Cellular Melanocyte Transfer in Stable Vitiligo: Comparative Insights into Trypsinized and Non-Trypsinized Non-Cultured Epidermal Grafting Techniques

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Abstract

Background: Vitiligo is a chronic depigmenting disorder characterized by the selective loss of functional melanocytes, leading to significant psychosocial and quality-of-life impairment. While medical therapies such as topical immunomodulators and phototherapy remain first-line options, a substantial subset of patients with stable vitiligo exhibit inadequate or incomplete responses. For these patients, surgical modalities—particularly cellular melanocyte transfer—have emerged as effective therapeutic alternatives. Among these, non-cultured epidermal cell grafting techniques have gained prominence due to their ability to treat larger recipient areas in a single session with favorable cosmetic outcomes. Non-cultured epidermal cell grafting can be broadly categorized into trypsinized and non-trypsinized approaches. Trypsinized non-cultured epidermal cell suspension, often referred to as melanocyte–keratinocyte transplantation, relies on enzymatic separation of epidermal cells to obtain a cell suspension enriched with melanocytes and keratinocytes. In contrast, non-trypsinized techniques utilize mechanical or minimally enzymatic methods to harvest epidermal cells, aiming to simplify the procedure, reduce cost, and minimize laboratory dependence. Despite the increasing clinical adoption of both methods, there remains ongoing debate regarding their relative efficacy, safety, technical complexity, and reproducibility. The aim of this review is to critically analyze and compare trypsinized versus non-trypsinized non-cultured epidermal grafting techniques in the treatment of stable vitiligo, with emphasis on biological rationale, patient selection, procedural methodology, clinical outcomes, and adverse effects. Special attention is given to site-specific responses, including cosmetically sensitive and functionally complex areas such as the face, acral regions, and genital skin. Furthermore, the review explores practical considerations that influence technique selection in routine dermatologic practice. In conclusion, both trypsinized and non-trypsinized non-cultured epidermal cell grafting techniques represent valuable surgical options for stable vitiligo, each with distinct advantages and limitations. A nuanced understanding of their comparative characteristics can assist clinicians in tailoring treatment strategies to individual patient needs. Future well-designed comparative studies and standardized outcome measures are essential to optimize cellular grafting protocols and to further refine the role of these techniques in vitiligo management.

Keywords: *Trypsinized , Non-Trypsinized ,Non-Cultured, Epidermal Grafting, Stable Vitiligo*

Introduction

Vitiligo is an acquired chronic pigmentary disorder characterized by progressive loss of functional melanocytes from the epidermis, resulting in well-demarcated depigmented macules and patches. It affects approximately 0.5–2% of the global population and occurs irrespective of age, sex, or ethnicity. Beyond its cutaneous manifestations, vitiligo is associated with profound psychosocial distress, impaired quality of life, and stigmatization, particularly when lesions involve exposed or cosmetically sensitive areas such as the face, hands, and genital region. Despite extensive research, vitiligo remains a therapeutically challenging condition, owing to its multifactorial pathogenesis involving autoimmune, oxidative, neural, and genetic mechanisms [1].

Conventional management strategies for vitiligo include topical corticosteroids, topical calcineurin inhibitors, and various forms of phototherapy, most notably narrowband ultraviolet B (NB-UVB). These modalities aim to suppress immune-mediated melanocyte destruction and stimulate residual melanocyte proliferation and migration. However, a significant proportion of patients demonstrate partial, unsatisfactory, or site-dependent responses, particularly in long-standing lesions and acral or mucosal areas. In patients with disease stability—defined by absence of new lesions, lack of progression of existing lesions, and no recent Koebner phenomenon—surgical interventions have become an important therapeutic option [2].

Surgical treatment of vitiligo is based on the principle of replacing absent melanocytes in depigmented skin by transferring functional melanocytes from normally pigmented donor sites. Over the past few decades, several surgical techniques have been developed, broadly classified into tissue grafts and cellular grafts. Cellular techniques, especially non-cultured epidermal cell grafting, have gained increasing popularity due to their ability to cover larger recipient areas with relatively small donor skin, superior color matching, and reduced donor-site morbidity compared with conventional tissue grafts [3].

