstrated in their study that expression of LXR-
α/b at both mRNA and protein level was significantly higher in perilesional skin as compared to the normal skin of vitiligo patient.

The new hypotheses:

**The melanocyte growth factor deficient theory** – Defective growth and passage capacity of melanocytes derived from uninvolved and perilesional skin could be due to decreased concentration of melanocyte growth factors in vitro.\(^{38}\)

**Decreased melanocyte lifespan hypothesis** -- Several cytokines, such as interleukin-1 and interferon-gamma, mainly produced and released by keratinocytes, may induce apoptosis of melanocytes due to deficiency in survival signals by interfering with the melanocyte membrane tyrosine kinase receptor, C-KIT.\(^{39}\) Reduced levels of C-KIT receptors in vitiligo melanocytes or of growth factors could induce premature apoptosis and decreased melanocyte survival.

**New Integrated Theory of Non Segmental Vitiligo (NSV):** A Melanocytorrhagic disorder\(^{40}\)-

The new integrated theory takes into account melanocyte detachment and transepidermal elimination, neural-biochemical and autoimmune hypotheses. This new theory proposes that NSV is a primary melanocytorrhagic disorder with altered melanocyte responses to friction and possibly other types of stress, inducing their indolent detachment and subsequent transepidermal loss. Further it was shown that melanocytorrhagy and apoptosis is seen only in patches of unstable vitiligo.\(^{41}\)

1. **Melanocyte defective adhesion:** - The melanocyte adhesion system is less well organized and far weaker than the system which firmly holds epidermal keratinocytes bound to each other and to the basement membrane. No melanocyte – keratinocyte adhesion structures can be detected by electron microscopy.

2. **Loss of dendricity:** Dendrites are critically important for melanosome transfer, because one melanocyte contacts several keratinocytes in the epidermis through dendritic cell processes. Moreover, ultra structural observations suggest that dendrites, independently of structural junctions, may dramatically increase the adhesion and anchoring of melanocytes within the basal layer of the epidermis. It has been suggested that the loss of dendricity induced either by oxyradicals (impaired redox status hypothesis) or by increased release of catecholamines (neuralbiochemical hypothesis) exaggerates
transepidermal loss induced by minor mechanical trauma. This loss of dendricity could also affect melanosome transfer and contribute to depigmentation.

3. **Weakening of melanocyte attachment and melanocyte detachment after friction:**

Ultrastructural abnormalities of the basement membrane have been observed frequently in vitiligo, namely multiple replication or layering of the basement membrane directly beneath melanocytes and focal gaps in the basement membrane. As a result of the weakening of their basal anchoring, melanocytes could be detached by mechanical or chemical injury. Human skin is repeatedly exposed to mechanical stimuli usually grouped under the term friction. During friction, an alternation of stretching and relaxation sometimes results in epidermal disruption, degeneration of keratinocytes and widening of intercellular spaces. Extracellular granular material deposits are found ultrastructurally in NSV skin after severe frictional injury of normal skin. Altered synthesis of extracellular matrix components (such as tenascin) may be produced by damaged keratinocytes.

4. **Transepidermal elimination of melanocytes:** After their detachment, melanocytes are seen in a mid-spinous location as early as 8 hours after friction. Twenty-four hours later, some melanocytes reach the stratum corneum.

The ultimate consequence of all the pathogenic mechanisms described above is melanocyte destruction, and therefore the final outcome is absence of pigmentation. Initially only epidermal melanocytes are affected, but as the condition progresses, the most important pigment cell reservoir, the hair follicle, may also become involved and leucotrichia develops, thus making repigmentation difficult. How and to what extent this phenomenon occurs is dependent on the individual response of the affected patient and the aggressiveness of the pathogenic process.

**HISTOPATHOLOGY**

There is a marked absence of melanocytes and melanin in the epidermis. Histochemical studies show a lack of dopapositive melanocytes in the basal layer of the epidermis. Recent immunohistochemical studies with a large panel of antibodies show only an occasional melanocyte in lesional skin. Electron microscopy studies confirm the loss of melanocytes, which appear to be replaced by Langerhans’ cells. Areas around the margins of vitiligo show abnormalities of keratinocytes as well as degenerating melanocytes. In inflammatory vitiligo,
where there is a raised erythematous border, there is an infiltrate of lymphocytes and histiocytes. This infiltrate is also found in the marginal areas of some biopsies. 

Mechanisms of repigmentation in vitiligo

Some vitiligo patients show spontaneous repigmentation even though all have a permanent melanocyte loss. Spontaneous repigmentation of the vitiligo patches is a regular feature when exposed to sun. After therapy, repigmentation can occur in four ways: follicular, marginal, diffuse and combined. Repigmentation usually occurs in the follicular pattern, suggesting that follicular melanocytes colonize vitiliginous skin. In most patients of repigmenting vitiligo, studies also argue for a proliferation of melanocytes, followed by their migration; however less commonly, repigmentation might occur from residual intraepidermal melanocytes. Based on follicular repigmentation, the existence of a melanocyte reservoir has been postulated. The existence of a population of intraepithelial cells that have immunopathological characteristics of mature melanocytes within the upper epidermal region has been shown. These KIT (+), BCL-2(+), TRP-1(-) cells may contribute to the precursor melanocyte reservoir of human skin.

During repigmentation, melanocytes migrate from the outer root sheath of the hair follicle to the basal layer of the epidermis just above the basement membrane. Because keratinocytes are attached to each other by desmosomes and to the basement membrane by hemidesmosomes, migration of melanocytes involves several complex processes that are not yet understood.

