Analysis of grafting techniques

Procedure: In this method a split-thickness skin graft is harvested from the donor site, either thigh or buttocks. It is then meshed either manually or in an Ampligreffe or any other suitable meshing apparatus.[40, 41] Meshing of the graft causes an expansion in its size to 4 or 6 times its original one. The meshed graft is then applied on the dermabraded recipient skin and bandaged as in any other form of tissue grafting. The main advantage of this technique is that the graft can take care of a vitiligo lesion that is 4-6 times that of its original size. Additionally meshing allows the graft to be applied on areas over joints and other areas with difficult contours.

This technique is increasingly being practiced in India and is a simple, cost-effective procedure with good cosmetic results.

Principle : In this technique of vitiligo, grafting the split-thickness or ultra-thin skin graft is cut or smashed into very small pieces and applied to the dermabraded recipient skin.[42, 43]

The donor: recipient ration is approximately 1: 10.

Procedure: A split-thickness or ultra-thin skin graft is first taken from the donor area preferably thigh or buttocks. It is then smashed/cut into thin pieces. The cutting process is continued till the graft is converted into a uniform mesh or paste. This mesh is then mixed with either hyaluronic acid or antibiotic ointments and is then spread evenly over the dermabraded recipient area as in any other form of tissue grafting.[42] The recipient area is then covered with a collagen dressing and this dressing is removed after 7-8 days. The advantage of this method is that a relatively larger area can be covered by a small sized graft. The results are almost similar to those

achieved with non-culture epidermal cell suspension (NCES) technique. Additionally, no expensive reagents or laboratory support is required as in NCES procedure. Some difficult to treat areas like the hairy skin, the joints and bony prominences can also be treated with this technique. The disadvantage is that it is difficult to spread the grafted tissue evenly on to the recipient area.

Figures 34. 7 and 34. 8 shows good results with smash graft on joints.

Three main cellular grafting techniques are described in the world literature. These are non-culture epidermal cell suspension technique, cultured melanocyte transplant and non-culture follicular suspension technique

Synonyms: non culture melanocyte transplant, non-culture melanocyte-keratinocyte cell transplant (NCCT), basal cell suspension technique.

Principle: The different cellular components of a STSG are separated and a suspension is prepared out of these cellular components. The suspension contains epidermal keratinocytes and melanocytes' this is applied on to a dermabraded recipient area. The donor: recipient ration is 1: 10.

In this cellular grafting procedure a split-thickness skin graft is harvested from a suitable donor area and this is treated with 5 ml of Trypsin-EDTA solution for about 45-60 minutes in an incubator at 37°C. This step separates the cells of the epidermis from the underlying dermis. The next step is the neutralization of Trypsin which is achieved either by using 2 ml of 0. 5% trypsin inhibitor solution or washing the graft with DMEM or any other suitable medium repeatedly. The treated graft is then taken in a petridish

with the epidermal side downwards and the dermal cells are teased out of the graft with forceps. The overlying dermal tissue is discarded and the solution with the cellular component is centrifuged for about 10 minutes at the end of which the cells pellet are seen suspended at the bottom of the centrifuge tube and the epidermal pieces are floating at the top, which is discarded. The cell pellet is then mixed with a about 0. 8 ml of Dulbecco's Modified Eagle's Medium (DMEM) medium (also called M2 melanocyte medium) and the suspension thus obtained is transferred to a 1 ml tuberculin syringe. After the recipient bed is created, the cell suspension is spread thinly and evenly with a spatula on to the dermabraded recipient skin after removing needle. The area is then dressed with collagen dressingto hold the transplanted cells and the dressings are removed after 1 week. As an alternative to the DMEM medium, patient's own serum or hyaluronic acid can be used as it improves the viscosity of the cellular suspension.[53]

This technique requires expensive laboratory equipment and is usually practiced only at research centres.

Principle: It replenishes melanocytes selectively by creating a melanocyte rich suspension. The donor: recipient ration can be as high as 1: 100

Procedure : The epidermis undergoes trypsinization and the melanocytes and keratinocytes are dissociated. The melanocytes are further seeded in a melanocyte medium containing growth factors and cultured over 15 to 30 days. The cultured melanocytes (free suspension or epidermal sheets) are then transplanted on to dermabraded recipient skin.

