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The Histologic and Genetics Aspects of skin grafting

A Search
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Requirements for the Degree of Bachelors degree of **Biology**

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بسم الله الرحمن الرحيم

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ

وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

(صدق الله العلي العظيم)

(المجادلة: ١١)

SUPERVISOR CERTIFICATION

I certify that the preparation of this project entitled **The Histologic and Genetics Aspects of skin grafting** prepared by **Njlaa Hatem Naaem** was under my supervision at General Biology Branch.

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Date: 2025 /   /

Dedication

To my dear brother,

The unwavering support who never left my side,

The soul who gave me strength in every moment of weakness—You have always been my inspiration and pride.

I dedicate this work to you in appreciation of your constant support and presence in my life.

And to my research supervisor,

Asst. Lect. Zainab Zamil,

With deep gratitude for your time, effort,

and valuable guidance,

I dedicate this research to you in recognition of

.your dedication and sincere academic support

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(وآخِرُ دَعْوَاهُمْ أَنِ الحمدُ للهِ رَبِّ الْعَالَمِينَ)

All praise is due to Allah, by whose grace good deeds are completed. With His blessings, I have completed this research, which represents the culmination of my academic efforts throughout my university journey.

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Abstract

Skin grafting is a crucial procedure for treating deep burns, chronic wounds, and major surgical defects. It involves the transplantation of skin tissue from the same patient or an external source to the affected area. It is one of the most vital techniques used in the restoration of damaged skin tissues. The current study was conducted to highlight the genetic and histological aspects related to skin grafting. The genetic aspect is discussed by examining the role of genetic engineering in skin grafting. Furthermore, the study explores the contribution of genetic engineering in developing bioengineered skin tissues that are more immunologically compatible for clinical use. The histological aspect focuses on the structural composition of the transplanted skin and its integration with the recipient site, emphasizing the importance of vascularization and tissue scaffolding in promoting healing and cellular regeneration.

In conclusion, the success of skin grafting depends on the complex interaction of genetic and histological factors, which underscores the need for further research and advancement in genetic and tissue engineering to enhance therapeutic outcomes and expand clinical applications in the future.

Chapter One
Introduction

1.1. Introduction

The skin is not only the largest organ of the human body but also the first line of defense against harmful influences such as mechanical forces, microorganisms, or radiation. It maintains thermoregulation and fluid balance as well as acts as a sensory organ that is able to register pressure, temperature, and pain, due to specific receptors. The integrity of human skin plays an essential role in maintaining physiological homeostasis of the body. A large skin loss caused by e.g., burns, can cause a disturbance of this integrity (Church, 2006; Sorg, 2017).

Skin grafting is the primary choice for closing large burn wounds, helping restore skin integrity effectively and promptly (Rowan et al., 2015). To date, autologous skin grafting is commonly considered as the gold standard for the coverage of large skin defects. While the usage of meshed split thickness skin grafting is the best option for the treatment of extensive burns, unmeshed sheet grafting is used for small burns and in aesthetically important regions. Full-thickness skin grafting achieves the best aesthetic and functional results in burn injury reconstruction (Brusselaers, 2010; Jeschke. 2020)

In 1970, Janzekovic demonstrated her concept of early excision and wound coverage with autologous split-thickness skin grafts (Janzekovic 1970). This method is considered as the current gold standard in surgical burn treatment, even today (Jeschke 2018; Jeschke 2020).

The RNA content of graft epithelial cells changes little in the first few days postgraft. By the fourth day postgraft RNA content increases greatly in the basal layers of epithelium, paralleling the hyperac- tivity of epithelial cells caused by acceleration of protein synthesis during a period of rapid cellular replication. By the 10th day postgraft the RNA level returns to normal. (Scothorne and Tough , 1952).

The human skin is a promising conduit for genetic engineering, as it is the largest and most accessible organ, epidermal autografts and tissue-engineered skin equivalents have been successfully deployed in clinical applications, and skin epidermal stem/progenitor cells for generating such grafts are easy to obtain and expand in vitro (Yue et al., 2017).

The aim of the study is to clarify the importance of skin grafting in addition to the histological and genetic aspect.