Treatment options

A number of therapeutic options for vitiligo are available but there is still a need for a treatment that is promptly effective. There is no curative treatment for this condition. Management of vitiligo is a real challenge for a dermatologist.

Medical therapies:

Corticosteroids (Topical, intralesional and systemic), Oral mini pulse, PUVA (topical and systemic), NBUVB, calcipotriol and tacrolimus are used most widely. Some of the less commonly used medical modalities include phenylalanine, khellin, topical minoxidil, levamisole and melagenina. Recently oral minocycline was shown to be effective in treating vitiligo.
Most of these therapies aim to restore melanocyte function by their anti-inflammatory or immunomodulatory action and by preventing melanocyte auto destruction so that normal skin appearance and function is restored.

**Surgical therapies:**

All patients with vitiligo should be initially treated with medical methods. Surgical methods are important solutions for stable vitiligo refractory to medical treatment. High repigmentation rates are obtained with all procedures so far described in most anatomic locations, but they are of little help for acral areas and bony prominences. Unilateral vitiligo is the clinical form with the best response to grafting and transplant methods, and a good proportion of patients with stable bilateral disease also respond adequately. Nevertheless, appropriate patient selection is important to achieve maximal results. However none of the surgical modalities developed so far is uniformly effective in all patients and body sites and there is need for constant research and innovations for better surgical therapeutic options for vitiligo.

**Aims of various surgical procedures:**

A) **Camouflage Tattooing:** Introduction of artificial pigments into the lesions for permanent camouflage.

B) **Excision:** Removal of the depigmented areas, e.g. excision with primary closure and covering with thin Thiersch's graft.

C) **Melanocyte transplantation:** commonly used methods of autologous transplant of melanocytes are

**Tissue grafts:**

1. Thin and ultra-thin split thickness grafts (STSG)
2. Suction blister epidermal grafts (SBEG)
3. Mini punch grafts (MPG)
4. Hair follicular grafts (HFG)

**Cellular grafts:**

5. Noncultured epidermal cell suspension (NCES)
6. Cultured “pure” melanocytes (CM)
7. Cultured epithelial grafts (CE)
8. Autologous noncultured extracted hair follicle outer root sheath cell suspension also called follicular cell suspension (FCS)

D) Therapeutically wounding the lesion to stimulate the melanocytes from the periphery and the black hair follicles to proliferate, migrate and re-pigment the lesion, e.g. therapeutic dermabrasion, laser ablation, cryosurgery (liquid nitrogen spraying), needling and local application of phenol or trichloroacetic acid.\textsuperscript{56}

Every method has its own advantages and disadvantages. As there are no specific data available from the prospective studies in this field, it is not easy to recommend which surgical approach to vitiligo offers the best result.

Several points need to be assessed in patients when surgical treatment is planned.\textsuperscript{23}

\textbf{Stable Disease:}\textsuperscript{58}

The most important factors indicating stability are

1. No progression of lesions for at least 1 year.
2. Spontaneous repigmentation.
3. A positive minigrafting test disclosing repigmentation around four to five minigrafts (1.0 or 1.2 mm), implanted 3 to 4 mm apart within an achromatic lesion, is an indication of future recovery by surgery. So far, this test is the most accurate evidence of vitiligo stability.
4. Absence of new koebner phenomenon (KP), including the donor site for the minigrafting test.\textsuperscript{23}
5. Unilateral vitiligo is almost a synonym of stable disease with an excellent repigmentation response.

However, these criteria may be challenged by clinical observations in which KP and minigraft testing are discordant. Data obtained from minigraft testing in case series suggest that the minigraft test provides a reflection of the stability of defined individual lesions, which does not necessarily reflect global stability of the disease.\textsuperscript{59}

‘Vitiligo global issues consensus conference, 2011’ convened by Vitiligo European Task Force (VETF), concluded that assessment of ‘overall’ stability is inaccurate and unreliable, whereas individual lesion stability is more reliable, especially when used in the context of surgical intervention.\textsuperscript{21}
Methods and Size of Lesions:
Depending on the size of the treated area, the method may vary. Simple methods such as minigrafting and suction epidermal grafting are useful for small or medium sized lesions. On the contrary, for extensive depigmented defects, cellular transplants may be required.

Age:
Because of the invasive nature of surgical procedures, they are not recommended in children; nevertheless, highly motivated adolescents can be treated under sedation or general anesthesia. Also, it is not surprising to see patients beyond the age of 50 years who may be interested in surgical repigmentation.

Psychological Aspects:
Some patients with high emotional trauma because of depigmentation may seek advice for invasive procedures. However, a psychological evaluation may be needed to ascertain the real need for surgical treatment.

Photographic Record:
Illustrations are recommended to help in determining the percentage of improvement, quality of repigmentation and possible side effects.

Patient's Expectations:
Repigmentation is not always comparable with normally pigmented skin and the final results vary considerably from patient to patient. However, most individuals are pleased with the achieved results; minor imperfections are far less important than the noticeable repigmentation of vitiliginous skin, mainly in ethnic skin patients with a dark complexion; sometimes surgical repigmentation may look even better than what is observed in many patients after medical therapy.

Method and Donor Site:
Appropriate training with a specific method is an important prerequisite for surgical therapy. Donor site should be as hidden as possible and the gluteal region may be suitable for this purpose in most patients.

Serial Procedures:
Most procedures require more than one intervention and several sessions may be needed to accomplish full recovery or to complete repigmentation of minor depigmented defects. Combination methods may be of value for this purpose.