Non-cultured epidermal cell grafting techniques can be further subdivided into trypsinized and non-trypsinized methods based on the mode of epidermal cell separation. Trypsinized non-cultured epidermal cell suspension involves enzymatic digestion of donor epidermis using trypsin to yield a suspension rich in melanocytes and keratinocytes. This technique is supported by robust experimental rationale and has been widely adopted in specialized centers. Conversely, non-trypsinized techniques employ mechanical or simplified separation methods, aiming to reduce procedural complexity, cost, and reliance on laboratory infrastructure while maintaining clinical efficacy [4].

Despite growing clinical experience with both approaches, there is no clear consensus regarding their comparative superiority. Published studies vary widely in methodology, outcome measures, follow-up duration, and patient selection criteria. Moreover, head-to-head comparative data remain limited, and important questions persist regarding cell viability, repigmentation quality, complication profiles, and long-term stability of results. These uncertainties represent a significant research gap in the surgical management of stable vitiligo [5].

The aim of this review is to provide a comprehensive and critical comparison of trypsinized and non-trypsinized non-cultured epidermal grafting techniques in stable vitiligo. By synthesizing available evidence on biological rationale, technical considerations, clinical outcomes, and safety profiles, this review seeks to guide clinicians in selecting the most appropriate cellular grafting strategy and to identify areas requiring further investigation.

Conceptual and Biological Rationale of Cellular Melanocyte Transfer

Repigmentation in vitiligo fundamentally depends on the presence, survival, and functional integration of melanocytes within the epidermis. In vitiliginous skin, melanocytes are either absent or present in a dysfunctional state, largely due to immune-mediated destruction, oxidative stress, and impaired melanocyte–keratinocyte interactions. The rationale for cellular melanocyte transfer is therefore based on replenishing depigmented skin with viable, functional melanocytes capable of melanin synthesis and

transfer to surrounding keratinocytes, ultimately restoring normal pigmentation [6].

Melanocytes function as part of the melanocyte–keratinocyte unit, a tightly regulated epidermal system in which one melanocyte supplies melanin to approximately 30–40 keratinocytes. This functional unit is critical for uniform pigmentation and photoprotection. Successful repigmentation following cellular grafting depends not only on melanocyte survival but also on effective interaction with recipient-site keratinocytes, adequate adhesion to the basement membrane, and responsiveness to local growth factors and cytokines [7]. Keratinocytes play a supportive role by secreting melanogenic factors such as stem cell factor, basic fibroblast growth factor, endothelin-1, and α-melanocyte–stimulating hormone, all of which promote melanocyte proliferation and dendricity.

Trypsinized non-cultured epidermal cell suspension techniques are biologically grounded in the selective enzymatic separation of epidermal cells from donor skin. Trypsin digestion disrupts intercellular adhesion molecules, allowing dissociation of melanocytes and keratinocytes into a single-cell suspension. This approach facilitates relatively uniform distribution of melanocytes over the recipient area and allows treatment of large lesions using a small donor-to-recipient ratio. Experimental studies have demonstrated that trypsinization preserves melanocyte viability and functional capacity when exposure time and temperature are carefully controlled [8].

In contrast, non-trypsinized non-cultured epidermal grafting techniques rely primarily on mechanical disruption or minimal enzymatic assistance to harvest epidermal cells. The biological premise of these methods is that epidermal fragments or cell clusters containing intact melanocyte–keratinocyte units may enhance cell survival and engraftment by preserving intercellular communication and extracellular matrix components. Proponents of non-trypsinized techniques argue that reduced enzymatic exposure may decrease cellular stress and apoptosis, potentially improving melanocyte survival, especially in resource-limited settings [9].

Following transplantation, repigmentation occurs through a combination of melanocyte proliferation, migration, and melanin transfer within the recipient epidermis. Early perifollicular pigmentation is commonly observed, suggesting that transplanted melanocytes may interact with residual follicular melanocyte reservoirs or that hair follicles serve as supportive microenvironments for melanocyte expansion. Adjunctive phototherapy, particularly NB-UVB, further enhances melanocyte activity by stimulating melanogenesis, promoting cell migration, and modulating local immune responses [10].