Chapter Two

Literatures Review

2.1. Skin Grafting history

Skin grafting dates back to ancient India, where Sushruta pioneered reconstructive techniques using skin flaps around 600 BCE (Vyas, 2021). Skin grafting has a long history dating back 3500 years ago and has undergone significant development over time (Hauben,1982). Ancient civilizations such as the Indians, Egyptians, and Romans used rudimentary techniques to apply skin from one body part to another. Sushruta, an ancient Indian physician, is considered the “father of surgery” and described techniques for reconstructing damaged noses and ears using skin grafts. Gaspare Tagliacozzi also developed techniques for reconstructing noses using skin grafts in the sixteenth and seventeenth centuries. In the nineteenth century, advance- ments in anesthesia and aseptic practices enabled more com- plex skin grafting procedures to be performed (Freshwater,1978). In the twentieth century, plastic and reconstructive surgery became established medical specialties leading to further refinement of skin grafting techniques. Innovations in tissue culture and graft preservation have improved the success and outcomes of skin grafting procedures. Additionally, tissue engineering and regenerative medicine have introduced new possibilities for the development of laboratory-grown (Trent,1998). Today, skin grafting is a widely used procedure in various surgical fields, with continuous improvement in techniques and technologies providing better outcomes and enhanced patient care.

2.2. Physiology of Skin Grafting

The survival of skin grafts is a complex process that involves different phases. In order to undestand them it is necessary to remember how the skin is irrigate Skin consists of 2 basic layers, the epidermis and dermis. The dermis is divided into a papillary and reticular layer. Within the dermis resides the skin's neurovascu- lar supply. The subcutaneous tissue beneath the skin contains the superficial fascia and subcutaneous fat (Losquadro, 2017)

The skin vasculature consists of a deep dermal/subcutaneous plexus and a superficial plexus (Losquadro, 2017). Both plexi are connected via communicating vessels (Ablove, 1997).

For the first 24–48 hours, the graft initially derives oxygen and nutrients from the underlying bed by diffusion (plasmatic diffusion or imbibition). The graft can increase in weight by up to 40%. During imbibition, the graft and the wound bed are held together via a layer of fibrin. The fibrin is eventually replaced by granulation tissue (gensen, 2008). The second phase, inosculation, is defined by an anastomosis of the preexistent vessels of the graft and the wound base. This phase occurs during the second and third postoperative days (Leal_khouri , 2012)

For graft-take tooccur, the recipient site must be capableof producing capillary buds. Since capillaryout growth is needed both to produce granulation tissue and to nourish askin graft, areas that granulate well (muscle and deep fascia), accept grafts readily. Other surfaces, such astendon denuded of its fascia covering, exposed bone, or cartilage, are incapableof producing granulation tissue and, therefore, are unable to nourish a skingraft (Lyon's, 1982). Also the skingraft must remain in close approximationto the recipient bed dur- ing thephase of capillary ingrowth. The presenceof air, serum, or fluid between the- graft and recipient bed creates a barrierthrough which the capillaries cannotgrow fast enough to prevent necrosis ofthe graft. Revascularization involves the growth, proliferation, and connection of vessels from the recipient base and sidewalls. The rate of revascularization is dependent on the thickness of the graft and the vascularity of the recipient bed. As a general rule, the thinner theskin graft, the faster it establishes a blood supply (Lyon's, 1982)

Within 4 to 7 days, full circulation has been restored to the graft. Restoration of lymphatic circulation also occurs within 7 days. Reinnervation of the graft begins approximately 2 to 4 weeks after grafting; however, full sensation may require several months or even years to return to normal (Adam's, 2005).

2.3. Definition of skin grafting

Skin grafting is a medical procedure used to replace damaged skin, often due to burns, injuries, or diseases. It involves transferring skin from a donor site on the patient or from a donor. The technique has evolved from early rudimentary methods to advanced practices, offering better outcomes, as shown figure (2-1).