Difficult Areas for Surgical Treatment:

With surgical procedures much improvement is achieved, particularly in unilateral vitiligo, but certain areas are difficult to repigment, such as joints, lips, eyelids, genitalia, cutaneous folds, the dorsum of hands and feet, especially fingers and toes. In some of these areas, inadequate immobilization prevents a good take of grafts and repigmentation is difficult to achieve; some of these areas may need regrafting, and recovery is possible in some patients. Nevertheless, other factors not known at present may prevent a good repigmentation response.

Success rates of different surgical options:

Among all procedures, suction blister epidermal grafts and thin and ultra-thin split-thickness grafts seem to be the most effective procedures, with overall success rates of 80.3% (CI 76.4–84.2%) and 77.9% (CI 72.2–83.6%) respectively. But, a recent randomized study directly comparing NCES and SBEG showed NCES is significantly better than SBEG. Among cellular grafts, all techniques seem to be equally effective with success rates of 61.1% (CI 56.1–66.1%), 63.6% (CI 57.2–70%), and 63.6% (CI 55.8–70.6%) for noncultured epidermal cell suspension, cultured melanocytes and cultured epidermis respectively. The mean repigmentation with noncultured extracted outer root sheath cell suspension is about 65.7%.

Cases with more extensive vitiligo vulgaris, involving greater than 30% body surface area, are generally considered unsuitable for transplantation procedures as chances of retention of the pigment are less. Extensive areas may be best treated with cellular grafts. Theoretically, culture methods would provide an unlimited number of cells/tissue for transplantation, while NCES would provide up to 8–10 times donor-to-recipient expansion. Therefore, it seems that larger areas may be treated with cellular grafts and thin and ultra-thin split-thickness grafts and moderate areas may be treated with cellular grafts and minigrafts. Smaller areas may be easily treated with suction blister epidermal grafts which gives good aesthetic results and is technically less challenging. Overall, better results are reported in focal and segmental vitiligo (75%-95%) than in generalized vitiligo. Young, dark complexioned patients have better results. Comparatively, acral areas, malleoli, knees, and elbows are less responsive to surgery. Smaller
patches respond better. Addition of PUVA/PUVASOL therapy enhances repigmentation and increases the repigmentation rate (90-95%).

**Adverse events:**

No serious adverse events have been reported with any of the transplantation methods. Cellular grafts appear to have the least frequency of adverse events. Adverse events reported at recipient sites are infection, milia, scarring, and rejection of the graft. Complications at donor sites reported are infection, milia, scarring and pigmentary changes. Cultured melanocytes and NCES have a mean of 0.01 and 0.08 adverse events at recipient site respectively, and 0.01 and 0.009 at the donor site respectively. Tissue grafts are reported to be associated with more adverse effects and the maximum number of adverse events on the recipient site is seen with MPG (0.7) and STSG (0.5).

**Response:**

The treated area appears bright pink immediately after removal of the dressing. The earliest pigmentation was noticed 3 weeks post surgery. Many patients showed hyperpigmentation which gradually blended with the surrounding skin over 6–8 months. The donor area healed rapidly and soon became indistinguishable from the surrounding skin. Occasionally, the donor area healed with hyperpigmentation.

**NONCULTURED EPIDERMAL CELL SUSPENSION**

The technique of noncultured epidermal suspension was pioneered by Gauthier et al61. The suspension was prepared by incubating the donor skin obtained from the scalp in trypsin 0.25% for 18 hrs. The suspension was injected into blisters raised by cryotherapy. Eight out of the 12 patients treated had > 70% repigmentation at the vitiligo site. It was proposed that the presence of keratinocytes in the suspension supplies essential growth factors for melanocyte growth. They stated that this technique could emerge as simple and effective alternative to the costly cultured melanocyte transplantation technique.61

Olsson and Juhlin62 first used the M2 medium for suspension of the noncultured epidermal cells. A total of 20 vitiligo patients were included and results showed 100% repigmentation in all 3 patients with segmental vitiligo and 80 % repigmentation in 12 patients of stable generalized vitiligo. The research group found the transplantation as effective as transplantation of cultured melanocytes. However, several practical problems surfaced with the procedure. Usage of
cryotherapy to raise blisters damaged the melanocytes resulting in hypopigmentation. Significant run-off of suspension from recipient site was associated with the high fluidity of suspension. Blisters were difficult to raise at the bony prominences by the use of cryotherapy.

Van Geel et al\textsuperscript{63} added hyaluronic acid to the cellular suspension to improve the viscosity and fixation, CO\textsubscript{2} laser was used to obtain a depth-controlled and precise dermabrasion at the recipient site and adjuvant PUVA or UVB therapy was added 3 weeks after grafting to stimulate and homogenize the repigmentation. First, a pilot study was conducted in 4 patients all of whom achieved 80\% pigmentation. The therapeutic value of this procedure was increased with the further evaluation in 28 patients in a double blind placebo controlled study. 70\% or more repigmentation was observed in 55\% of the patients.