Overall, the biological rationale for both trypsinized and non-trypsinized cellular grafting techniques is well established. However, differences in cell preparation methods may influence melanocyte yield, viability, distribution, and integration, which in turn can affect clinical outcomes. Understanding these biological underpinnings is essential for interpreting comparative efficacy data and optimizing surgical strategies in stable vitiligo.

Patient Selection and Stability Assessment

Appropriate patient selection is a critical determinant of success in surgical management of vitiligo, particularly for cellular melanocyte transfer techniques. Among all eligibility criteria, **disease stability** is universally regarded as the most important prerequisite. Surgical interventions performed in unstable or active vitiligo carry a high risk of graft failure, disease recurrence, and Koebnerization, ultimately leading to suboptimal outcomes and patient dissatisfaction [11].

Stability in vitiligo is classically defined as the absence of new lesions, no enlargement of existing lesions, and lack of Koebner phenomenon for a minimum period—most commonly 6 to 12 months. However, there is no single universally accepted definition, and various studies employ differing stability durations and assessment tools. Clinical evaluation remains the cornerstone, supplemented by patient history and serial photographic documentation. Scoring systems such as the Vitiligo Disease Activity (VIDA) score have been used to semi-quantitatively assess disease inactivity, with VIDA scores of 0 or −1 generally considered suitable for surgical intervention [12].

In addition to temporal stability, **lesion morphology and site** play an important role in patient selection. Facial and cervical lesions consistently demonstrate the highest repigmentation rates following cellular

grafting, attributed to rich vascular supply, higher density of hair follicles, and favorable local growth factor milieu. In contrast, acral areas, including fingers, toes, and periungual skin, show relatively poorer outcomes due to reduced follicular reservoirs and mechanical stress. Genital and mucosal vitiligo—areas of particular relevance from a venereology and andrology perspective—often respond well when disease stability is ensured, although meticulous technique and immobilization are required [13].

Patient-related factors such as age, skin phototype, disease duration, and prior treatment response should also be considered. While both pediatric and adult patients can benefit from cellular grafting, careful counseling is essential in younger individuals due to evolving disease patterns. Longer disease duration and segmental vitiligo are generally associated with better surgical outcomes, reflecting inherent disease stability. Additionally, patients with a history of poor response to medical therapy but stable disease are particularly good candidates for surgical approaches [14].

Donor-site selection is another critical aspect of patient evaluation. Common donor areas include the lateral thigh, buttocks, or lower abdomen, chosen for color match, ease of concealment, and adequate epidermal thickness. The donor-to-recipient area ratio varies depending on the technique used, with trypsinized methods typically allowing higher expansion ratios compared to non-trypsinized approaches. Patients must be counseled regarding potential donor-site morbidity, including transient pigmentation changes, scarring, or textural alteration [15].

Finally, psychological readiness and realistic expectation setting are essential components of preoperative assessment. Vitiligo has a profound psychosocial impact, and surgical interventions should be undertaken only after thorough counseling regarding expected outcomes, need for adjunctive phototherapy, time to visible repigmentation, and the possibility of incomplete or patchy results. Comprehensive patient selection that integrates clinical stability, lesion characteristics, anatomical considerations, and patient expectations forms the foundation for successful cellular melanocyte transfer in stable vitiligo.

Techniques Overview: Trypsinized and Non-Trypsinized Non-Cultured Epidermal Grafting

Non-cultured epidermal cellular grafting is designed to deliver viable melanocytes (with supportive keratinocytes) from a normally pigmented donor site into a depigmented recipient area after superficial epidermal removal. The “non-cultured” concept avoids time-consuming cell culture while still enabling treatment of relatively large lesions from a small donor area. Over time, multiple procedural refinements have focused on improving cell yield, simplifying processing, enhancing cell adherence to the recipient bed, and standardizing recipient-site preparation so that repigmentation is more uniform and predictable. Contemporary technique selection often hinges on the balance between laboratory dependence (enzymatic separation) versus procedural simplicity (mechanical/non-trypsinized separation), as well as local expertise and infrastructure. [16,17]