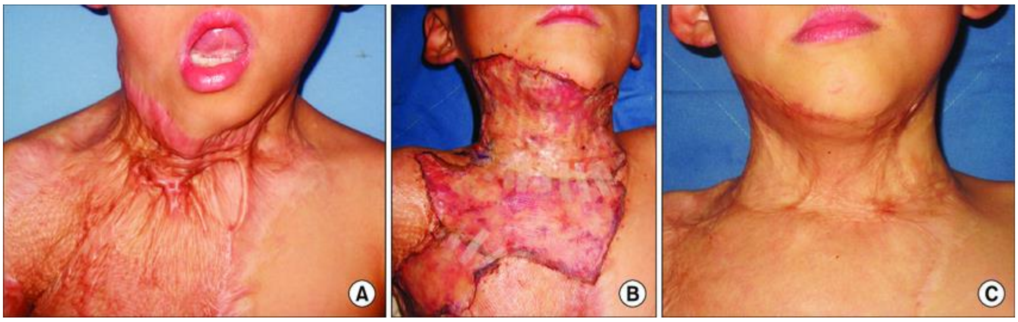

Figure (2-1) Skin grafts (Seo et al., 2014)

Skin grafting is vital for restoring the skin's protective function, promoting healing, preventing infections, and reducing scarring. It plays a significant role in cosmetic and functional recovery for patients with severe burns or skin defects. This procedure also accelerates recovery and improves quality of life. Skin grafting is essential in modern medicine to treat extensive skin damage and enhance long-term outcomes (Klasen, 2015).

2.4. Types of skin grafting

There are several type of skin grafting from which:

1. Split-thickness skin grafts (STSG) STSG are indicated for large defects (>5 cm), slow- or nonhealing chronic wounds, or as a temporary cover when monitoring a wound bed for potential cancer recurrence. (Brenner et al., 2017). This type of skin graft is useful when the definitive reconstruction of a wound is delayed, either for surveillance of an aggressive cancer or when granulation tissue is needed on the bed of the recepient site (Zhang and Meine, 2011), as shown figure (2-2).

Figure (2-2): Showing split thickness skin grafting (Borda et al., 2024)

2. Full-thickness skin grafts (FTSG) are very useful in dermatologic surgery, especially after the removal of a skin cancer, areas that are conducive to FTSG include nasal ala and tip, helix, medial can- thus, lower eyelid, digits, and extremitiesm, as shown figure (2-3).

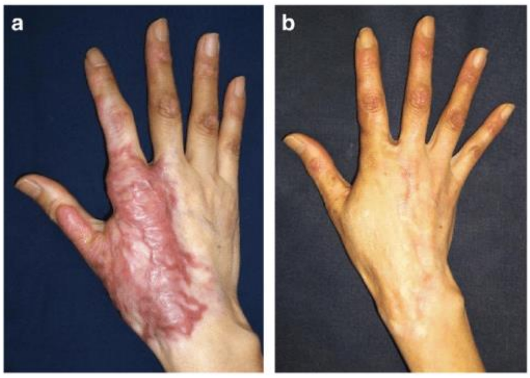

Figure (2-3): Showing full-thickness skin grafts

2.5. The Tissue Subjected to Skin Grafting

Tissue grafts can be categorized as autograft, allograft, or xenograft.

1. Autograft is described as the transfer of skin without the aid of a blood supply from the donor location to the recipient site. The epidermis and portions of the underlying dermis from the donor location are transferred using split-thickness skin grafts (STSGs). The whole layer of skin is harvested for full-thickness skin grafts (FTSGs) and used as the transplant (Seo et al., 2014).
2. Allograft

   The current uses of allograft skin among burn and plastic surgeons are threefold: the temporary coverage of freshly excised wounds, use as an overlay on widely expanded autografts, and its use to improve recipient bed quality and vascularity for a period of time prior to autograft application. In addition to burns, allograft is used in cases of skin loss caused by trauma (full thickness wounds with bone and/or tendon exposed), surgery (after the debridement of extensive, necrotizing soft tissue infections), donor sites for autologous skin grafts, reconstruction of critical facial defects, as temporary coverage (after laser-resurfacing and dermabrasion), and in the cases of disease (as temporary coverage for toxic epidermal necrolysis, congenital bullous epidermolysis, diabetic ulcers, venous ulcers, pressure and trophic ulcers) (Saxena, 2009).