It is concluded that the transplantation of noncultured epidermal cell suspension is an efficacious and safe procedure. The technique requires special laboratory equipment. However, in comparison to cultured melanocytes, this is an inexpensive and simple OPD procedure requiring 4-6 hrs. Large areas, 8-10 folds the size of donor skin, can be treated with this procedure. A temporary color mismatch is observed in all patients, which improved over 5-6 months. No scarring is observed at the donor or recipient site. They stressed on proper aseptic precautions and the use of prophylactic antibiotics to prevent postoperative infections. The use of post-operative UVB therapy helped to achieve uniform pigmentation. In one patient it was observed that leucotrichia adjacent to depigmented eyebrow was also repigmented supporting retrograde migration of pigment cells.\textsuperscript{63, 64}

Transplantation of noncultured melanocytes is the method that results in least hyperpigmentation. This is because the cells are diluted and are transplanted in smaller numbers than in other methods. Halo phenomenon that noted in transplantation of cultured melanocytes is noted even in grafting of noncultured epidermal cell suspension. The technique shows excellent results in segmental vitiligo, focal stable vitiligo and piebaldism. Age and gender seem to have no significant effect on repigmentation. Acral and periorificial vitiligo has the poorest response.\textsuperscript{65}

Mulekar et al\textsuperscript{66} conducted an extensive study recruiting 49 patients with segmental vitiligo and 15 with focal vitiligo. They used Hams F-12 medium for suspension. 95-100\% repigmentation was observed in 41 patients of segmental vitiligo and 11 patients of focal vitiligo. The percentage of patients with segmental vitiligo showing an excellent response was 84\%, while 6\% of the patients had a good response to treatment. 10\% of patients failed to produce any pigmentation. In
focal vitiligo 73% showed an excellent response, while 20% had poor repigmentation at the end of the respective follow-up period. Response to the treatment on the lips was not encouraging. Patients with both focal and segmental vitiligo had retained the pigment at the end of respective follow-up periods (5 yrs). Repigmentation failed to be produced in 10% of patients in the segmental vitiligo group and 20% in the focal vitiligo group.

Pandya et al. abraded the recipient area with a high speed motor dermabrader and the denuded area was covered with saline moistened gauze piece. The suspension was poured evenly from the pipette and covered with a collagen dressing. This is covered with a small gauze piece moistened with MK medium. The dressing was kept in place by a Tegaderm dressing.

In Swedish procedure of melanocyte transplantation pioneered by Olsson and Juhlin, they used phosphate buffered saline (PBS) to wash the denuded area and they put PBS moistened gauze over denuded area.

NON-CULTURED DERMAL CELL SUSPENSION

Dermal mesenchymal cells were originally isolated from the dermis of juvenile and adult mice by Toma et al., afterwards, same group indentified such a cell population in human skin. Georg Bartsch firstly indentified and characterized dermal mesenchymal cells (DMCs). DMCs had multi-lineage differentiation potential into adipocytes, osteocytes and chondrocytes. The surface antigenic profile of DMCs was positive for CD90 but differs in regard to negativity for CD34. Zhou et al investigated the factors determining the efficiency of autologous melanocyte transplantation of vitiligo patients by focusing on perilesional skin homing CD8+ T lymphocytes, and studied the potential effects of dermal mesenchymal cells (DMCs) on CD8+ T cell activities in vitro. The patients with high number of perilesional CD8+ T cells were associated with poor repigmentation rate and a significant lesser number of CD8+ T cells was infiltrating in patients with excellent or good re-pigmentation responses. Also, skin homing CD8+ T cells proliferation was significantly inhibited when co-culture with DMCs at 1:1 ratio as the percentage of proliferative CD8+ T cells dropped from 94.72% to 39.50% (p<0.05) after DMCs co-culture. In the co-culture system, DMCs significantly inhibited skin homing CD8+ T proliferation and induced those cells apoptosis. These data confirm that DMCs induces
significant immunosuppressive abilities against skin homing CD8+ T lymphocytes and may help improve the efficacy of melanocytes transplantation. Non-cultured Dermal Cell Suspension (NDCS) is a novel method to increase the efficacy of non-cultured epidermal cell suspension (NCES).

**Justification for the proposed study**

NCES is proved to be efficacious for vitiligo surgery in terms of extent of pigmentation and colour matching. Here, we have divided patients into 2 stability groups depending on the time elapsed since the appearance of last lesion or the increase in the size of existing lesions: Group 1 (3 months to 6 months stability) and Group 2 (>1 year stability). As stable disease is defined as the stability > 1 year and NCES is well proven surgical method in stable vitiligo so in this study we are doing NDCS in addition to NCES to see the effects of dermal cells in repigmentation and stability of the vitiligo patches. So we are treating the vitiligo patches with the modality with proven efficacy in one patient, and in other patient we are combining NDCS with NCES which may increase the re-pigmentation efficiency of patients with NCES.

Also, there are studies showing keratinocyte damage in vitiligo. Bhawan J et al\(^{70}\) performed light and electron-microscopic studies on the amelanotic and adjacent normal-appearing skin in patients with vitiligo. The amelanotic skin revealed complete loss of pigment and absence of melanocytes. Kumar R et al\(^{71}\) proposed that oxidative stress may also manifest as the ultrastructural and functional changes observed in keratinocytes extracted from the perilesional and normal skin of NSV patients. The histological analysis of the perilesional margin surrounding the patients’ de-pigmented skin revealed infiltration of activated T cells and other lymphocytes.\(^{8}\) Further studies confirmed that these surrounding T cells were skin-homing, and were apparently cytotoxic to nearby melanocytes.\(^{9}\) There are notable morphological alterations like swollen mitochondria with disrupted cristae, a pathognomonic feature of apoptosis in keratinocytes.