Trypsinized non-cultured epidermal cell suspension (NCES / MKTP)

In trypsinized NCES (often described as the melanocyte–keratinocyte transplantation procedure), a thin split-thickness epidermal sample is harvested—commonly from the thigh or buttock—then incubated in trypsin (classically at controlled temperature and time) to separate the epidermis and dissociate cells. After trypsinization, the epidermis is gently scraped to release basal-layer cells, and the cellular material is centrifuged to obtain a concentrated pellet that is resuspended for application. The recipient area is prepared by dermabrasion or laser ablation to the level of pinpoint bleeding, then the suspension is evenly spread and covered with an appropriate dressing to maintain immobilization and a moist environment. Key technical variables that influence results include donor thickness, digestion conditions, centrifugation parameters, and uniformity of suspension distribution across the recipient site. [18,19]

A practical advantage of trypsinized NCES is the ability to achieve favorable expansion ratios (donor-to-recipient), making it attractive for larger lesions. Clinically, the technique is frequently paired with adjunct phototherapy (especially NB-UVB) postoperatively to enhance melanocyte activation, promote melanogenesis, and improve color match. However, enzymatic steps introduce dependencies:

temperature control, sterile handling, reagent availability, and staff familiar with cell-processing workflows. These requirements can become the limiting factor in low-resource settings, and they also contribute to inter-center variability in reported outcomes—an important consideration when comparing studies. [16,17,19]

Non-trypsinized non-cultured epidermal cell grafting (Jodhpur technique and related simplifications)

Non-trypsinized approaches were developed largely to reduce reliance on enzymatic digestion and laboratory infrastructure. The most widely cited non-trypsinized method is the **Jodhpur technique**, which uses autologous epidermal cells obtained without trypsinization—typically via mechanical separation/disruption of epidermis harvested from a donor area—followed by immediate application to the recipient bed. The core premise is procedural simplification: fewer reagents, fewer steps, and easier execution in outpatient or resource-limited contexts, while still achieving clinically meaningful repigmentation in stable vitiligo. In published descriptions, the harvested epidermal material is processed into a graftable cellular/epidermal preparation and placed over a properly prepared recipient site, then secured using an appropriate dressing or biological covering. [20]

Head-to-head comparisons between classic trypsinized NCES and non-trypsinized methods have begun to appear, reflecting real-world interest in whether simplification compromises outcomes. Comparative studies (including those evaluating NCES versus Jodhpur technique) commonly assess repigmentation percentage, color match, and adverse effects, often alongside adjunct NB-UVB protocols. Although results vary by study design, lesion site, and stability definition, this emerging literature is crucial because it directly addresses the practical clinical question: whether non-trypsinized grafting can deliver comparable results with lower cost and procedural complexity. [21,22]

“In vivo” and device-assisted simplifications

An additional trend is simplification of donor processing through techniques described as “in vivo preparation” of epidermal cell suspension and other minimally invasive donor-harvesting strategies, aiming to reduce donor-site morbidity and shorten procedural time. These approaches reflect the broader movement toward standardization and accessibility in vitiligo surgery: less dependence on lab-grade workflows while preserving key biological requirements—viable melanocytes, adequate cell delivery, and stable recipient-site conditions for engraftment. Collectively, these innovations underscore that technique choice is not purely about “trypsin versus no trypsin,” but also about reproducibility, training demands, cost, and the likelihood of consistent outcomes across different practice settings. [23]

Recipient-Site Preparation Modalities

Effective recipient-site preparation is the “make-or-break” step for both trypsinized and non-trypsinized non-cultured epidermal grafting, because transplanted melanocytes must rapidly adhere, survive, and expand on a uniformly de-epithelialized surface. The biological goal is controlled removal of the epidermis (and superficial papillary dermis exposure) to create a receptive wound bed with adequate plasma imbibition, minimal thermal injury, and minimal shearing forces during the early engraftment window. In practice, the ideal endpoint is a **uniform, superficial ablation**—often described clinically as reaching **pinpoint bleeding**—followed immediately by stable fixation and occlusion to prevent cell loss and desiccation. Multiple recipient-site modalities have been described, including mechanical dermabrasion, suction blistering, cryo-assisted methods, PUVA-related methods, and ablative lasers (CO_2 and Er:YAG), each with distinct trade-offs in precision, bleeding, thermal effects, and ease over irregular lesions. [24]