This is a novel cellular graft technique by using the hair follicle outer root sheath cell for transplant. Cosmetic results obtained with this procedure are almost similar to those seen with NCES technique.

Principle: This is another cellular grafting technique wherein the melanocytes present in the hair follicles are utilized in repigmenting resistant vitiligo. The outer root sheath of the hair follicle is a rich source of inactive melanocyte. Theseinactive melanocytes function as stem cells and hence can be harvested and used in vitiligo.

Procedure: The procedure is almost similar to NCES technique but here extracted hair follicles are used instead of a split-thickness skin graft. The hair follicles can be extracted by the follicular unit extraction (FUE) method. The hair follicle is decontaminated by washing with antibiotics. Enzymatic dissociation of ORS is done by addition of trypsin and incubated at 37 C. Mechanical disruption of the ORS is done by vortexing and the ORS cells are separated from the hair shaft by a cell strainer. The dissociated cells are examined microscopically for viability and the cell suspension can be transplanted onto the prepared recipient site. This technique is in a nascent stage, however it has shown good repigmentation comparable to NCES .

The surgical techniques discussed above have various advantages and disadvantages. (Table 34. 3) [50, 58]

Table 34. 3: Advantages and Disadvantages of grafting techniques

1. Sur Advantages Disadvantages

   gic

al Tec hni que s		
MPG	2. Easiest of all the grafting procedures. 3. Performed on difficult to treat sites like the finger tips and toes, areolae or palms and soles. 4. Lesions with geographic borders can be managed	6. Adverse effects include cobble-stoning, polka dot appearance, perigraft halo and color mismatch. [17, 21] 7. The procedure is not suitable for cosmetically important areas like the face.

	5. Perigraft halo that remains after split-thickness grafting or smash can be managed with minigraftin g. [23]	
STSG	1. Split-thickness skin grafting has the highest success rate among all the techniques of tissue as well as cellular grafting. 24, 25 2. A relatively large area can be treated in as single session.	8. The size of the donor graft required is equal to or even more than the area of the recipient area to be treated. 9. Taking a thin graft of uniform thickness with minimal dermal tissue needs a

		lot of experience and training. 10. Thicker grafts can lead to scarring both at the donor and recipient sites. 11. Milia formation, tire patch or stuck-on appearance, cosmetic mismatch of pigmentation, perigraft halo of depigmentation, hyperpigmentation of the graft can be seen especially in dark skinned individuals.[27, 28]
	3. The grafted areas achieve a rapid repigmentation after the procedure	
UTSG	12. The cosmetic	15. Perigraft halo and

effect achieved is excellent

13. There is no scarring at the donor site and repeated grafts can be taken from a single donor site on multiple occasions.

14. There is no milia formation and no chances of scarring at the recipient site.

hyperpigmentation in dark individuals common

16. Good surgical skills and expertise needed

17. Large donor area required

SBG	18. The cosmetic result achieved is usually excellent as only the epidermis is grafted without any underlying dermal tissue. 19. Difficult areas like the lips, the areolae can be grafted satisfactorily with this type of grafting. [34, 35]	20. The time taken for the blisters to form is too long and becomes really inconvenient for the patient. 21. Time taken to perform the procedure is also accordingly longer. 22. Patients usually complain of pain once the blister is formed on the donor area. 23. If blister does not form, one may have to switch to other techniques
Smash	24. Relati	27. Difficult to

	vely larger area can be covered by a small sized graft (1: 10 donor recipient ratio)	spread the grafted tissue evenly on to the recipient area.
	25. no expensive reagents or laboratory support is required 26. Can be used on hairy areas or joints	28. Perigraft halo and hyperpigmentation in dark individuals common
NCES	29. Relati vely larger area can be treated in a single session and with a	31. Expensive 32. Storage facilities for reagents/ incubator needed 33. Time

	much smaller size of donor graft.[44-52] (1: 10 donor recipient ratio)	
	30. In addition, the repigmentation achieved matches the recipient skin closely leading to a better cosmetic result.	consuming 34. Involves a learning curve for the operating surgeon. [49]
CMT	35. A large area can be	36. Expensive laboratory support and set-

	up required.
	37. The cost is high
treated in a single session (1: 100 donor recipient ratio)	38. There is a risk of mutagenicity, especially with use of culture media, such as tetradecanoylphorbol acetate (TPA).