The success of vitiligo surgery also depends on the anatomical area of vitiligo patch. Acral areas, joints, lips show poor response to any therapeutic method. In the literature, various anatomical areas are selected randomly for comparing different surgical methods. We will select different patches on the similar anatomical sites for the comparison of outcome. This overcomes the issue of koebnerisation, lack of quiescence after surgery at areas like joints and the innate resistance of acral areas\(^{72}\) to vitiligo surgery.
AIMS AND OBJECTIVES

**AIM:** To compare the effect of autologous noncultured epidermal cell suspension v/s autologous non-cultured epidermal cell suspension and non-cultured dermal cell suspension in combination as a novel surgical method in stable vitiligo.

**Primary outcome:**

1. To assess the difference in extent of repigmentation by both methods.

**Secondary outcomes:**

1. To assess the outcome in either procedure with regards to duration of disease stability.
2. Pattern of repigmentation attained post-procedure.
3. Color matching of repigmented area between two procedures done in between the groups.
5. Adverse events if any.
This is a prospective study where the cohort will be recruited from the patients attending Pigmentary and Dermatosurgery Clinic of Department of Dermatology, Venereology and Leprology; Postgraduate Institute of Medical Education and Research, Chandigarh, India. Totally 40 subjects of vitiligo satisfying inclusion/exclusion criteria will be assigned into one of 2 stability group of 20 each depending on the time elapsed since the appearance of last lesion or the increase in the size of existing lesions: Group 1 (clinical stability > 3 months but < 6 months), Group 2 (clinical stability > 1 year). These groups will be further divided into 2 subgroups i.e. Group1A and Group1B; Group2A and Group2B respectively consisting of 10 patients each randomly assigned to the subgroup. In Group1A and Group2A subgroup, NCES will be done and in Group1B and Group2B, combination of NCES + NDCS will be performed. Follow ups will be done at day 8 of surgery and 4, 8, 16, 24 weeks. Patient assessment will be done by using digital photography in the same settings with respect to patient positioning, background, lighting and camera settings; and questionnaire to know extent of repigmentation.

INCLUSION CRITERIA:

1. Subjects with clinical diagnosis of focal, segmental or generalized vitiligo which has been stable for more than 3 months and not responding to medical treatment, or residual patches of vitiligo vulgaris after medical therapy will be included for the study. Maximum size of vitiligo patches to be selected for surgery will not be >100cm².

EXCLUSION CRITERIA:

1) Age less than 18 years
2) Pregnancy
3) Patient with actively spreading vitiligo
4) Appearance of new lesions
5) History of Koebnerisation
6) History of hypertrophic scars or keloidal tendency
7) Bleeding disorders
8) Patients with unrealistic expectation
At the first visit, a proforma will be filled noting the baseline characteristics, history and clinical examination findings. An informed consent will be taken before the procedure is performed.

**TECHNIQUE OF TRANSPLANTATION:**

*Noncultured Epidermal Cell Suspension Method*[^23]

**Harvesting the graft:**

1. About one-tenth the size of recipient area will be selected as the donor site, usually on non-cosmetically important site like the thighs.
2. Donor area will be shaved, cleaned with betadine and surgical spirit and anaesthetized with mixture of 2% lignocaine and normal saline, NS (1:1).
3. Split thickness skin graft will then be taken with the help of a shaving blade held firmly by a straight artery forceps.
4. Haemostasis is established and the area will be dressed with Bactigras gauze.
5. Suitable antibiotic and analgesic will be prescribed.

**Preparing noncultured epidermal cell suspension:**

1. Split thickness skin specimen will be transferred under aseptic conditions to a container with NS and transferred to laboratory. There, the skin graft will be transferred to Trypsin-EDTA solution (0.25% trypsin and 0.02% EDTA) in a Petri dish and incubated overnight at 4°C in 5% CO₂ to separate the epidermis from the dermis.
2. Afterwards, the Trypsin-EDTA solution will be removed and PBS will be added and pipetted well so as to separate the cells from the tissue.
3. The solid waste of tissue will be removed and the suspension will be centrifuged at 1000 rpm for 5 minutes.
4. The supernatant will then be discarded and the pellet, containing cells from the stratum basale and lower half of the stratum spinosum that are rich in melanocytes will be taken.
5. The melanocytes will be stained with trypan blue and counted simultaneously with Neubauer’s chamber under the light microscope. This will help to identify whether the melanocytes are viable as the dead cells would pick up the blue stain.

6. Phosphate buffer saline is added to make suspension of non-cultured epidermal cells.

**Non-cultured Dermal Cell Suspension**

1. Skin punch will be collected in phosphate buffer saline (PBS) with antibiotics (penicillin and streptomycin).

2. The epidermis will be cut off from dermis carefully using a surgical blade (Epidermal part will be used for the epidermal cell suspension).

3. Dermis will be then cut into small pieces and incubated in collagenase (1mg/ml) overnight at room temperature.

4. Next day content will be diluted with PBS and centrifuged at 1000rpm for 5 minutes.

5. Pellet will be washed three times with PBS to remove collagenase activity.

6. The cells will be stained with trypan blue and counted simultaneously with Neubauer’s chamber under the light microscope. This will help to identify whether the cells are viable as the dead cells would pick up the blue stain.

7. Phosphate buffer saline is added to make suspension of non-cultured dermal cells and will be used for the autologous transplantation.

**Transplantation procedure:**

1. The recipient site will be shaved, cleaned with betadine and surgical spirit and anaesthetized with mixture of 2% lignocaine and NS (1:1).

2. Dermabrasion will be done until tiny pinpoint bleeding spots are seen which imply that the dermo-epidermal junction has been reached. Dermabrasion will be extended 5mm beyond margins to prevent halo phenomenon.