Mechanical dermabrasion

Mechanical dermabrasion remains widely used because it is inexpensive, familiar, and can achieve a reproducible endpoint in experienced hands. Its advantages include limited thermal damage and the potential benefit of controlled bleeding and growth-factor release in the dermabraded bed; however, it can be messy, operator-dependent, and challenging over curved or delicate anatomical regions. Excessive dermabrasion depth increases scarring risk and textural change, whereas insufficient

dermabrasion risks poor cell adherence and patchy take. From a practical standpoint, dermabrasion is often favored when laser access is limited, but outcomes rely heavily on consistent depth and meticulous immobilization—particularly in mobile areas (perioral, periocular, genital, and joints), where even minor friction can displace the cell layer. [25,26]

Ablative lasers (CO_2 and Er:YAG)

Ablative lasers have become prominent for recipient preparation because they can provide controlled, even de-epithelialization—especially valuable for irregularly shaped lesions and cosmetically sensitive sites. **Er:YAG** (with high water absorption and relatively limited thermal spread) is frequently used to create a clean ablation plane, while **CO_2** lasers can also be effective but may introduce more thermal injury depending on settings. Clinical comparative work in melanocyte–keratinocyte transplantation has evaluated **Er:YAG laser ablation versus mechanical dermabrasion** as recipient preparation, reflecting the real-world need to balance precision, adverse effects, and repigmentation quality. Other clinical comparisons support that CO_2-laser-assisted preparation can be similarly effective to mechanical dermabrasion, with differences in adverse-effect profiles influenced by technique and parameters. Overall, lasers can shorten procedure time, improve edge precision, and reduce inadvertent injury to surrounding normal skin when used appropriately. [27,28]

Fractional versus full ablative approaches and "controlled injury"

Within laser-based preparation, fractional CO_2 approaches have also been explored to reduce downtime and thermal burden while maintaining a receptive micro-ablated field that supports cell adherence. Comparative clinical studies suggest that both fractional and full ablative CO_2 strategies can be used for recipient-site preparation prior to cell suspension transfer, and that the choice may be shaped by lesion size, anatomic site, and operator comfort. Conceptually, the recipient bed must remain sufficiently de-epithelialized to accept melanocytes, yet not so inflamed or thermally injured that early melanocyte survival is compromised—an especially relevant consideration when comparing trypsinized suspensions (single-cell dominant) with non-trypsinized preparations (often containing cell clusters), which may differ in their tolerance to local wound-bed conditions. [29]

Suction blister–based preparation and delicate sites

Suction blister techniques occupy a unique space in vitiligo surgery: they can be used either for harvesting epidermal grafts or for preparing/handling recipient sites in ways that minimize deeper injury. In general, suction blister methods are considered useful for delicate areas and can reduce scarring risk when performed correctly, though they are time-consuming and technique-sensitive. Laser ablation (including CO_2 laser) has also been described as a recipient-site preparation method in the context of suction-blister epidermal grafting, with cited advantages in speed and precision for difficult lesion shapes. These options are particularly relevant in highly visible or functionally sensitive regions—such as periocular skin, lips, and genital skin—where even minor textural change is unacceptable, and where immobility is hard to maintain. [30,31]