3. **Xenograft**

   A xenograft is a skin graft taken from a dissimilar species (Hermans, 2014). In the human□to□mouse xenograft model, MSTCs from human plantar skin are implanted into full□thickness wounds on immunodeficient mice (Fuchs et al., 2024).

2.6. When to Use Soft Tissue Grafts

A connective tissue graft is used to convert thin to thick tissue. A common clinical situat you ion for the use of a connective tissue graft is when removing an anterior tooth that has lost labial bone and has thin overlying tissue, where gingival recession must be avoided. The patient with a high smile line and thin gingiva will have gingival recession if the tooth is removed and the thin gingiva is left without conversion. For these situations, the tooth is removed, the socket grafted with allograft or non-sintered xenograft, and a connective tissue graft is placed under the facial gingiva to convert thin to thick tissue (Michael and Block, 2017).

2.7. Complications of Skin Grafting

Complete or partial graft failure is the primary complication seen with FTSGs. Causes for failure includehematoma, graft-bed contact disruption, infection, smok- ing, and excessive electrocoagulation of the wound (Adams, 2005).

If the skin graft develops necrosis, it should not be debrided, the necrotic tissue serves as a natural dressing that allows new skin formation under it. There can be some contour alterations made by the healing process, especially on the nose (elevation), that can improve by themselves in a period of 6 months, if after that period the alterations remain dermabrasion or intralesional steroids can be used. The acute complications of STSG are the same of those presented by FTSG (hematoma, seroma, graft movement). Long term complications of STSG are related to skin graft contraction that may distort free margins, impair function, develop graft fragility or alter the final aesthethic result. If infection of the graft is suspected or developed a skin culture needs to be performed and proper antibiotics should be given.

In composite grafts short term potential complications, include bleeding, infection, and necrosis of the graft. This last complication presents initially as a yellow- ish whiteness that remains and then is followed by the aparition of a black eschar. Should the graft become necrotic, the escharshould not be debrided. The necrosis may only be superficial, with the underlying dermis and cartilage still viable. (Adams and Ratner, 2005). Long term complications of composite grafts are consequence of an inappropriate anchorage of the graft to the recipient site, trauma or extreme contractural forces, all of which can cause displacement or deformation of the graft.

2.8. Preparation of Skin Grafting

The following are some necessary prerequisites needed to carry out the procedure of wound grafting successfully:

- Watson knife, Goulian blade
- scalpel
- electrocautery
- dermatome
- mineral oil
- skin mesher
- suture or staples, as shown figure (2-4).

Figure (2-4) Shows some necessary prerequisites needed to carry out the procedure of wound grafting successfully (Adil et al., 2023).

An appropriate team includes a well-trained surgeon, an anesthesiologist, a scrub tech, and a scrub nurse. Preparation of the wound bed is essential to a successful procedure. Early excision and debridement to healthy tissue are vital to the graft's healing. The standard of care for successful grafting is a 95% graft take. Removing eschar and necrotic tissue promptly lowers the risk of developing a wound infection. This is essential to preventing the loss of the graft. Early excision has also been proven to decrease the number of contractures, scarring, and skin tightness, and patients often are rehabilitated faster. The eschar is removed in layers until healthy tissue is identified by diffuse punctate bleeding with a Watson knife or Goulian blade. This is known as tangential excision. Fascial excision allows for faster resection and provides a plane ready for grafting but can lead to subpar cosmetic results. Wounds over joints need early excision and grafting to help prevent excessive contracture (Jones et al., 2017). Before placing the graft, all granulation tissue should be excised as it can have a significant bacterial burden and prevent adherence to the graft. Skin margins should be freshened to expose healthy dermis. Hemostasis must be ensured to prevent the formation of a hematoma, failing the graft (Elseth, 2020).