3. The denuded area will be washed with PBS and covered with a PBS moistened gauze piece.

4. The noncultured epidermal cell suspension (in Group1A and Group2A) and the combination of NCES+NDCS (in Group1B and Group2B) will be carefully transferred to a tuberculin syringe.
5. With 18g needle attached to this syringe, few small drops of suspension will be placed over
the denuded surface which will be then spread evenly with the help of needle.
6. This will be covered with sterile Vaseline gauze or Bactigras after washing with NS. Once
again small drops of suspension will be placed over this gauze and spread evenly.
7. After washing with NS a meshed collagen sheet (Kollagen M) will be put over the gauze with
suspension.
8. This will be then covered by a small gauze piece moistened with PBS.
9. Tegaderm will be placed over this so that an artificial blister will be formed which holds
melanocytes with PBS over the recipient site. At difficult areas like lips surgical glue will be
used to put Tegaderm in place.
10. Over this, surgical pad is put and the dressing will be stabilized by placing the elastic plaster
(Dynaplast).

The patient will be observed for 1 hour after procedure and then allowed to go home. Post-
surgery, patient will be given antibiotics and analgesics for 5 days. The dressing will be removed
at the first follow-up visit after 5-7 days in the hospital.

**FOLLOW UP:**
The patients will be asked to follow up at the clinic on day 8, week 4, week 8, week 16 and
week 24 after the transplantation procedure and percentage of repigmentation will be assessed by
blinded physician (Dr. Davinder Parsad) subjectively by serial digital photographs in the same
settings with respect to patient positioning, background, lighting and camera settings and
objectively by serial paper markings. No intermittent treatment will be given during this post-
surgery period.

Repigmentation will be assessed as follows:

- ≤25% Minimal repigmentation
- 26-50% Mild repigmentation
- 51-75% Moderate repigmentation
- 76-90% Marked repigmentation
- >90% Excellent repigmentation
Also, the repigmentation pattern will be noted as ‘diffuse’, ‘perifollicular’ or ‘migrating from the borders’. A note will also be made of the colour matching of repigmented skin as ‘somewhat lighter than’, ‘same as’ or ‘somewhat darker than’ normal skin.

At each visit, patient will also be asked and observed about any adverse events like:

**Recipient site**

- infection
- milia
- scarring
- rejection

**Donor site**

- Infection
- Milia
- Scarring
- Hypopigmentation
- Hyperpigmentation

Patient will also be asked to fill a questionnaire about the satisfaction with the procedure.

Hence, both objective and subjective evaluation of the results shall be done.
The statistical analysis will be carried out using Statistical Package for Social Sciences (SPSS version 22.0 for Windows). Normalcy of continuous data will be checked using Kolmogorov-Smirnov Test. Mean/SD will be calculated for all normally distributed continuous variables e.g. age, duration of disease, BP, pulse, BMI, Hb, bleeding/clotting time, size of graft, %BSA affected etc. For not normally distributed continuous variables median (IQR) will be calculated. Qualitative or categorical variables will be described as frequencies and proportions. Unpaired t test will be applied to compare mean of continuous data e.g. age, duration of disease, BP, pulse, BMI, Hb, bleeding/clotting time, size of graft, %BSA affected, duration of disease stability etc. between two study groups (Group 1 and Group 2). Categorical/qualitative variables e.g. presence of pallor/edema/clubbing/cyanosis/icterus/lymphadenopathy (yes/no), areas affected (head and neck/ trunk/ upper limb/ lower limb/ hands/ feet/ mucosae), leucotrichia (present/absent), mucosal involvement (present/absent), history of past illness, personal history (smoking/alcohol/addictions), type of precipitating factors, present status (stable/ unstable), Koebnerisation (present/ absent), extent of repigmentation, side effect at recipient/donor site, pattern of repigmentation attained post-procedure (diffuse/perifollicular/migrating from the borders), color matching of repigmented area (somewhat darker/somewhat lighter/same), patient satisfaction post-procedure level, adverse event, etc. will be analyzed using Chi-Square/Fisher’s Exact test between two groups (Group 1A vs Group 1B and Group 2A vs Group 2B). Extent of repigmentation will be analyzed within groups using McNemar-Bowker test between two time periods (at 8 days/4 wks/8 wks/16 wks/24 wks). All statistical tests will be two-sided and will be performed at a significance level of \( \alpha = 0.05 \) with 95% confidence interval.
ETHICAL JUSTIFICATION

This planned study is to be undertaken in stable vitiligo patients not responding to medical treatment. Informed consent will be obtained from all patients and it will be explained them that surgical treatment is for the existing lesions of vitiligo and new lesions of vitiligo may still appear in future. Patients will not be denied of medical treatment and only those who failed medical treatment will be chosen for surgery. To detect any adverse effect at the earliest, periodic visits of the patient along with active intervention are planned at regular intervals. All necessary steps would be undertaken to ensure safety and convenience to the patients during entire study period. Moreover, the patients who may deny participating would be excluded from study without asking any reason thereof.

Nowadays surgical modalities have become treatment of choice for stable vitiligo not responding to medical treatment. NCES have been shown to be safe and efficacious in the repigmentation of stable vitiligo patches.