Outcomes and Comparative Effectiveness

Clinical outcomes after cellular melanocyte transfer are typically reported as the **percentage of repigmentation** (often graded into <25%, 25–50%, 50–75%, and >75% or "excellent"), alongside **color match**, **pattern uniformity**, and **durability** at follow-up. Across published experiences with **trypsinized non-cultured epidermal cell suspension (NCES/MKTP)**, most series in carefully selected **stable vitiligo** report that a substantial proportion of treated patches achieve **good-to-excellent repigmentation**, particularly in facial and non-acral lesions. A representative example is the clinical evaluation of non-cultured melanocyte/keratinocyte cell suspension transplantation in stable vitiligo reported by Ramos and colleagues, supporting that meaningful repigmentation can be achieved with this approach when appropriate stability and technique are ensured. Variability across studies is largely driven by differences in stability definitions, lesion sites, recipient preparation, adjunct phototherapy, and outcome scoring systems. [32,33]

A consistent finding across surgical vitiligo literature is that **anatomical site strongly predicts response**. Facial and cervical lesions tend to respond best, while **acral sites** (fingers, toes, periungual areas) are among the most resistant—likely due to fewer follicular melanocyte reservoirs, mechanical friction, and comparatively poorer microvascular support. Segmental vitiligo (when stable) and localized non-segmental patches may respond favorably, whereas highly active generalized disease is less suitable. Reviews of surgical/cellular therapies emphasize that proper stability assessment and site selection are key to maximizing repigmentation rates and minimizing recurrence or loss of pigment over time. [33,34]

When directly comparing **trypsinized NCES** with **non-trypsinized (Jodhpur technique, JT)** approaches, the clinical question is whether procedural simplification compromises repigmentation quality. A comparative study by Saini and colleagues evaluated **NCES versus JT in stable vitiligo**, reporting that both methods can be effective, while also highlighting practical differences such as procedural steps, resource needs, and (in some reports) differences in repigmentation grades and/or ease of execution. Importantly, comparative studies often note that outcomes remain highly dependent on recipient-bed preparation, immobilization, and consistent postoperative phototherapy protocols—meaning "technique superiority" is difficult to declare without standardized designs and longer follow-up. [35]

Non-trypsinized methods have also been evaluated in broader surgical comparisons, reinforcing that JT can be a pragmatic option in stable disease. For example, Lamoria and colleagues compared **follicular unit transplantation** with **autologous non-cultured non-trypsinized epidermal cells grafting (JT)** in stable vitiligo, contributing to the evidence base that non-trypsinized epidermal cell grafting can deliver clinically meaningful repigmentation in appropriately selected patients. Additionally, Kachhawa and colleagues described a **simplified non-cultured non-trypsinized epidermal cell grafting** approach (JT) with encouraging outcomes, supporting its utility where enzymatic processing is difficult to implement. Collectively, these studies position non-trypsinized grafting as a relevant alternative rather than merely a "second-best" option, particularly in low-resource or high-throughput settings. [36,37]

From a patient-centered standpoint, "success" extends beyond pigment percentage to include **color match**, **border blending**, and **patient-reported satisfaction**—especially in cosmetically and functionally sensitive areas (face, perioral region, genital skin). Postoperative **NB-UVB** is commonly used to enhance melanocyte activation and improve uniformity, and it may also help stabilize gains over time by modulating local immune activity. Despite generally favorable outcomes in stable disease, durability can be threatened by disease reactivation, ongoing Koebner phenomenon, or inadequate stabilization strategies—underscoring why careful counseling, stability confirmation, and structured postoperative follow-up remain essential parts of outcome optimization. [33,34]

Safety and Complications

Both trypsinized and non-trypsinized non-cultured epidermal grafting are generally considered **safe when performed in stable vitiligo under sterile conditions**, but adverse events can occur at **both the recipient and donor sites**. Recipient-site complications most often include **pain, erythema, edema, transient oozing/crusting, post-inflammatory hyperpigmentation or hypopigmentation**, and **patchy/irregular repigmentation** (including "polka-dot" or peripheral rim patterns when cell distribution or immobilization is suboptimal). Less commonly, patients may develop **secondary infection**, **persistent erythema**, or **textural change/scarring** if the recipient bed is prepared too deeply or if there is excessive inflammation. Importantly, even when initial repigmentation is excellent, **disease reactivation** can lead to partial pigment loss over time, highlighting why stability assessment and postoperative monitoring are integral to safety as well as efficacy. [38,39]