2.9. Technique or Treatment of Skin Grafting

Full-thickness skin grafts include both the epidermis and the dermis. The donor site selection is carefully considered for optimal cosmetic outcome and control of discomfort. Cosmetic outcome is based on desired pigmentation, texture, and skin thickness (Hazani et al., 2012). These skin grafts minimize the degree of contracture across joints and on the face and fingers (Koljonen et al., 2011). Full-thickness grafts are chosen from the flank, groin, hypothenar eminence, pre- and post-auricular areas, or the forearm. The skin is shaved, and the tumescent is injected into the tissue below the intended donor site. The skin is then gently harvested using a scalpel, customizing the shape to that of the area to be grafted. Sharp dissection is preferred as electrocautery can damage the donor graft very easily. The graft removes some of the subcutaneous tissue. It is then taken to the back table and “defatted” to remove the excess tissue. The donor site is then closed primarily. When placing the full-thickness skin graft, it is sutured along the edges using rapidly dissolving sutures. Many surgeons use quilting sutures to help hold the graft in place, preventing both shear and empty space in which a hematoma or seroma could develop. Larger grafts (>3 cm diameter - Stephenson) risk failure because the surface area is too large for adequate imbibition (Elseth, 2020), as shown figure (2-5)

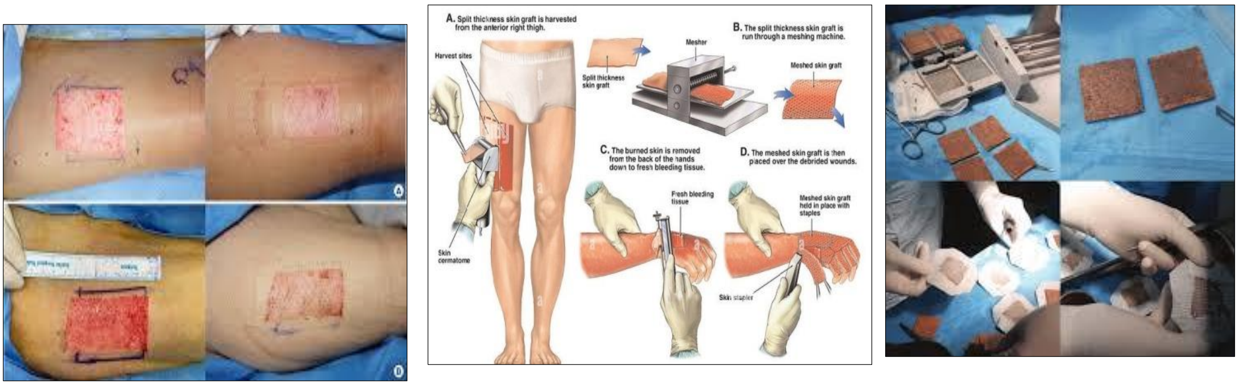

Figure (2-5) Showing technique of Skin Grafting (Dahmardehei et al., 2020; Jun et al., 2020).

2.10. Genetic Aspects of Skin Graft:

Somatic gene therapy is a promising approach for treating otherwise terminal or debilitating diseases. The human skin is a promising conduit for genetic engineering, as it is the largest and most accessible organ, epidermal autografts and tissue-engineered skin equivalents have been successfully deployed in clinical applications, and skin epidermal stem/progenitor cells for generating such grafts are easy to obtain and expand in vitro. Here, we develop skin grafts from mouse and human epidermal progenitors that were engineered by CRISPR-mediated genome editing to controllably release GLP-1 (glucagon-like peptide 1), a critical incretin that regulates blood glucose homeostasis. GLP-1 induction from engineered mouse cells grafted onto immunocompetent hosts increased insulin secretion and reversed high-fat-diet-induced weight gain and insulin resistance. Taken together, these results highlight the clinical potential of developing long-lasting, safe, and versatile gene therapy approaches based on engineering epidermal progenitor cells (Yue et al., 2017), as shown figure (2-6)