By this study our aim is to compare transplantation of autologous non cultured epidermal cell suspension vs its combination with non-cultured dermal cell suspension in the treatment of stable vitiligo. By combining non-cultured dermal cell suspension (NDCS) with NCES will increase the efficiency of repigmentation in stable vitiligo. Non cultured dermal cell suspension is also a safe procedure as a single skin biopsy with a 4.0 mm punch will be taken from the donor area. These surgical modalities are affordable to most of the patients and impart not much financial burden. This in the long run will be helpful to the patients in terms of cost-effectiveness and ultimately the outcome. Thus, the cost and risk of undergoing an invasive procedure by the patient can be ethically justified.
BIBLIOGRAPHY


CONSENT PROFORMA

A COMPARATIVE STUDY BETWEEN TRANSPLANTATION OF AUTOLOGUS NON-CULTURED EPIDERMAL CELL SUSPENSION AND COMBINATION OF AUTOLOGUS NON-CULTURED EPIDERMAL CELL SUSPENSION AND NON-CULTURED DERMAL CELL SUSPENSION IN STABLE VITILIGO.

Name of the participant: ____________________________________________

Name of the Principal (Co-) Physician: ______________________________

Name of the Institution: ____________________________________________

I, age CR. No. exercising my free power of choice, hereby give my consent to be included as a subject in “a comparative study between transplantation of autologous non-cultured epidermal cell suspension v/s transplantation of autologous non-cultured epidermal cell suspension and non-cultured dermal cell suspension in combination - a novel surgical method in stable vitiligo.”

I have been explained in a language understandable to me, the nature of the treatment, its expected benefits and possible side effects and I am willing to undergo any necessary investigations.

I have been informed that for academic and scientific purposes, the white patches will be photographed before and after the study.

I will allow the use of my photographs for presentation and publication purposes with the understanding that I will never be identified by name.

I hereby give permission to the investigators to release the information obtained from me, as a result of participation in this study, to the sponsors, regulatory authorities, government agencies, and ethics committee. I understand that they may inspect my original records.

I am aware that I will have to come to PGIMER, Chandigarh for follow up at least 5 times over a period of 24 weeks (weeks 1, 4, 8, 16 and 24) for the proper conduct of study.

I am also aware of my right to opt out of the study any time during the course trial without having to give the reason for doing so.

My signature on this form indicates that I:

- Have carefully read and understood the information provided in this form
- Have been explained the nature of this study and give my consent for inclusion in the study.

Name and signature of patient Name and signature of physician
APPENDIX II
PATIENT INFORMATION SHEET

PROTOCOL NO:

Physician: Dr. Vishal Thakur, Dr. Davinder Parsad, Dr. M. Sendhil Kumaran

Name of Participant:

TITLE “A COMPARATIVE STUDY BETWEEN TRANSPLANTATION OF AUTOLOGOUS NON-CULTURED EPIDERMAL CELL SUSPENSION AND COMBINATION OF AUTOLOGOUS NON-CULTURED EPIDERMAL CELL SUSPENSION AND NON-CULTURED DERMAL CELL SUSPENSION IN STABLE VITILIGO.”

You are invited to take part in this research study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

You are being asked to participate in this study being conducted in PGIMER Chandigarh because you satisfy our eligibility criteria which are:

1. Age more than 18 years
2. No contraindication to the use of the agents to be used in the study, which means absence of any disease or condition likely to get worsened by the drugs under study like disease unstable Vitiligo, history of Koebnerisation, history of hypertrophic scars or keloidal tendency, bleeding disorders.

You will be one of the 40 patients we plan to recruit in this study. You will be treated with one of the two modalities of treatment on the patch of Vitiligo.

What is the purpose of research?

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Now a day surgical modalities have become treatment of choice for stable vitiligo not responding to medical treatment. Autologous non-cultured epidermal cell suspension has been shown to be safe and efficacious in the repigmentation of stable vitiligo patches. We are combining dermal cell suspension with NCES to increase the efficiency of repigmentation. But this method is associated with significant pain during anesthesia and sometimes with scarring.

We have obtained permission from the Institutional Ethics Committee for conducting this study.

The study design

All the Vitiligo patches in the study will be divided into two groups. You will be receiving one of the interventions on patches. The patches will be randomly assigned to receive any one of the treatment.

Randomization improves the scientific quality of research.

Study Procedures

Following abrasion of the superficial areas of the skin this cell suspension is transplanted into vitiligo area. Melanocytes in the cell suspension home into the dermabraded area and causes pigmentation in 2-6 months. The surgery and preparation of cell suspension is undertaken in strict aseptic precautions to minimize the chances of infection.

Once you are enrolled in the study, you will be required to follow the instructions.

You will be told about your visit schedules and you will have to report to the hospital.

The planned scheduled visits involve visits at 4, 8, 16, 24 weeks.

You will be required to visit the hospital 4 times during study.

You are not allowed to take any medications other than the ones prescribed by your investigator. If you need to take some treatment (drug/physiotherapy/other) you must consult your investigator before taking that treatment. In addition, if you notice any physical or mental change(s), you must contact the persons listed at the end of the document.
You may have to come to the hospital for examination and investigations apart from your scheduled visits, if required.

**Possible risks to you**

Some of the common adverse effects of surgery include complications / side effects

- Infection
- Milia
- Scarring
- Rejection
- Donor site
- Hypopigmentation
- Hyperpigmentation

**Possible benefits to you**

You are not expected to get any benefit from being on this research study, other than the treatment benefit and free investigations/tests.

**Compensation**

You will not receive any compensation or travel grant.

**Possible benefits to other people**

This study will be helpful in future to provide safe, cost effective and easy available treatment for stable vitiligo in future.

If you do not wish to participate, you have the alternative of getting the standard treatment for your condition.

**Cost to the participant**

You will not be required to pay for the medications or lab tests.

In case of any adverse event occurring due to the study medications, you will be provided free treatment at our Institute and proper referral if necessary.