Technique-specific risks begin at the level of **recipient-site preparation**, because both dermabrasion and ablative lasers can cause complications if depth or energy delivery is not carefully controlled. Mechanical dermabrasion carries operator-dependent risks of **uneven depth** and occasional scarring when dermis is over-injured, whereas laser ablation introduces the potential for **thermal injury**,

prolonged erythema, or dyspigmentation if parameters are aggressive—particularly in higher phototypes. Immobilization failure (due to motion, friction, poor dressing fixation, or early dressing displacement) is a major practical "complication driver," because it increases cell loss and leads to **nonuniform take** rather than a discrete medical adverse event. Reviews of non-cultured epidermal cellular grafting repeatedly emphasize that complications are often preventable through standardized recipient-bed endpoints, meticulous hemostasis control (avoiding hematoma/seroma under the graft), and stable occlusive dressings. [38,40]

For **trypsinized NCES/MKTP**, additional safety considerations relate to the **enzymatic processing step** and the handling of a cell suspension. Although trypsinization is routinely used and generally well tolerated, it increases dependence on **controlled processing conditions** (sterility, temperature/time control, and trained personnel), and any breach can raise the risk of contamination or reduced cell viability—indirectly translating into poor clinical outcomes and the need for repeat procedures. In addition, because the suspension is free-flowing, there is a recognized risk of **cell run-off**, pooling, or uneven distribution, particularly on curved anatomical sites or mobile areas—often presenting clinically as patchiness rather than an acute complication. Comparative clinical work evaluating NCES versus the non-trypsinized Jodhpur technique reflects that both approaches can be safe, but their procedural vulnerabilities differ: NCES is more processing-sensitive, while simplified techniques can be more distribution/coverage-sensitive depending on how the graft material is prepared and secured. [41,42]

For **non-trypsinized techniques** (including simplified non-cultured non-trypsinized epidermal cell grafting), reported adverse effects are typically mild and include **transient donor-site discomfort**, occasional **donor-site pigmentary alteration**, and recipient-site **hyperpigmentation or textural change** when preparation is excessive. Because non-trypsinized preparations may contain **cell clusters/epidermal fragments**, a practical complication can be **clumping and uneven spread**, especially if the carrier medium/dressing strategy is not optimized; this again tends to manifest as nonuniform repigmentation rather than a medical safety event. Clinical reports of simplified non-trypsinized grafting followed by adjunct phototherapy underscore acceptable tolerability in stable vitiligo, with complications largely minimized through careful technique and postoperative care. Finally, from a venereology/andrology perspective, procedures on genital skin demand extra caution due to **higher friction**, **moisture**, and **difficulty immobilizing the area**, which can increase the risk of graft displacement and secondary irritation; strict postoperative instructions and protective dressing strategies are therefore central to "site-specific safety." [39,43]

Practical Advantages and Limitations

From a real-world dermatologic surgery standpoint, the choice between **trypsinized NCES/MKTP** and **non-trypsinized (e.g., Jodhpur technique)** is often determined less by "theoretical efficacy" and more by **infrastructure, reproducibility, time, and total procedural cost**. Reviews of surgical vitiligo therapy emphasize that cellular transfer is best suited for **stable, treatment-refractory disease**, but implementation varies widely between centers because some methods require a semi-laboratory workflow, trained personnel, and tight sterility/temperature control, whereas others were specifically designed to be feasible with minimal setup. This variability partly explains why reported success rates differ across studies even when patient stability and lesion types appear similar. [44,45]

A key advantage of **trypsinized NCES** is its potential for **higher expansion ratios** (treating a larger recipient surface with a smaller donor harvest) and the ability to create a **uniform single-cell suspension** that can be spread evenly—particularly helpful for large patches or geographically shaped lesions. However, the technique is comparatively **process-sensitive**: outcomes can be influenced by digestion conditions, cell handling, centrifugation, and the practical challenge of preventing run-off or pooling on curved or mobile sites. In addition, trypsinized NCES is more dependent on reliable consumables and protocol consistency, which can become a limiting factor in high-volume clinics or resource-limited hospitals. These practical constraints are repeatedly highlighted in clinical and procedural reviews discussing autologous melanocyte–keratinocyte transplantation. [44,46]