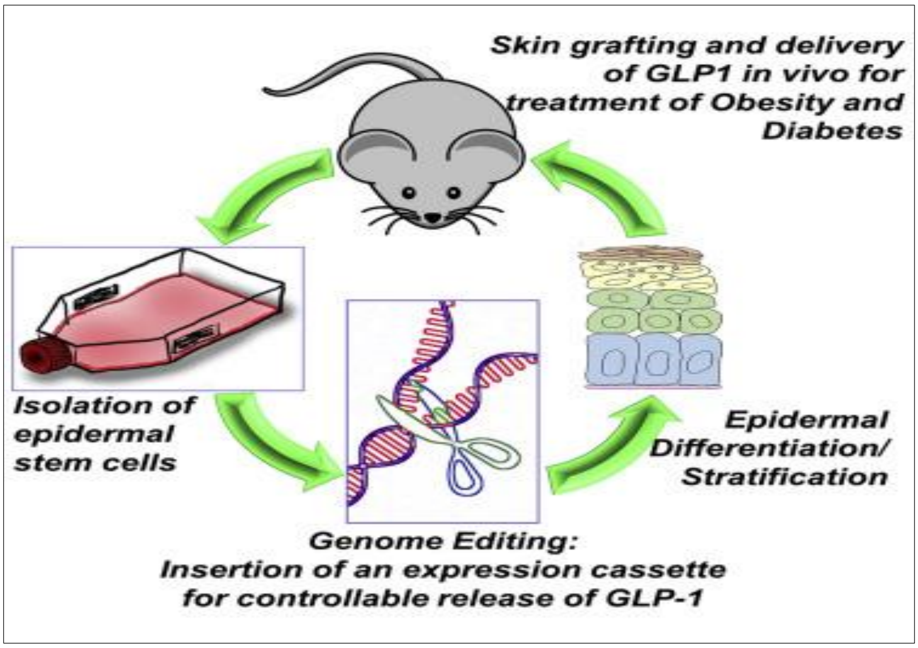

Figure (2-6) Showing CRISPR-based editing of epidermal stem cells provides inducible GLP-1 expression

Two different gene delivery methods can be used to achieve the genetic modification of skin: ex vivo and in vivo. The ex vivo approach consists of the isolation and in vitro propagation of skin cells (either keratinocytes or fibroblasts or both). Then, cells are genetically modified before grafting them back to the patient as part of a tissue-engineered skin equivalent. In the in vivo setting, direct gene transfer is accomplished either through the delivery of plasmidic DNA (by direct injection, biolistic "gene gun" or electroporation) or using viral vectors (such as lentivirus, retrovirus and adenovirus) (Carretero et al., 2006).

Alcohol use disorder (AUD) is one of the foremost public health problems. Alcohol is also frequently co-abused with cocaine. There is a huge unmet need for the treatment of AUD and/or cocaine co-abuse. We have developed and used a skin stem cell-based gene delivery platform and found that production of the glucagon-like peptide-1 (GLP1) from the grafted genetically modified skin reduced development and reinstatement of alcohol-induced drug-taking and seeking, voluntary oral alcohol consumption and alcohol-induced increase in dopamine (DA) levels in the nucleus accumbens (NAc). Moreover, we have developed a novel co-grafting procedure for both modified human butyrylcholinesterase (hBChE)- and GLP1-expressing cells. Skin grafts-derived hBChE and GLP1 reduced acquisition of drug-taking and toxicity induced by concurrent alcohol and cocaine injections. These results imply that gene delivery through skin transplants may add a new option to treat drug abuse and co-abuse.We previously developed a skin stem cell-based long-term gene delivery approach in mouse models that enables us to genetically engineer the skin stem cells with the CRISPR/CAS9 technology and transplant these engineered cells to normal animals via skin grafting, Doxycycline inducible expression of GLP1 in grafted animals can reverse diet-induced obesity and diabetes ((Kong et al., 2021)), as shown figure (2- 7).

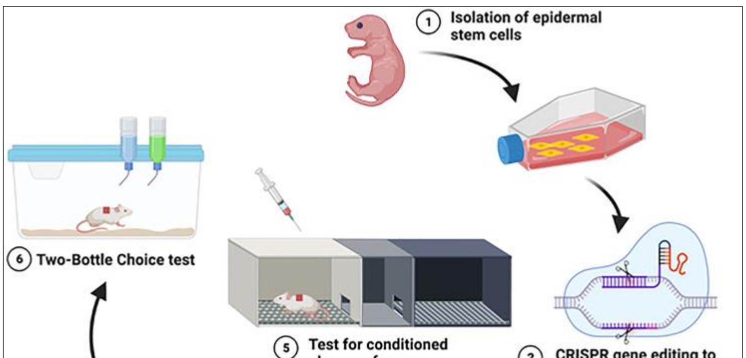

Figure (2-7) Shows using a skin cell-based gene delivery platform to reduce alcohol and/or cocaine abuse.