**What should you do in case of injury or a medical problem during this research study?**

Your safety is the prime concern of the research. If you are injured or have a medical problem as a result of being in this study, you should contact one of the people listed at the end of the consent form. You will be provided the required care/treatment. You will be entitled to your legal rights besides this.
Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information by signing this document; you will be allowing the research team investigators, other study personnel, sponsors, institutional ethics committee and any person or agency required by law like the Drug Controller General of India to view your data, if required.

The results of clinical tests and therapy performed as part of this research may be included in your medical record. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity. How will your decision to not participate in the study affect you? Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. Your doctor will still take care of you and you will not lose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start?

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. However, it is advisable that you talk to the research team prior to stopping the treatment. You may be advised about how best to stop the treatment safely. If you withdraw, you may be asked to undergo some additional tests to which you may or may not agree. Though advisable that you give the investigators the reason for withdrawing, it is not mandatory.

Can the investigator take you off the study?

You may be taken off the study without your consent if you do not follow instructions of the investigators or the research team or if the investigator thinks that further participation may cause you harm.

Right to new information

If the research team gets any new information during this research study that may affect your decision to continue participating in the study, or may raise some doubts, you will be told about that information.
Contact persons

For further information / questions, you can contact us at the following address:

Principal Physician:
Dr. Vishal Thakur. Ph: 9878576002
Department of Dermatology
PGIMER Chandigarh

Co-physician:
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Professor
Associate Professor
Department of Dermatology
Department of Dermatology
PGIMER Chandigarh
PGIMER Chandigarh
APPENDIX III
DATA RECORD SHEET

Name:             Age/ Sex:                 CR. No. :
Occupation:       Pt. No
Address:
Chief complaints:
Total duration:
Site of onset:
Progression of disease:
Precipitating factors:
Present status: stable/ unstable
Koebnerisation: present/ absent
History of past illness: H/o similar disease/ autoimmune disorder (pernicious anemia, hyperthyroidism, hypothyroidism, alopecia areata, autoimmune hemolytic anemia and myasthenia gravis)/ atopy / diabetes/ hypertension/ tuberculosis / photosensitivity / any other disease.
Treatment history:

<table>
<thead>
<tr>
<th>Treatment taken</th>
<th>Response</th>
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1. Topical

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2. Systemic

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3. Phototherapy

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4. Indigenous

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**Personal history:**

1263 Smoking
1265 Alcohol
1266 Addictions

**Family history**

1269 **GENERAL PHYSICAL EXAMINATION:**
1270 Pulse:       BP:     Weight:
1271 Pallor:      Edema:   Clubbing:
1272 Cyanosis:    Icterus:  Lymphadenopathy:

**SYSTEMIC EXAMINATION:**
1275 CVS
1276 RS
1278 P/A

**CUTANEOUS EXAMINATION:**
1283 % BSA involved:
1284 Areas affected:
1285 Head and neck/ trunk/ upper limb/ lower limb/ hands/ feet/ mucosae
1286 Leucotrichia: Present/Absent
1287 Mucosal involvement: Present/Absent

42
INVESTIGATIONS:

<table>
<thead>
<tr>
<th>Investigations</th>
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<tbody>
<tr>
<td>Hb</td>
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<tr>
<td>Total count</td>
<td>N</td>
<td>L</td>
<td>E</td>
<td>M</td>
<td>B</td>
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<tr>
<td>Differential Count</td>
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<td>Platelets</td>
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<td>RBS</td>
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<td>Bleeding time</td>
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<td>Clotting time</td>
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<td>HBsAg</td>
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<tr>
<td>HIV</td>
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Donor site:

Size of the split thickness graft:

Site of treated area:

- a) NCES
- b) NDCS + NCES

Size of treated area:

- a) NCES
- b) NDCS + NCES
**CLINICAL EVALUATION:**

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Day 8</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>16 weeks</th>
<th>24 weeks</th>
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<tbody>
<tr>
<td>Extent of pigmentation</td>
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<tr>
<td>&lt;25%</td>
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<td>26-50%</td>
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<td>51-75%</td>
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<td>75-90%</td>
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<td>&gt;90%</td>
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<td>Color match of grafted area with the normal skin</td>
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<tr>
<td>- somewhat darker</td>
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<td>- somewhat lighter</td>
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<td>- same</td>
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<td>Pattern of repigmentation</td>
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<tr>
<td>- Diffuse</td>
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<td>- Perifollicular</td>
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<td>- Migrating from the borders</td>
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<td>Complications / side effects</td>
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<tr>
<td>a. <strong>Recipient site</strong></td>
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<td>- infection</td>
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<td>- rejection</td>
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<td>b. <strong>Donor site</strong></td>
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<td>- Infection</td>
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<td>- Milia</td>
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<tr>
<td>- Scarring</td>
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<tr>
<td>- Hypopigmentation</td>
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<td>- Hyperpigmentation</td>
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<td>Evaluation</td>
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<td>Repigmentation at 24 weeks</td>
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APPENDIX IV

PATIENT SATISFACTION QUESTIONNAIRE

1. Patients’ Global Assessment:

1) Grade the change in pigmentation in the transplanted area. (0 to 10)

2) Are you satisfied with the obtained result? (0 to 10)

3) Do you find the treatment worthwhile? (0 to 10)

4) Would you choose this treatment again? (yes / no)

For question 1, ‘0’ means ‘much worse’ and ‘10’ means ‘much improved’.
For question 2 and 3, ‘0’ means ‘not at all’ and ‘10’ means ‘very much’.