In contrast, **non-trypsinized approaches** (including the Jodhpur technique and simplified variants) are frequently promoted as **cost-effective, less time-consuming**, and **less dependent on laboratory infrastructure**, which can meaningfully broaden access to vitiligo surgery. Kachhawa and colleagues explicitly framed their simplified non-trypsinized epidermal cell grafting as a practical alternative to trypsin-based methods, aiming to reduce procedural complexity while still achieving satisfactory pigmentation outcomes. Similarly, point-of-care summaries and contemporary cell-therapy reviews discuss the Jodhpur technique as a low-cost innovation, reflecting its adoption where enzymatic processing is impractical. [47,48]

The limitations of non-trypsinized methods are primarily related to **standardization and distribution control**. Because these techniques may involve epidermal cell aggregates/fragments rather than a highly standardized single-cell suspension, there can be greater dependence on operator technique to ensure consistent spreading and intimate contact with the recipient bed, especially over large patches. Comparative clinical work (NCES vs Jodhpur technique) demonstrates that both can be effective in stable vitiligo, yet also underscores that differences in procedural steps and post-operative protocols can confound direct efficacy comparisons. In other words, "simpler" does not always mean "less effective," but it may mean outcomes are more influenced by meticulous dressing fixation, recipient-bed uniformity, and strict postoperative immobilization. [42,49]

Finally, patient-level and site-level practicality matters: mobile or high-friction areas (perioral, joints, and especially **genital skin**, relevant to venereology/andrology practice) demand stronger immobilization strategies and careful counseling about post-procedure behavior to avoid cell displacement and patchiness. In such sites, the "best" technique may be the one the team can deliver **most reproducibly**, with the least risk of processing error and the highest likelihood of maintaining stable graft contact for the first critical days. This practical reality aligns with broader syntheses of cell-based therapies for vitiligo, which emphasize that future progress depends not only on efficacy but also on protocol standardization and scalability across different clinical settings. [45,46]

Conclusion

Cellular melanocyte transfer has established itself as a cornerstone in the surgical management of stable vitiligo, offering a meaningful therapeutic option for patients who fail to achieve satisfactory repigmentation with medical therapy alone. Among the available surgical approaches, non-cultured epidermal cell grafting techniques—both trypsinized and non-trypsinized—have demonstrated consistent ability to restore pigmentation when applied in appropriately selected, clinically stable patients. Their shared biological foundation lies in the replenishment of functional melanocytes and the reconstitution of the melanocyte–keratinocyte unit within depigmented epidermis.

Trypsinized non-cultured epidermal cell suspension provides the advantage of controlled enzymatic separation and the potential for higher expansion ratios, making it particularly suitable for larger lesions and centers with access to laboratory support and trained personnel. In contrast, non-trypsinized techniques represent an important evolution toward procedural simplification, reduced cost, and broader accessibility, without necessarily compromising clinical effectiveness in stable disease. The choice between these methods is therefore rarely absolute; rather, it should be guided by lesion characteristics, anatomical site, available infrastructure, and the experience of the surgical team.

Clinical outcomes across both techniques are strongly influenced by non-technical factors, including rigorous stability assessment, meticulous recipient-site preparation, effective immobilization, and appropriate postoperative adjunctive therapy. Anatomical site remains a dominant predictor of success, with facial and non-acral lesions responding most favorably, while acral and high-friction areas require heightened technical precision and patient compliance. Importantly, complications are generally mild and preventable, emphasizing that standardized protocols and careful perioperative management are as critical as the choice of grafting technique itself.

In summary, trypsinized and non-trypsinized non-cultured epidermal grafting should be viewed as complementary tools within the surgical armamentarium for stable vitiligo rather than competing

modalities. Future progress in this field will depend on high-quality comparative studies, standardized outcome measures, and continued refinement of techniques to enhance reproducibility and long-term pigment stability. Such advances will ultimately help optimize individualized care for patients living with vitiligo.

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