In addition to the grafting techniques, various other methods also have been used including tattooing, excision with primary closure and therapeutic wounding.

Tattooing: In tattooing, artificial pigments are introduced into the depigmented lesions for permanent camouflage. This can be done with a hand held pin vise or an electrical device.

Excision with primary closure: The depigmented areas are removed and the wound is sutured; this technique is useful for small vitiligo lesions.

Therapeutic wounding: Wounding of the lesions stimulate the melanocytes from the periphery of the lesion as well as from the hair follicles which migrates and re-pigments the lesion. Various modalities which are used for

therapeutic wounding include dermabrasion, laser ablation, cryosurgery, needling, and local application of phenol or trichloroacetic acid. [59]

Besides the technical aspects of various procedures (detailed in the earlier section), the other important practical aspects include:

- Choice of technique (Which procedure should be performed, where and why?)
- General pre and post-operative considerations,
- Role of phototherapy,
- Complications and their management.

The choice of technique depends on the dermatosurgeons' skills, experience and the availability of facilities in the dermatosurgery set up. However, the factors which are considered while planning a vitiligo surgery also determine the choice of surgical technique employed. Based on these factors, an algorithmic approach to choosing an appropriate surgical technique in stable vitiligo can be evolved. (Illustration 34. 3

In cases of pediatric segmental vitiligo, NCES is suitable. It can be followed by phototherapy for faster results. Tissue grafting techniques are usually not recommended due to constraints of immobility in this population subset. In adult, population both tissue grafting and cellular techniques can be employed based on the site and total area of depigmentation.

Segmental and focal vitiligo are most amenable to surgical treatment. Amongst the non- segmental type, lesions located on the glabrous skin are suitable for surgical intervention. The acromucosal types are usually not responsive.

The location of the lesion plays an important role in determining the choice of grafting technique. (Table 34. 4)

Table 34. 4: Anatomical location and choice of grafting technique

Anatomical Location	Choice of grafting technique
Eyelids	SBG, NCES
Lips	SBG, NCES
Genitals	SBG, NCES, UTSG
Acral/ palms, soles	MPG
Areola	STSG, SBG
Hairy areas	STSG, Smash
Joints	Smash

Small areas (1-4 cm) – All techniques work well in vitiligo involving small areas (1-4 cm) and technique should be chosen based on anatomical location and cost to the patient. In cases of large areas, NCES, smash or UTSG is preferred.

The general pre and post-operative aspects have been outlined in Box 34. 6. Specific pre and post-operative procedural aspects have been dealt with in the description of procedures.

Box 34. 6: General pre and post-operative aspects

Pre- operative aspects:

Counselling, Photographs and Informed consent

Serological investigations: Complete blood count, Blood sugars, Bleeding time, Clotting time , Prothrombin time, screening for HIV and Hepatitis B

Shaving of donor and recipient area, pre medication (antibiotics)

Proper marking of the donor and recipient area, assess the approximate size or number of grafts required

Post- operative aspects

Proper dressing/ Immobilization

Antibiotics/ Anti-inflammatory medications

Check recipient site after 1-3 days

Change dressing at donor and recipient site after 8-10 days

Phototherapy to be started after 1-2 weeks

depending on response

Topical immunomodulators/ Topical steroids or oral immunosuppressants considered later if there is poor repigmentation or uptake of graft

The role of phototherapy (narrow band UVB) post vitiligo surgery has been well demonstrated with various tissue grafting and cellular techniques. Phototherapy can be started within 1-2 weeks following surgery. Concurrent use of narrow band UVB exerts a stimulatory and proliferative effect on the grafted melanocytes; thus post- surgery phototherapy enhances and accelerates the repigmentation.

In cases of UVB therapy which is initiated after split thickness skin grafting, repigmentation can occur within two weeks and a better colour match at the recipient site is seen. Repigmentation with cellular techniques has been observed within 3-4 weeks after surgery and can progress till 6 months and this can be enhanced with phototherapy. Use of excimer laser has also shown good results post punch grafting.