Chapter Three

3. Discussion:

Skin graft is one of the most indispensable techniques in plastic surgery and dermatology. Skin grafts are used in a variety of clinical situations, such as traumatic wounds, defects after oncologic resection, burn reconstruction, scar contracture release, congenital skin deficiencies, hair restoration, vitiligo, and nipple-areola reconstruction. Skin grafts are generally avoided in the management of more complex wounds. Conditions with deep spaces and exposed bones normally require the use of skin flaps or muscle flaps (Shimizu-Hayashi & Kishi, 2012). In this study, we reviewed the technical of skin grafting and provide importance -of- technique in treatment of skin disorder because burns or some disease and evidence supporting the efficacy of cutaneous gene therapy for skin disorders in human and mouse model. In addition to expression of a hBChE and GLP1. Skin grafts-derived hBChE and GLP1 reduced acquisition of drug-taking and toxicity induced by concurrent alcohol and cocaine injections.Thus, the applicability of cutaneous gene therapy by CRSPIR (Cas9) and skin grafting is very importance and broad. Zimmer et al mentioned that the CRISPR-Cas9 gene editing holds promise for treating pediatric epidermolysis bullosa (EB), a group of genetic disorders characterized by severe skin fragility. The application of CRISPR-Cas9 in correcting genetic mutations associated with various types of EB. Preclinical studies have demonstrated the potential of CRISPR-Cas9 to target and correct mutations in genes such as COL7A1, LAMB3, and COL17A1, which are implicated in different EB subtypes. Although the combination of CRISPR gene therapy and skin grafting is important, at the same time, gene therapy using CRISPR for skin grafts may carry the risk of undesirable outcomes. Meuli & di Summa mentioned that the Combined with current genome editing tools such as the CRISPR-Cas9 system, such genetically engineered skin grafts could not only provide coverage for the wound but could also be used as a therapy for the underlying condition that caused the wound to appear in the first place, in non-traumatic cases. The major issue with such genetically engineered tissues is the same as with the direct application of growth factors to the wound: the similarity of several cellular pathways in wound healing and in oncogenesis carries a significant risk of iatrogenic-induced carcinomas. While providing thrilling opportunities for fundamental research groups, as the occurrence of cancer in an experiment can be seen as a potential target for treatment as well, this seriously limits the application and trials of such products on living humans for the time being.

Chapter Four
Conclusion & Recommendations

4.1. Conclusion:

1. Skin grafting is a vital procedure for compensating skin loss caused by burns, injuries, or diseases, as it restores the protective function of the skin.

2. The success rate of skin grafts varies depending on the graft type, the patient's health condition, and the surgical technique used.

3. Autografts have the highest success rate compared to allografts or xenografts due to better tissue compatibility.

4. Proper preparation of the recipient bed is critical for graft survival, including thorough debridement and achieving hemostasis.

5. Advanced genetic techniques, such as CRISPR-modified epidermal stem cells, show promising potential in enhancing graft outcomes and treating chronic conditions.

6. Common complications such as infection, bleeding, partial or complete graft failure, and contraction can be minimized with modern and precise surgical practices.

4.2. Recommendations:

1. We recommend that Autologous skin grafts should be prioritized to reduce graft rejection and ensure higher success rates.

2. We recommend that the medical staff be well-trained in the latest techniques for graft bed preparation and accurate surgical execution.

3. We recommend that genetic and stem cell engineering should be incorporated into future grafting strategies to expand their therapeutic uses.

4. We recommend that the Implement early diagnostic tools to predict potential graft failure and intervene promptly.

5. We recommend further practical research to explore the combination of skin grafting and gene therapy in dermatological and systemic diseases.

6. We recommend following strict infection control protocols during all grafting procedures to prevent graft loss due to contamination.

Chapter Five
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